Coconut Milk, Soy Milk, and Fish Flour as *Bacillus sphaericus*'s Alternative Media Material Used in the Control of *Anopheles sp*.

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ABSTRACT---- ***Some materials can be used to suppress mosquito larvae. One is entopathogenic microbial-based biopesticides, such as Bacillus thuringiensis for the control of Aedes aegypti larvae and Bacillus sphaericus to suppress Culex and Anopheles larvae. B.sphaericus does not require carbohydrates in its growth, because it lacks the enzymes necessary to enter carbohydrates and convert them into energy sources. However, various materials rich in protein and fat are reported to support their growth, even from simple sources. Coconut milk without water, fish flour and soy milk are common food ingredients consumed by the people of Indonesia. The purpose of this research is to know the ability of simple media with 30% v / v coconut milk formula, 30% v / v soy milk, 30% w / v fish flour as a simple medium for B. sphaericus growth. The results showed that a simple medium with a formula of 30% coconut milk showed the most fertile B. sphaericus growth of 296 x 108 cells / ml, fish flour 30% w / v 291 x 108 cells / ml and soy milk 30% v / v 89 x 108 Cell / ml. The results of Bioassay B. sphaericus test on coconut milk media, Anopheles sp larvae that died from 24 hours 15 larvae, at 42 hours and 72 hours killed 20 larvae.***

Keywords--- Alternative media, B.sphericus, Anopheles sp.

1.INTRODUCTION

The incidence of malaria in NTB population according to Riskesdas results in 2013 there are five districts / cities with the highest incidence of malaria. A proven effective and safe approach in larval control is suppressing the development of mosquito larvae stage. The use of biopesticides has proven to be effective and safe to apply. One is entopathogenic microbial-based biopesticides, such as Bacillus sphaericus to suppress Culex and Anopheles larvae (California Department of Public Health, 2008). A variety of protein and fat rich ingredients are reported to support *B. Sphaericus* growth. Yadav et al in 2011 reported some simple materials that could be used to grow *B. sphaericus* entopathogenik in the form of a liquid medium while maintaining its toxicity properties. Products of entopathogenic bacteria based biopesticides (*B. thuringiensis* and *B. sphaericus*) are mostly produced overseas which are hard to find and their breeding needs special media.

Suryadi et al (2015) isolated *B. sphaericus* from several locations on Lombok Island that could kill Culex, Anopheles and Aedes mosquito larvae within 24 to 48 hours which are expected to support the development of local-based biopesticides. Formula biopestisida with base material of bacterium *B. sphaericus* local isolate of Lombok Island which can be developed using simple media for growth of *B. sphaericus* isolate from Lombok Island of Coconut Milk 30% v / v, Milk Soybean 30% w / v, Sea Fish Flour with concentration 30% w / v.

Coconut milk without water addition is the usual food consumed by the people of Indonesia. Coconut milk without water addition contains energy of 324 kilocalories, 4.2 grams of protein, 5.6 grams of carbohydrates, 34.3 grams of fat, 14 milligrams of calcium, 45 milligrams of phosphorus, and 2 milligrams of iron. Also in the Coconut milk without water addition contained vitamin A as much as 0 IU, vitamin B1 0.02 milligrams and vitamin C 2 milligrams. Fish flour is a common food ingredient consumed by the people of Indonesia. Fish flour contains 316 kilocalorie energy, 60.1 gram protein, 22.4 grams of carbohydrate, 6.5 grams of fat, 3196 milligrams of calcium, 1976 milligram phosphorus, and 16.6 milligrams of iron. Also in Fish flour contained 1083 IU of vitamin A, vitamin B1 0 milligram and vitamin C 0 milligram. The results obtained from the research on 100 grams of fish flour, with the amount of edible as much as 100%. Soy milk contains 38% of Soya protein; 18% Unsaturated Poly Acid Fat; 15% Carbohydrates; 15% Fiber Phytonutrients such as lecithin, Isoflavones and Saponins; Minerals, such as Calcium, Magnesium, Iron, Phosphorus,

Potassium, Selenium and Zink. Protein Digestibility Corrected Amino Acid Score (PDCAAS) values 1.0.

The purpose of this research is to know the ability of simple media with 30% v / v coconut milk formula, 30% v / v soybean milk, 30% w / v fish meal as growth media of *B. sphaericus* local isolate of Lombok Island used in control *Anopheles sp*. The medium is a material composed of a mixture of nutrients / substances used to grow microbes. The composition and levels of nutrients in a medium for microbes must be balanced so that microbial growth can be as good as possible Although the requirements of microbial nutrients are very diverse, their names as living creatures have the same basic needs, which include water, carbon, energy, minerals and growth factors. (Murray, 2005)

Coconut milk without water addition is the usual food consumed by the people of Indonesia. Coconut milk without water addition contains energy of 324 kilocalories, 4.2 grams of protein, 5.6 grams of carbohydrates, 34.3 grams of fat, 14 milligrams of calcium, 45 milligrams of phosphorus, and 2 milligrams of iron. Also in the Coconut Coconut Flat without Water also contained vitamin A as much as 0 IU, vitamin B1 0.02 milligrams and vitamin C 2 milligrams.

Fish flour is a common food ingredient consumed by the people of Indonesia. Fish flour contains 316 kilocalorie energy, 60.1 gram protein, 22.4 grams of carbohydrate, 6.5 grams of fat, 3196 milligrams of calcium, 1976 milligram phosphorus, and 16.6 milligrams of iron. Also in fish flour contained 1083 IU of vitamin A, vitamin B1 0 milligram and vitamin C 0 milligram. Soy milk contains 38% Soya Protein; 18% Acid Unsaturated Fatty Fats; 15% Carbohydrate; 15% Fiber Phytonutrients such as lecithin, Isoflavones and Saponins; Minerals, such as Calcium, Magnesium, Iron, Phosphorus, Potassium, Selenium and Zink. Protein Digestibility Corrected Amino Acid Score (PDCAAS) values 1.0.

B. sphaericus is a Gram-positive, rod-shaped bacterium capable of forming endospores of terminals with enlarged sporangium (swollen sporangium) that can be isolated from the soil (Baumann et al 1991). Most strains of *B. sphaericus* grow using acetate as a source of carbon present in the soil and decomposed plant remains. *B. sphaericus* requires thiamin or biotin (or both) and some strains require glutamate. These bacteria can not grow on a medium containing only glucose as the only carbon source. Its inability to use glucose can serve as one of the biochemical characters to detect *B. sphaericus*. This is due to the absence of enzyme systems that allow the transport of glucose and sucrose into cells (Baumann et al, 1991). *B. sphaericus* is generally able to kill mosquito larvae from the genus Culex and Anopheles, but is less able to kill the genus larvae Aedes (Berry et al., 1993) *B. sphaericus* bacteria can be grown on several growth media types. Commonly used media are NYSM media (Myers and Yousten, 1978) and MBS (Kalfon et al, 1984).

Anopheles adult mosquito is a vector that causes malaria. Anopheles species proven as vectors are *Anopheles aconitus*, *Anopheles nigerrimus*, *Anopheles sundaicus*, *Anopheles leucosphyrus* and others. Anopheles Female mosquitoes can survive for a month (Damar T, 2008). Anopheles mosquito larva has a head and mouth used for feeding, a piston and a stomach, has no legs yet. The larvae do not have a respiratory tract and for body positions are parallel to the surface of the water. Larvae breathe with a vent on the abdomen because it is on the surface. Food larvae of algae, bacteria and other microorganisms on the surface. Larvae are found in clean water and brackish water that has salinity, mangrove swamps, wetland water, grass-covered ditches, riverbanks, and puddles of rainwater

2. METHOD

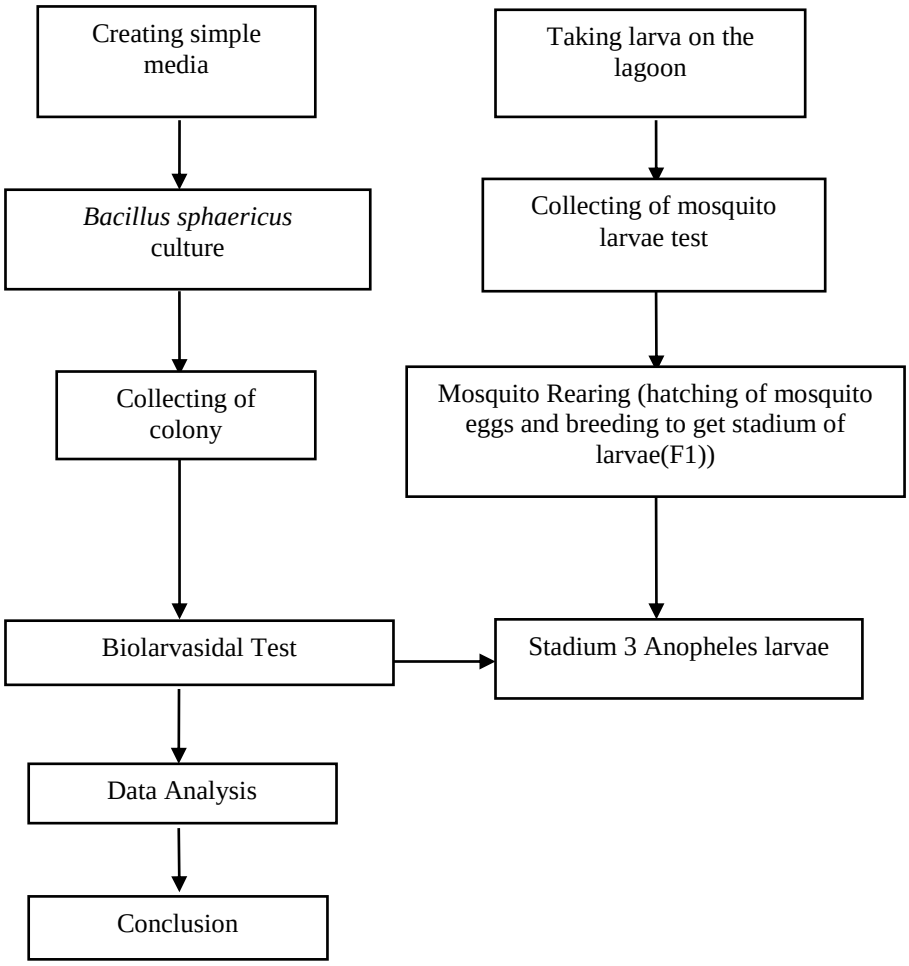
This research is an exploratory research in laboratory. The research unit used was *Bacillus sphaericus* bacteria, Simple Growth Media: 30% v / v coconut milk, 30% v / v soy milk, 30% w / v fish meal, Larva *Anopheles sp.*

Large unit of research needed to know the ability of simple media in growing *Bacillus sphaericus* of local isolate of Lombok Island in *Anopheles sp*. larvae control determined by using sample at least 3 times replication in laboratory study according to Kemas hanafiah (2010). For a simple media ability test in growing *Bacillus sphaericus* local isolate of Lombok Island in *Anopheles sp*. larvae control of each media formula replication 3 times. Thus, the major research units are 4 kinds of media (NYSM, 30% w / v fish meal, 30% v / v soy milk and 30% v /v)x 3 replication = 12 research units.

Materials and media used in research:

1) Isolate *B. sphaericus*: Isolate *B. sphaericus* used in this study was *B. sphaericus* isolate MNT isolated by Suryadi et al (2015) capable of killing *Culex* mosquito larvae, *Anopheles sp*, and *Aedes* instar III done in the laboratory.
2) Media type used: Standard medium NYSM (Nutrient Broth 8.0 g / L; Yeast extract 0.5 g / L; MgCl2 0.2 g / L; MnCl2 0.01 g / L; and CaCl2 0.1 G / L) (Myers and Yousten, 1978), are used as controls.
3) Medium made formula of 30% v / v coconut milk, 30% v / v soy milk, and 30% w/v Fish Flour.

The following procedures are performed for the research are:



Tests were performed by implanting *B. sphaericus* isolates on four mediums: NYSM Solid medium, Medium modest natural coconut milk 30% v / v broth, Medium Soy milk 30% w / v broth and Medium Flour Fish 30% w / v broth. After that, Colonization of *Anopheles* mosquito larvae to get instar larvae of mosquito instar III (F1) which will be tested Bioaasay (Biological test) then do Bioassay Procedure (Biological Test) which aims to test to get LC (Lethal Concentration). Done by:

a) Cultures of *B. sphaericus* used were cultured in a liquid medium with an incubation time of 72 hours. Each containing 200 ml of sterile aquadest added with 20 larvae of *Anopheles Sp* instar III and spherically-diluted *B. sphaericus* culture.

b) The test is done at room temperature with exposure time 24, 48 and 72 hours.

c) Medium control using a container containing 200 ml of sterile aquadest mixture containing 10% v / v growth medium (without bacterium *B. sphaericus*) and 20 *Anopheles sp* instar III larvae.

d) Water control using a container containing 200 mL of sterile aquadest and 20 larvae of *Anopheles sp* instar III.

e) The number of dead larvae in each container is then recorded.

f) If the larvae death occurs in the first container (medium control) and second (water control), then the test should be repeated.

g) The test should be repeated, if 10% of the test and control larvae have been transformed into pupae, as this condition illustrates that the larvae are in the non-eating conditions.

Testing should be repeated, if there are deaths in the control group more than 20%. The mortality of the test larvae should be corrected with the Abott formula if there are deaths in the control group of 5 -20%.

Formula Abott:

$$\frac{\text{Mortality of treatment group} - \text{Mortality control group X } 100\%}{100 - \text{mortality control group}}$$

(Umniyati, 2008)

Data from the observation of colony growth on each simple media were analyzed descriptively. The biolarvasidal ability data of *B. sphaericus* grown from each simple medium were the number of dead larvae, cell / endosporal concentration and number of replicates in each container then tabulated and analyzed using Probit Analysis with the help of MINITAB 16 software to obtain LC50 and LC90 values from Each isolated *B. sphaericus* bacterium obtained (Dulmage et al 1991 and Minitab 17 Support, 2015).

3. RESULTS

Result of calculation of bacterium *B. sphaericus* to Calculate Colony Method of pour plate from each treatment medium as shown in table 5.1 as follows:

Table 5.1. Calculation of the number of *B. sphaericus* bacteria to Calculate Colony Method of pouringplate

no.	Media Type	Number of Bacteria x 10^8 cells / ml			Total	Average x 10^8 cells / ml
		P1	**P2**	**P3**		
1.	*NYSM*	363	352	346	1.061	353
2.	Fish Flour 30% w/v	298	285	292	875	291
3.	Soy Milk 30% v/v	86	89	92	267	89
4.	Coconut milk 30 % v/v	304	296	289	889	296

Information:
P1 : Dilution 1
P2 : Dilution 2
P3 : Dilution 3

From the data table 5.1 shows that *B. sphaericus* grow most fertile on simple natural media Santan with concentration of 30% v / v that is the number of *B. sphaericus* bacteria 296 x 108 cells / ml, then followed by fish meal flour with concentration 30% w / V 291 x 108 cells / ml and Soy milk 30% v / v 89 x 108 cells / ml. The growth of bacterial cells on NYSM standard media is 353x 108 cells / ml.

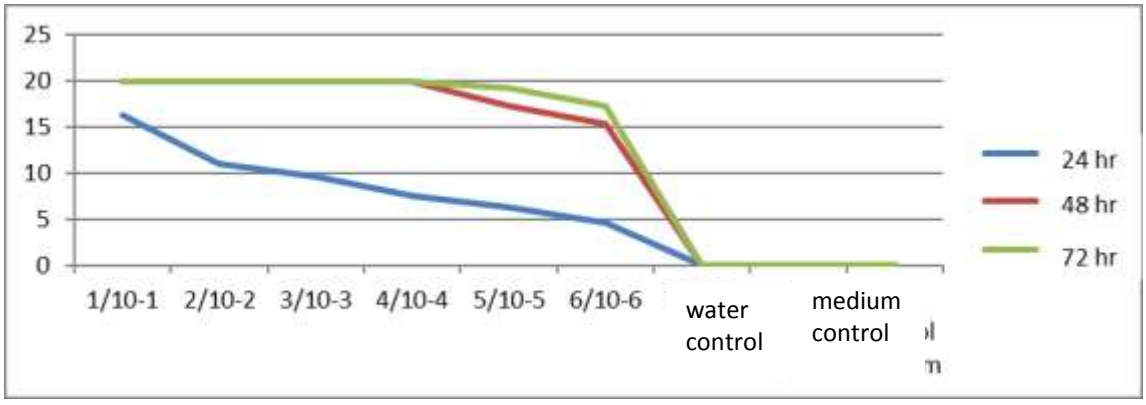
Table 5.2. Calculation of the number of *B. sphaericus* bacterial cells from each simple medium

No	Media type	Number of Bacteria x 10^8 cells / ml			Total	% Endospora
		Vegetative cells	**Vegetable cells with endospores oval terminal**	**Endospora**		
1.	*NYSM*	204	477	1287	968	65,39%
2.	Fish Flour 30% w/v	314	504	1027	1845	55,66%
3.	Soy Milk 30% v/v	344	577	855	1776	48,1%
4.	Coconut milk 30 % v/v	232	568	1089	1889	57,6%

Table 5.2 shows that the percentage endospora bacterial cell of *B. sphaericus* on standard media of NYSM is 65,39%, whereas for simple media the percentage of endospora is mostly formed in coconut milk 30% v / v (57,6%) followed by fish meal medium 30% W / v (55.66%) and Soy Milk 30% v / v (48.1%).

Table 5.3. Mean larva mortality with *Bacillus sphericus* bacteria from NYSM media 24 hours, 48 hours and 72 hours Petridish No / Dilution Mean larva mortality in time 24 hours 48 hours 72 hours

Petridsh No / Dilution	**Mean larva mortality in time**		
	24 hours	**48 hours**	**72 hours**
1/ 10^{-1}	16	20	20
2/ 10^{-2}	11	20	20
3/ 10^{-3}	10	20	20
4/ 10^{-4}	8	20	20
5/ 10^{-5}	6	17	19
6/ 10^{-6}	4	15	17
7/ 10^{-7}	0	0	0
8/ Water Control	0	0	0
9/ Medium Control	0	0	0

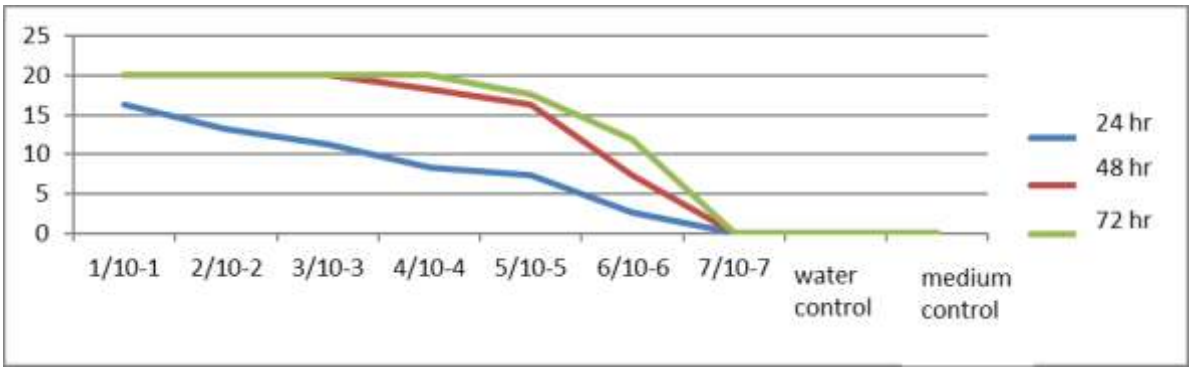


Graph 5.1. Average of Bioassay Larvae test results with *Bacillus sphericus* bacteria from NYSM media observation for 24 hours, 48 hours and 72 hours.

Table 5.3 and graph 5.1 show that on NYSM standard media, *B. sphaericus* has been able to kill larvae 16 larvae at 24 hours observation, and observation time of 48 hours and 72 hours of dilution 10-1, 10-2,10-3, and 10-4 shows larvae that died 20 larvae (100%).

Table 5.4. Average of Bioassay Larvae test results with *Bacillus sphericus* bacteria from fish meal medium 30% w/v observation of 24 hours, 48 hours and 72 hours

Petridsh No / Dilution	**Mean larva mortality in time**		
	24 hours	**48 hours**	**72 hours**
1/ 10^{-1}	16	20	20
2/ 10^{-2}	13	20	20
3/ 10^{-3}	11	20	20
4/ 10^{-4}	8	18	20
5/ 10^{-5}	7	16	18
6/ 10^{-6}	3	7	12
7/ 10^{-7}	0	0	0
8/ Water Control	0	0	0
9/ Medium Control	0	0	0

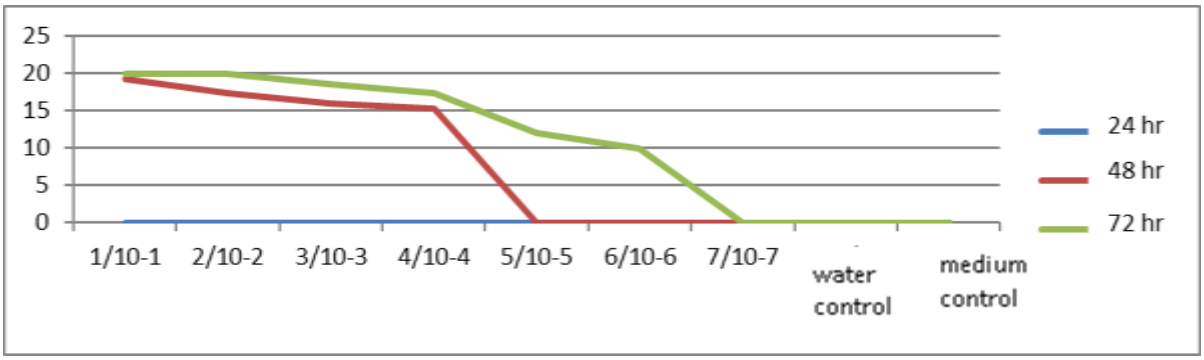


Graph 5.2. Average of Bioassay Larva test result with *Bacillus sphericus* bacteria from fish meal medium 30% w/v observation of 24 hours, 48 hours and 72 hours

Table 5.4 and graph 5.2 show that on 30% w / v fish meal medium, *B. sphaericus* has been able to kill larvae 16 larvae at 24 hours observation, and observation time of 48 hours and 72 hours of dead larvae of 20 larvae (100%) of Dilution 10-1, 10-2, and 10-3.

Table 5.5. Average of Bioassay Larvae with *Bacillus sphericus* from Soy Milk 30% v / v observation of 24 hours, 48 hours and 72 hours Grail No / Dilution Mean larva mortality in time 24 hours 48 hours 72 hours

Petridsh No / Dilution	**Mean larva mortality in time**		
	24 hours	**48 hours**	**72 hours**
1/ 10^{-1}	0	19	20
2/ 10^{-2}	0	17	20
3/ 10^{-3}	0	16	19
4/ 10^{-4}	0	15	17
5/ 10^{-5}	0	0	12
6/ 10^{-6}	0	0	10
7/ 10^{-7}	0	0	0
8/ Water Control	0	0	0
9/ Medium Control	0	0	0

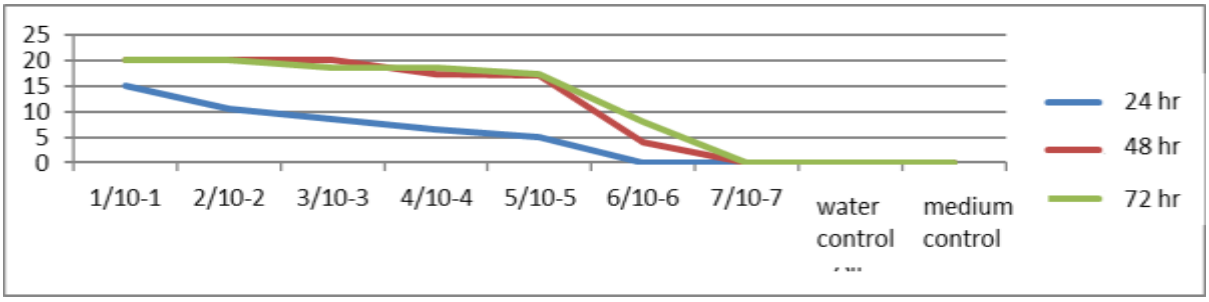


Graph 5.3 Average Bioassay test results Larvae with *Bacillus sphericus* bacteria from medium Soy milk 30% v/v observation of 24 hours, 48 hours and 72 hours

Table 5.5 and graph 5.3 show that on 30% v / v soy milk media, the new *B. sphaericus* showed its toxicity to *Anopheles sp* larvae after 48 hours of observation of 19 larvae and at 72 hours observation was able to kill larvae 20 larvae (100%) of Dilution 10-1, and 10-2.

Table 5.6. Average of Bioassay Larva test result with *Bacillus sphericus* bacteria from medium coconut milk 30% v/v observation of 24 hours, 48 hours and 72 hours

Petridsh No / Dilution	**Mean larva mortality in time**		
	24 hours	**48 hours**	**72 hours**
1/ 10^{-1}	15	20	20
2/ 10^{-2}	11	20	20
3/ 10^{-3}	9	20	20
4/ 10^{-4}	7	17	19
5/ 10^{-5}	5	17	17
6/ 10^{-6}	0	4	8
7/ 10^{-7}	0	0	0
8/ Water Control	0	0	0
9/ Medium Control	0	0	0



Graph 5.4. Average Bioassay test results Larva with *Bacillus sphericus* bacteria from medium coconut milk 30% v/v observation of 24 hour, 48 hour and 72 hour

Table 5.6 and Graph 5.4 show that in coconut milk 30% v / v media, *B. sphaericus* has been showed the toxicity to *Anopheles sp* larvae since 24 hours of observation that is amount of larvae that died 15 larvae and on observation 42 hours and 72 hours was able to kill larvae 20 Larvae (100%) from dilution 10-1, 10-2 and 10-3

Table 5.7. Calculation results of Letal Concentration (LC 50 and LC90) Bioassay of Biolarvasidal *Bacillus sphericus* from NYSM media.

Concentration of	3.53E+08	cells/ml
Endospora	65.39	%
LC50-24 hours	1.20E+07	cells/ml
LC50-48 hours	9.97E+02	cells/ml
LC50-72 hours	5.65E+02	cells/ml
LC90-24 hours	4.49E+07	cells/ml
LC90-48 hours	3.78E+03	cells/ml
LC90-72 hours	2.51E+03	cells/ml

Table 5.7 shows that LC_{-50} of 24 hours (1.20E + 107), meaning within 24 hours to kill 50% larvae required 1.20 x 10^7 cells / ml of *B. sphaericus* bacteria. LC_{-50} of 48 hours (9.97E + 02) within 48 hours to kill 50% larvae required 9.97 x 10^2 cells / ml of *B. sphaericus* and LC_{-50} 72 hours (5.65E + 02) bacteria within 72 hours to kill 50 % Larvae required 5.65 x 10^2 cells / ml of *B. sphaericus* bacteria, while LC_{-90} of 24 hours (4.49E + 07) within 24 hours to kill 90% larvae required 4.49 x 10^7 cells / ml of *B. sphaericus* bacteria, LC_{-90} of 48 hours (3.78E + 03) within 48 hours to kill 90% larvae required 3.78 x 10^3 sel / ml of *B. sphaericus* and LC_{-90} of 72 hours (2.51E + 03) bacteria within 72 hours to kill 90% of larvae required 2.51 x 10^3 sel / ml of *B. sphaericus* bacteria. On the NYSM media getting lower in accordance with the long observation. The lower LC_{-50} and LC_{-90} possessed by a bacterium at the time of observation 48 hours and 72 hours, the higher the bacterial toxicity.

Table 5.8. Result of calculation of Letal Concentration (LC 50 and LC90) Bioassay of Biolarvasidal *Bacillus sphericus* from fish meal medium 30% w/v.

Concentration of	2.91E+08	cells/ml
Endospora	55,66	%
LC50-24 hours	9.08E+06	cells/ml
LC50-48 hours	6.28E+03	cells/ml
LC50-72 hours	9.56E+02	cells/ml
LC90-24 hours	3.64E+07	cells/ml
LC90-48 hours	2.61E+04	cells/ml
LC90-72 hours	2.73E+03	cells/ml

Table 5.8 shows the lethal concentration (LC) test of *B. sphaericus* inoculated on simple medium Fish flour 30% w / v is LC50-24 hours (9.08E + 06) that means within 24 hours to kill 50% larvae required 9.08 x 106 sel/ml of *B. sphaericus* bacteria, LC50-48 hours (6.28E + 03) in 48 hours to kill 50% larvae required 6.28 x 103sel / ml of *B. sphaericus* bacteria, LC50-72 hours (9.56E + 02) in 72 Hour to kill 50% larvae required 9.5 x 102 sel / ml bacterium *B. sphaericus*. In the calculation of LC90-24 hours (3.64E + 07) within 24 hours to kill 90% larvae required 3.64 x 107 cells / ml of *B. sphaericus*, LC90-48 hours (2.61E + 04) bacteria in 48 hours to kill 90% larvae required 2.61 x 104 sel/ml of bacterium *B. sphaericus* and LC90-72 hours (2.73E + 03) in 72 hours to kill 90% larvae required 2.73 x 103sel / ml of *B. sphaericus* bacteria.

Table 5.9. Result of calculation of Letal Concentration (LC 50 and LC90) Bioassay Biolarvasidal *Bacillus sphericus* from Soy Milk medium 30% v/v.

Concentration	**8.90E + 08**	**Cells / ml**
Endospora	48.00	%
LC50-24 hours	-	Cells / ml
LC50-48 hr	1.02E + 06	Cells / ml
LC50-72 hours	3.51E + 03	
LC90-24 hours	-	Cells / ml
LC90-48 hours	5.25E + 06	Cells / ml
LC90-72 hours	6.71E + 04	Cells / ml

Table 5.9 shows the lethal concentration (LC) test of *B. sphaericus* which is inoculated on a simple medium 30% v / v soy milk is LC50-24 hours (-) and LC90-24 hours (-) means in time 24 *B. sphaericus* is incapable killing Anopheles sp. larvae. Letal Concentration (LC) by endospores *B. sphaericus* inoculated on a simple medium fish flour 30% v / v appears after 48 hours of observation. LC50-48 hours (1.02E + 06) within 48 hours to kill 50% larvae required 1.028 x 106sel / ml bacterium *B. sphaericus*, LC50-72 hours (3.51E + 03) within 72 hours to kill 50% larvae required 3.51 x 103sel / ml of *B. sphaericus* bacteria. In the calculation of LC90-48 hours (5.25 + 06) within 48 hours to kill 90% of larvae required 5.25 x 106sel / ml of *B. sphaericus* and LC90-72 hours bacteria (6.71 + 04) within 72 hours to kill 90% larvae required 6.71 X 104sel / ml of *B. sphaericus* bacteria.

Table 5.10. Result of calculation of Letal Concentration (LC 50 and LC90) Bioassay Biolarvasidal Bacillus sphericus from medium Santan 30% v/v.

Concentrations	**Concentrations 2.96E + 08**	**Cells / ml**
Endospora	57.6	%
LC50-24 hours	1.25E + 07	Cells / ml
LC50-48 hours	8.22E + 03	Cells / ml
LC50-72 hours	4.43E + 03	Cells / ml
LC90-24 hours	4.22E + 07	Cells / ml
LC90-48 hours	2.92E + 04	Cells / ml
LC90-72 hours	2.26E + 04	Cells / ml

Table 5.10 shows the lethal concentration (LC) test of *B. sphaericus* which is inoculated on a simple medium coconut milk 30% v / v is LC50-24 hours (1.25E + 07) meaning that in 24 hours to kill 50% larvae is needed 1.25 x 107sel / ml of *B. sphaericus* bacteria, LC50-48 hours (8.22E + 03) in 48 hours to kill 50% larvae required 8.22 x 103 sel / ml of *B. sphaericus* bacterium, LC50-72 hours (4.43E + 03) in 72 hours to kill 50% larvae required 4.43 x 103sel / ml bacterium *B. sphaericus*. On the calculation of LC90-24 hours (4.22E + 07) in 24 hours to kill 90% larvae required 4.22 x 107 cells / ml of *B. sphaericus* bacteria, LC90-48 hours (2.92E + 04) in 48 hours to kill 90 % larvae required 2.92 x 104sel / ml of *B. sphaericus* and LC90-72 hours (2,26E + 04) bacteria in 72 hours to kill 90% of larvae required 2.26 x 104sel / ml of *B. sphaericus* bacteria.

4. DISCUSSION

B. sphaericus is a Gram-positive, rod-shaped bacterium capable of forming endospores of terminals (at the ends of cells) with enlarged sporangium (swollen sporangium) that can be isolated from the soil (Baumann et al 1991). The results showed that 30% v / v simple coconut medium, 30% w / v fish flour and 30% v / v soy milk can be used to grow the local Lombok *B. sphaericus* as evidenced by good colony growth. *B. sphaericus* grow most fertile on simple natural media coconut milk with concentration 30% v / v that is the number of *B. sphaericus* bacterium 296 x 108 cell / ml, then followed by fish meal flour with concentration 30% w / v 291 x 108 cell / ml and soy milk 30% v / v 89 x 108 cells / ml. The growth of bacterial cells on NYSM standard media is 353 x 108 cells / ml. The percentage of endospora of *B. sphaericus* bacterial cell on standard media of NYSM was 65,39%, whereas for simple media the percentage of endospora was most determined in coconut milk 30% v / v (57,6%) followed by medium of fish flour 30% w / v (55,66%) and Soy Milk 30% v / v (48,1%).

B. sphaericus does not require carbohydrates in its growth, because it lacks the necessary enzymes to enter carbohydrates and convert them into energy sources (Hu et al., 2008). However, various materials rich in protein and fat are reported to support their growth, even from simple sources. Yadav et al in 2011 reported some simple materials that could be used to grow *B. sphaericus* entopathogenik in the form of a liquid medium while maintaining its toxicity properties. In these bacteria are not found enzyme glucokinase activity, hexokinase, phosphoglukoisomerase, phosphofruktokinase and glucose-6-phosphate dehydrogenase. Extra cellular enzymes such as amylase, gelatinase, chitinase and lecithinase are not owned by *B. sphaericus*. *B. sphaericus* was able to grow on a medium containing citric and 5% NaCl, and showed oxidase and catalase activity (Vanlahlruaia et al, 2011).

The results also show that breeding of *B. sphaericus* from 3 types of simple media did not alter the morphology of vegetative cells and endospores produced. Because 3 types of medium is safe to use. From this result it can be seen that cell concentration is not directly proportional to the percentage of endospores. This means that with the length of incubation of 24 hours, 48 hours and 72 hours more and more endospora formed the number of vegetative cells less. The high percentage of endospores is related to *B. sphaericus.*

Killing power against Anopheles sp larvae. This is due to the toxins synthesized by *B. sphaericus* attached to the endospores. The high production of endospores, causing high kill power of *B. sphaericus*. Anopheles sp's larvae kill is highest by *B. sphaericus* grown on NYSM standard media. In coconut milk, *B. sphaericus* was able to kill the larvae of Anopheles sp. larvae from the first 24 hours (up to 80%). The lowest killing power was grown on 30% v / v soy milk medium. The highest killing power was achieved at 48 and 72 hours after the administration of *B. sphaericus*, reaching 100%. Dilution below 10-5 indicates a very low kill power of less than 40%, this is because the endospora is less and the concentration of the toxin is more dilute due to the dilution of the stratum.

B. sphaericus grown in growth medium soy milk did not show larval mortality at 24 hours observation. The ability to kill the new Anopheles sp larvae was seen in 48 and 78 hours of observation, which is 75% - 100%. This ability lasts up to 10-4 dilution. At 10-5 and 10-6 dilutions, the killing power occurs only after 72 hours of *B. sphaericus* aperture, which is between 50% -60%.

B. sphaericus grown on the NYSM standard medium showed a lower LC value compared to the LC value of the simple coconut milk medium 30% v/v, 30% w/v and 30% v/v soybean milk at 24 hours, 48 Hours, and 72 hours after observation. Of the three simple natural mediums are Coconut milk medium 30% v/v, Fish meal 30% w/v and 30% v/v Soya Milk. In this result, the cell concentration is not directly proportional to the LC value. However, the concentration of endospores is directly proportional to the LC value, due to the higher concentrations of endospores, resulting in higher toxins produced. The lower LC50 and LC90 possessed by a bacterium at the time of observation 48 hours and 72 hours, the higher the bacterial toxicity. The alternative medium best used for *B. sphaericus* local Lombok island entomopatogenik is medium coconut milk and flour of fish. Coconut medium is the medium that produces the highest toxicity of *B. sphaericus* to Anopheles Sp larvae and its toxicity can be detected from the first 24 hours after *B. sphaericus* is released on the medium test.

5. CONCLUSION

Simple medium with coconut milk formula with concentration of 30% v/v showed the most fertile *B. sphaericus* growth of 296 x 108 cells / ml, with the endospora percentage of 30% v/v (57,6%) and Bioassay *B. sphaericus* Has been able to kill larvae as many as 15 larvae at the time of observation 24 hours and on observation 42 hours and 72 hours have been able to kill larvae 20 larvae (100%). Then followed by fish meal flour with concentration 30% w/v showed growth of *B. sphaericus* that is 291 x 108 cell/ml with endospora percentage that is 30% w/v (55,66%) and result of Bioassay *B. sphaericus* test was able Killed larvaae as many as 16 larvae at 24 hours observation, and observation time of 48 hours and 72 hours larvae that died 20 larvae (100%). And the last one was in soy milk with a concentration of 30% v/v showed *B. sphaericus* growth of 89 x 108 cells / ml with endospora percentage of 30% v/v (48.1%) and new Bioassay *B. sphaericus* Showed their toxicity to Anopheles Sp larvae after 48 hours of observation of 19 larvae and 72 hours observation was able to kill 20 larvae (100%). The lowest LC50 and LC90 values were owned by *B. sphaericus* cultured in medium coconut milk 30% v/v, followed byfish meal medium 30% w/v andSoy Milk 30% v/v on observation 24 hours, 48 hours and 72 hours. The higher concentration of endospores, causing high toxins produced. The lower LC50 and LC90 possessed by a bacterium at the time of observation 48 hours and 72 hours, the higher the bacterial toxicity.

6. SUGGESTION

Simple medium formulas for the growth of Bacillus sphaericus Local Island of Lombok can be used by people in controlling Anopheles Sp larvae and can be developed on a double scale by the relevant Institution or industry without using imported media from abroad.

7. REFFERENCES

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