Patogenicity of *Bacillus thuringiensis* which Isolated from Tidal Ecosystem against Diamond Backmoth Larvae, *Plutella xylostella* Linn

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ABSTRACT---- ***The purpose of this research was 1) the exploration of Bacillus thuringiensis in soil, water, and organic matter contained in the tidal area of the South Kalimantan and Central Kalimantan; 2) Comparing the pathogenicity among isolates of B. thuringiensis were found against Plutella xylostella larvae; 3) To test the effectiveness of the highest pathogenicity B. thuringiensis isolates from isolates of exploration results against P. xylostella larvae. Soil samples taken from six areas of land in tidal area of the Barito Kuala, Banjarmasin, Banjar district, Province South Kalimantan, Pulang Pisau and Kapuas of Central Kalimantan Province. Isolation of bacteria and pathogenicity tests conducted in the the laboratory of Plant Pests and Diseases Department of the Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru. Research carried out for 8 months. Pathogenicity carried out by determining the level of LC_{50} value from each B. thuringiensis were found by probit analysis. Efficacy Test of B. thuringiensis implemented using completely randomized design, which consists of 5 treatments and 4 replicates ie a) 2 cc suspension of Bacillus thuringiensis/ l water; b) 3 cc suspension of Bacillus thuringiensis/l water; c) 4 cc suspension of Bacillus thuringiensis/ l water; d) Application of klorfluazuron insecticide, the trade name Atabron with a concentration of 2 cc / l water; e) Applied with water. Differences between treatment effect is determined by Duncan's Multiple Range Test (DMRT). The final conclusion is that: a) Results of exploration was found 11 (eleven) isolates of B. thuringiensis in the areas of tidal ecosystems; b) The higher the concentration of cells of B. thuringiensis more larvae of P. xylostella were dead; b) the high pathogenicity of B. thuringiensis isolates obtained on isolated from drains ecosystems (sewers) on forest with LC_{50} values of 2.41 x 10^7 cells / ml of water; d) The concentration of B. thuringiensis the most effective in reducing leaf damage is 4 cc / l.***

Keywords---Pathogenicity, *Bacillus thuringiensis*, Tidal ecosystem, *Plutella xylostella*

1. INTRODUCTION

The Mustard is one of the important vegetable crops in Indonesia. Mustard plant widely grown in lowland South Kalimantan, among which are the centers of vegetables in lowland in the district of Banjar, Banjarbaru, and Banjarmasin. Mustard plants are grown on tidal land in South Kalimantan and Central Kalimantan.

Many factors cause low production of mustard plants in South Kalimantan, one of which is the presence of pest infestation. One of them is a mustard leaf-eating larvae, *Plutella xylostella* Linn. Without controlled use of pesticides, in the dry season these pests can cause damage to 100% (4). One of the factors controlling *P. xylostella* larvae naturally available in nature is the bacterium *Bacillus thuringiensis* Berliner which endogenous and already adapt in nature. Therefore we need a study to find *B. thuringiensis* which effective to control of *P. xylostella* larvae on crop land. The first step in the development and utilization of *B. thuringiensis* is exploring the bacteria in nature, and then test the pathogenicity against insect pests.

Given the magnitude of destructive capability of *P. xylostella* larvae of the mustard plant, it is necessary to look for *B. thuringiensis* that have a high pathogenicity against these pests, and able adapt with tidal land environment.

The purpose of this study is:

1. Exploration of *B. thuringiensis* in soil, water, and organic matter contained in the tidal area in South Kalimantan and Central Kalimantan.
2. Comparing pathogenicity among isolates of *B. thuringiensis* were found against *P. xylostella* larvae.
3. To test the effectiveness of *B. thuringiensis* isolates the highest pathogenicity of isolates exploration results against *P. xylostella* larvae.

2. MATERIAL AND METHODS

Soil samples taken from six areas of land in Tidal area of the Barito Kuala district, Banjarmasin, Banjar in South Kalimantan Province, and Kapuas, Pulang Pisau district in Central Kalimantan Province. Isolation of bacteria and pathogenicity tests conducted in the laboratory of Plant Pests and Diseases Department of the Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru. Effectiveness trials conducted in the Village of Banua Anyar on Banjarmasin. Research carried out for 8 months.

The material used is composed of distilled water, Luria-Bertani Broth, 0,25 M sodium acetate pH 6,8, T3 medium (per liter: 3 g tryptone, 2 g Tryptose; 1,5 g yeast extract; 0,05 M sodium phosphate pH 6,8 dan 0,005 g MnCl2), Nutrient Agar, dan Nutrient Broth. The tools used include petri dishes, test tubes, Erlenmeyer, ose needle, phase contrast microscope, insect cage size 1 x 1 x 0.5 m and a size of 0.5 x 0.5 x 0.5 m.

Sampling Soil, Organic Materials and Water

The sample material was collected by scraping the surface of the soil, organic matter and water by using a sterile spatula and obtained approximately 10 g samples obtained from into 2-5 cm. All samples were placed in sterile clip plastic and stored at 4 ° C until further processing.

Isolation

Isolation of *B. thuringiensis* was performed according to the method performed by Travers *et al*. (14). One gram of each sample was resuspended in 10 ml of sterile distilled water and pasteurized at 80°C for 30 minutes. For the selection of *B. thuringiensis* one ml of each suspension was added to 10 ml of Luria-Bertani (Merck, Germany) broth (1.0% Tryptone, 0.5% Yeast Extract, 1.0% Sodium Chloride (NaCl), pH 7.0) given buffer with 0,25 M sodium acetate pH 6,8. The suspension was heated at 30 ° C for four hours and then heated at 80 ° C for 3 minutes. The suspension was diluted and cultured on the media T3 (per liter: 3 g tryptone, 2 g Tryptose; 1,5 g yeast extract; 0,05 M sodium phosphate pH 6,8 dan 0,005 g MnCl2), then incubated at 30 °C for 24 hours. Colonies that showed the same morphology were selected and examined under a phase-contrast microscope to determine the presence of parasporal inclusion and spores. All isolates of *B. thuringiensis* was transferred into the oblique Nutrient Agar medium and prepared for further testing.

Pathogenicity test

Bacteria were taken from the isolation results of the exploration activities. Insect pathogenic bacteria propagated using Nutrient Broth (NB) was to move the bacteria in pure culture media slant NA 2 days old media into the Erlenmeyer NB using aseptic loopful. Bacterial cultures were incubated for 48 hours at room temperature while shaking by using a shaker. Cultures prepared tested and used with diluent water. Implementation of preliminary tests conducted to determine the lethal concentration of test insects between 20% to 95%. Implementation of the preliminary test is as follows:

First of all cell suspensions prepared insect pathogenic bacteria in the water with five different concentrations. Pieces of mustard leaf spilled liquid of bacterial suspension evenly on the leaf surface. The leaf pieces and third instar larvae of *P. xylostella* which had been fasted for 3 hours put into a plastic cup and covered with a plastic lid which is a hole. When the leaves are treated has been exhausted by the test insects, then added another leaf that is not given to the bacterial suspension in plastic cups to avoid insect death because there is no food.

At untreatment insect, given the leaf pieces which untreated bacterial suspension. Each treatment concentration was given to each of 20 third instar larvae of *P. xylsotella*.

Death of insects recorded after 24 hours were treated, every 24 hours until it forms a pupa. Based on insect mortality data on preliminary experiments after 48 hours, set five different concentrations of bacterial suspension suspension with a range that can kill 20% - 95% of the population of *P. xylostella* larvae. Way of treatment as in the preliminary experiment, but the larvae used were as many as 30 larvae for each treatment concentration. Parameters observed that the number of dead larvae every 24 hours until it forms a pupa. Pathogenicity level is determined by calculating the LC_{50} values at 48 hours after treated by using probit analysis.

Effectiveness Test of *Bacillus thuringiensis* against larvae of *Plutella xylsotella*

Experiments used a completely randomized design, which consists of 5 treatments and 4 replicates ie a) 2 cc / l suspension of *Bacillus thuringiensis*; b) 3 cc / l suspension of *Bacillus thuringiensis*; c) 4 cc / l suspension of *Bacillus thuringiensis*; d) Application by the insecticide klorfluazuron the trade name Atabron with a concentration of 2 cc / liter; e) Applied with water (Without *Bacillus thuringiensis* cells). Application of treatment carried out with a spray volume of 500 l per ha, using handsprayer. *Bacillus thuringiensis* used propagated in media Nutrient Broth (NB).

T en seeds of mustard plants 10 days old are planted in soil in pots measuring 30 x 30 x 20 cm. Three days later, mustard crop was invested with third instar larvae of *P. xylostella* who had fasted for three hours. Mustard crop that has been invested with 30 *P. xylostella* larvae were then sprayed with a bacterial suspension of *B. thuringiensis* in accordance with the treatment. Three days after spraying *B. thuringiensis*, conducted observations of the number of larvae that die from the infection of *B. thuringiensis*, and intensity of attacks by the following formula:

$$P = \frac{\sum ni.\ vi}{Z.\ N} \times 100\ \%$$

where Is :
P : intensity of leaf damage
N : number of damaged leaves for each category of attacks
v : numerical value of the category of attacks
Z : numeric value of the highest attack category.
Categories of attack is :
0 = no attack at all
1 = damage > 0 - < 20 %
2 = damage 20 % - < 40 %
3 = damage 40 % - < 60 %
4 = damage 60 % - < 80 %
5 = damage 80 % - < 100 %

Data were analyzed using analysis of variance of completely randomized design. Differences between treatment effect is determined by Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

Exploration And Pathogenicity Test *Bacillus thuringiensis*

From the results of exploratory research on field obtained 11 isolates of *Bacillus thuringiensis* with the percentage of mortality as in Table 1. From the results of pathogenicity test data obtained in accordance with Table 2.

Table 1. Percentage of *Plutella xylostella* L. larvae mortality infected by *Bacillus thuringiensis* isolated from tidal land ecosystems

No. Isolate/Ecosystem	concentration (cell/ml)	Percentage of Mortality (%)
1. People Forest	1.59×10^{13}	93.3
	6.19×10^{12}	43.3
	3.61×10^{12}	30.0
	1.68×10^{12}	20.0
	3.87×10^{11}	13.0
	Without *B. thuringiensis* cells	0
2. Paddy / Animals	1.17×10^{12}	96.7
	8.73×10^{11}	70.0
	6.52×10^{11}	50.0
	3.95×10^{10}	26.7
	1.74×10^{9}	6.7
	Without *B. thuringiensis* cells	0
3. Rubber plantation	9.74×10^{12}	93.3
	6.79×10^{11}	66.7
	3.84×10^{11}	40.0
	1.26×10^{10}	16.7
	5.16×10^{10}	10.0
	Without *B. thuringiensis* cells	0
4. Paddy-Banana	8.60×10^{11}	90.0
	4.87×10^{10}	53.3
	2.17×10^{9}	26.7
	4.73×10^{8}	10.0
	1.35×10^{8}	6.7
	Without *B. thuringiensis* cells	0
5. People Forest	4.78×10^{12}	93.3
	2.80×10^{11}	50.0
	1.73×10^{10}	26.7
	1.43×10^{10}	20.0
	9.74×10^{9}	10.0
	Without *B. thuringiensis* cells	0

6. Planting of paddy	4.03×10^{10}	93.3
	4.03×10^{9}	80.0
	4.03×10^{7}	73.3
	1.54×10^{7}	30.0
	1.17×10^{6}	13.3
	Without *B. thuringiensis* cells	0
7. Gully	4.65×10^{8}	93.3
	4.12×10^{8}	83.3
	5.71×10^{7}	43.3
	5.71×10^{5}	10.0
	5.71×10^{4}	6.7
	Without *B. thuringiensis* cells	0
8. Drainage of paddy	4.68×10^{9}	96.7
	3.10×10^{9}	83.3
	5.53×10^{8}	53.3
	5.53×10^{7}	26.7
	5.53×10^{4}	20.0
	Without *B. thuringiensis* cells	0
9. Paddy-Beans	2.93×10^{13}	93.3
	3.6×10^{11}	66.7
	3.6×10^{10}	53.3
	3.6×10^{9}	46.7
	7.1×10^{8}	26.7
	Without *B. thuringiensis* cells	0
10. Rubber plantation	9.8×10^{10}	93.3
	8.65×10^{10}	66.7
	8.65×10^{9}	50.0
	8.65×10^{8}	33.3
	8.65×10^{7}	10.0
	Without *B. thuringiensis* cells	0
11. vegetable	1.1×10^{9}	90.0
	1.1×10^{8}	66.7
	9.1×10^{7}	50.0
	7.1×10^{7}	26.7
	6.1×10^{7}	10.0
	Without *B. thuringiensis* cells	0

Bacillus thuringiensis is found everywhere in diverse environments, especially in soil habitats (12, 15). The geographic distribution of *B. thuringiensis* in soil habitat are well documented. Bacteria naturally reportedly exist in the soil environment in South America (14, 6), North America (7, 9), Europe (11), West and South Asia (1), Far East (China, Korea and Japan) (8) , and Africa (7).

The result showed that the higher the concentration, the more the *P. xylostella* larvae died (Table 1), this is due to the greater concentration of *B. thuringiensis* increasingly large doses are ingested by the larvae. This is consistent with research Damo, who have been screened against *Helicoverpa armigera* by *B. thuringiensis* which found that the higher dose of *B. thuringiensis* the higher mortality of larvae (2). Research results Gazali *et al*., also found that the higher concentration of *B. thuringiensis*, the higher mortality of *P. xylostella* third instar larvae (5).

From the results obtained that the pathogenicity test, highest pathogenicity of *Bacillus thuringiensis* bacterium isolated from the land derived from ecosystems drains (Gully) on forest with LC50 values of 2.41×10^{7} cells / ml of water (Table 2). The high pathogenicity of *Bacillus thuringiensis* on waterways in forest ecosystems is due to buildup of bacteria *Bacillus thuringiensis* on the water channel and also environmental factors that favor the development of *Bacillus thuringiensis* populations in the ecosystem that the drains in forest ecosystems that exist in a closed exploration of light violet which can damage the bacterial cell.

According Poinar and Thomas, that the influence of abiotic factors in regulating the occurrence of infection. Abiotic factors that influence was ultraviolet light that can damage the *B. thuringiensis* spores and crystals, temperature and humidity can interfere the stability of *B. thuringiensis* (10).

Table 2. LC50 values of *Bacillus thuringiensis* isolates isolated on tidal land ecosystems

No.	**Ecosystem**	**LC_{50} (cells/ml)**
1.	Paddy/animals	1.72×10^{11}
2.	Paddy-Benana	2.23×10^{10}
3.	Planting of Paddy	4.52×10^{7}
4.	Drainage of paddy	5.30×10^{7}
5.	Paddy-Beans	1.59×10^{10}
6.	People Forest	5.36×10^{12}
7.	Rubber Plantation	$3{,}83 \times 10^{11}$
8.	People Forest	1.59×10^{11}
9.	Gully	2.41×10^{7}
10.	Rubber Plantation	5.68×10^{9}
11.	Vegetable	1.25×10^{8}

Effectiveness Test

From the test results of the effectiveness of selected *B. thuringiensis* was found that the application of klorfluazuron active ingredient pesticide and applications of *B. thuringiensis* can decrease the attack intensity of mustard leaf-eating larvae, *P. xylostella* (Table 3). Among the concentration of *B. thuringiensis* were applied, concentration of 4 cc / l has the ability to lower attack was the highest with than the concentration of 3 cc / l and 2 cc / l, because the higher the concentration, the higher the number of cells of *B. thuringiensis* are ingested by *P. xylostella* larvae.

Application of *B. thuringiensis* may result in decreasing intensity of attacks this is due to leaf damage due to *P. xylostella* larvae attack decreases. According Poinar and Thomas, infected insect symptoms of *B. thuringiensis* is causing the insect to stop eating, slow moving, from the mouth and anus out of fluid. In insects dead body watery, his skin color to black, soft, wrinkle, foul smelling, and after a few days to dry and shrink (10).

Table 3. Average intensity of leaf damage caused by *P. xylostella* larvae after applied by the solution of *B. thuringiensis* and klorfluazuron pesticides

No.	Treatment	Damage Intensity of leaf (%)
1.	Applied by water	67.5 a
2.	Applied by Klorfluazuron	21.8 b
3.	4 cc / l suspension of *B. thuringiensis*	25.5 c
4.	3 cc / l suspension of *B. Thuringiensis*	50.1 d
5.	2 cc / l suspension of *B. Thuringiensis*	56.3 e

Description: The average value of the percentage of the intensity of leaf damage in the same column followed by the same letters are not significantly different by Duncan's Multiple Range Test at 95% level.

Based on the observation that the number of dead larvae, it can be obtained that applications of *B. thuringiensis* solution treatment can reduce the number of *P. xylostella* larvae that can live and use of *B. thuringiensis* with a concentration of 4 cc / l can kill *P. xylostella* larvae more than the other dosage and the ability to kill each with synthetic organic pesticides klorfluazuron with a concentration of 2 cc / l (Table 4). This is consistent with studies Don-Fronk, found that application of *B. thuringiensis* microbial insecticide spraying at intervals of seven days is very effective in controlling pests of cabbage (3). According Sympathy, that the deposit of *B. thuringiensis* for seven days after the application is still able to kill larvae of *P. xylostella* and *Croccidolomia binotalis* (13).

Tabel 4. The average number of dead *P. xylostella* larvae caused by *B. thuringiensis* and klorfluazuron insecticide

No.	Treatment	The average number of dead larvae
1.	Applied by water	0.00 a
2.	Applied by Klorfluazuron	23.50 b
3.	4 cc / l suspension of *Bacillus thuringiensis*	22.25 b
4.	3 cc / l suspension of *Bacillus thuringiensis*	14.75 c
5.	2 cc / l suspension of *Bacillus thuringiensis*	12.50 c

Description: The average value of *P. xylostella* larvae died in the same column followed by the same letters are not significantly different by Duncan's Multiple Range Test at 95% level.

4. CONCLUSIONS AND RECOMMENDATIONS

From the results of the study concluded that:

a. Found 11 (eleven) isolates of *B. thuringiensis* results of exploration in areas Tidal ecosystems.
b. The more the concentration of *B. thuringiensis* cells, more dead larvae of *P. xylostella*.
c. The most high pathogenicity was found in isolates of *B. thuringiensis* isolated from ecosystems gully on forest with LC_{50} values of 2.41 x 107 cells / ml water
d. The concentration of *B. thuringiensis* the most effective in reducing damage leaves and kill *Plutella xylostella* larvae were concentration of 4 cc / l.

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