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Agroecosystem Stability and Breakdown Leaves on Mustard Cropping after Application by the *Bacillus thuringiensis*

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Abstract: ***The purpose of this research are: 1) To test the effect of the application of Bacillus thuringiensis reproduced using the artificial medium against mustard leaf damage caused by leaf-eating pests; 2) Comparing the stability of the mustard plant agroecosystem after applicated by various concentrations of B. thuringiensis. Tests using a randomized block design with 5 treatments and 4 replications. The treatments were a) Control (application by water); b) Application B. thuringiensis using a concentration of 2 cc/l of water; c) Application B. thuringiensis using a concentration of 4 cc/l of water; d) Application B. thuringiensis using a concentration of 6 cc/l of water; e) Application B. thuringiensis using a concentration of 8 cc/l of water. Observed variables consist of any number of arthropod species found and the intensity of leaf damage. Data were analyzed by analysis of variance using a randomized block design, while the difference between the effect of treatment is determined by LSD. agro-ecosystem stability is determined by determining the diversity index by Shannon and Weaver, Domination Index, Richness Index of species, and the similarity index. Research results can be summarized as follows: 1) Application B. thuringiensis can reduce the intensity of the damage caused by leaf-eating pests in crop mustard mustard; 2) The concentration of B. thuringiensis the most severe damage is low intensity is a concentration of 6 cc/l and 8 cc/l; 3) Planting mustard has the highest stability of mustard plant ecosystem treated B. thuringiensis applications with a concentration of 4 cc/l with a diversity index of 1.867.***

Keywords: Agrocosystem stability, *Bacillus thuringiensis*, Breakdown leaves, Mustard, Diversity, Arthropods

1. Introduction

Mustard is a vegetable crop that is much consumed by the people of Indonesia. Mustard plant widely grown in the province of South Kalimantan and Central Kalimantan. In South Kalimantan mustard plants are planted in low-lying areas such as Banjarbaru, Banjar District, Banjarmasin. Many factors cause low production of mustard plants in South Kalimantan, one of which is the disruption of insect pests. They are the mustard leaf-eating caterpillars, *Plutella xylostella* Linn, *Croccidolomia binotalis*, and Some types of grasshoppers. Without controlled use of pesticides, in the dry season these pests can cause damage to 100%. One of the factors controlling caterpillars and grasshopers naturally available in nature is the bacterium *Bacillus thuringiensis* that endogenous and already beradaftasi in nature. Therefore we need a research to find *B. thuringiensis* is effective in controlling *P. xylostella* caterpillars and other pest on crop land.

The first step in the development and utilization of *B. thuringiensis* is undertaking these bacteria in nature, pathogenicity test, looking for mass propagation media and then test the influence of mass propagation medium the pathogenicity of *B. thuringiensis* against insect pests. Given the magnitude of destructive ability of leaf-eating pests against mustard plant, it is necessary to find a suitable medium for propagation of *B. thuringiensis* and can increase the pathogenicity against these pests, and is able to adapt to the environment tidal land.

The purpose of this study were 1) To test the effect of the application of *B. thuringiensis* reproduced using the artificial medium against mustard leaf damage caused by leaf-eating pests; 2) Comparing the stability of the mustard plant agroecosystem after applicated by various concentrations of *B. thuringiensis*.

2. Methodology

The research was conducted in the laboratory of Plant Pests and Diseases, Faculty of Agriculture, Lambung Mangkurat University, as well as the farmer's land. The study lasted four months. Materials used consist 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 and 0.005 g MnCl2), nutrient agar, and nutrient broth, alcohol, spirits, C media (a mixture of corn, rice, and soybeans). The tools used include petri dishes, test tubes, erlenmeyer glass, needles ose, phase contrast microscopes, bunsen lamp.

2.1. Purification of *Bacillus thuringiensis*

To obtain pure *B. thuringiensis* performed as follows, one ml *B. thuringiensis* culture in nutrient broth media resuspended into 9 ml of sterile distilled water and pasteurized at 80 ° C for 30 minutes. For the selection of *B. thuringiensis* one ml each suspension was added to 10 ml Luria-Bertani (Merck, Germany) broth (1.0% Tryptone, 0.5% Yeast Extract, 1.0% Sodium Chloride (NaCl), pH 7.0) by buffer with 0, 25 M sodium acetate pH 6.8. The suspension was heated at 30 °C for four hours and then heated at a temperature of 80 °C for 3 minutes. The suspension is 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 and 0.005 g MnCl2), then incubated at 30 °C for 24 hour (Travers *et al*., 1987). Colonies that show 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 medium Nutrient Agar to be slanted.

2.2. Propagation of *Bacillus thuringiensis* Berliner

C Media (a mixture of corn, rice, and soybeans) until smooth, then filtered using a sieve, in order to obtain a solid powder. 100 g of powdered solid medium boiled in 500 ml of distilled water to obtain a suspension medium, then filtered using a sieve. Filtered results readily used for propagation of *B. thuringiensis*. Bacteria used comes from the isolation of the exploration activities that have the pathogenicity of *B. thuringiensis* highest among all the bacteria *B. thuringiensis* tested. Insect pathogens propagated using media Nutrient Broth (NB) was to move the bacteria in pure culture in media NA slant aged 2 days to NB media in erlenmeyer using ose needle aseptically. Bacterial cultures were incubated for 48 hours at room temperature while shaken using a shaker. Two milliliters of *B. thuringiensis* cultures in NB media put into 50 ml of C medium, then shaken by using a shaker for one week. *Bacillus thuringiensis* is ready for field trials.

2.3. Research methods

Tests used a randomized block design with 5 treatments and 4 replications. The treatments were a) Control (application by water); b) Application *B. thuringiensis* using a concentration of 2 cc / l of water; c) Application *B. thuringiensis* using a concentration of 4 cc / l of water; d) Application *B. thuringiensis* using a concentration of 6 cc / l of water; e) Application *B. thuringiensis* using a concentration of 8 cc / l of water. Testing was conducted in farmer's land plot with an area the size of 2 x 4 m, the observed variables consisting of the amount of each species of arthropods found in experimental plots each treatment.
Variables observed consisted of the amount of each species of arthropods found in experimental plots and the estimated percentage of leaf damage to each leaf cultivation, further defined the overall category of attack to get the intensity of mustard leaf damage for each experimental unit. Observations arthropods using a fitfall trap, a yellow trap, light traps and insect netting with 10 double swing. Fitfal trap laid diagonally much as 3 pieces, while the yellow traps and light traps placed in the middle of the experimental plots respectively of the piece.

The intensity of the damage is determined by the following formula:

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

Where :
P : intensity of leaf damage
N: the number of damaged leaves for each category of attack
v : numeric value category of attack
Z : numeric value category highest attack.

Categories attacks are :
0 = no attack at all
1 = damage> 0 - <20%
2 = damage 20% - <40%
3 = damage 40% - <60%
4 = damage 60% - <80%
5 = 80% damage - <100%

2.4 Data analysis

Data were analyzed using analysis of randomized block design variants, while the difference between the treatment effect is determined using LSD test. Agroekosistem stability is determined by considering Diversity Index, species richness Index, Dominance Index, and the similarity Index, as follows:

1. Dominance Index (C)
   $C = \Sigma (ni / N) 2$
   ni: total number of individuals of a species
   N: total number of individuals of all species
2. The diversity index (H ') by Shannon - Weaver (Southwood, 1978; Ludwig and Reynolds, 1988)
   H '= - ln Σ pi pi
   pi: proportion of the i-th species in the total sample
3. The similarity index (E) according to Pilou (Ludwig and Reynolds, 1988)
   E = H '/ ln S
   H ': diversity index
   S: kind entirely
4. Species richness Index (R) according to Margalef (Ludwig and Reynolds, 1988).
   $R = \frac{S-1}{Ln\ N}$
   S: kind entirely
   N: total number

3. Results and Discussion

3.1. Mustard Leaf damage

From the observation showed that the bacteria *B. thuringiensis* application can reduce the intensity of mustard leaf damage caused by leaf-eating pests of mustard. The highest damage was found in the mustard crop just sprayed with water or controls (24.70%), while crops that suffered damage which the smallest is the mustard crop is sprayed with *B. thuringiensis* with a concentration of 6 ml / l of water (8.80%) and 8 ml/l of water (7.60%) (Table 1).

Table 1: Average percentage of mustard leaf damage by leaf-eating pest of mustard

Treatments	The average percentage of leaf damage by the mustard leaf eaters (%)
Control (applied by water)	24.70 a
Application *B. thuringiensis* used a concentration of 2 cc / l of water	13.00 ab
Application *B. thuringiensis* used a concentration of 4 cc / l of water	11.20 bc
Application *B. thuringiensis* used a concentration of 6 cc / l of water	8.80 cd
Application *B. thuringiensis* used a concentration of 8 cc / l of water	7,60 d

Description: The average percentage of mustard leaf damage by insects or leaf eaters in the same column differ significantly based on the LSD test with a confidence level of 95%.

Applications effect of *B. thuringiensis* treatment by a concentration of 2 cc/l of water against of mustard leaf damage by pests compared to the application only of water (control). This is due to a concentration of 2 cc / l of water yet can be deadly of mustard leaf-eating pests optimally because of the number of doses consumed by the of mustard leaf-eating pests are still low, so most of the pest is still not death, but the effect of *B. thuringiensis* applications visible at the time of application *B. thuringiensis* enhanced concentration to 4 cc / l of water, and the effect increases with increasing the growing concentration of *B. thuringiensis*.

Effect of application *B. thuringiensis* against the decrease in the intensity of mustard leaf damage caused by leaf-eating pests of mustard looks after the concentration is increased to 4 cc/l of water and achieve a reduction in crop damage lowest of mustard is the of mustard crop concentration of the treated 6 cc/l of water and 8 cc/ l of water. This is due to the higher concentration of *B. thuringiensis*, the higher the cells or spores of *B. thuringiensis* were consumed leaf-eating pests. According Gazali *et. al.* (2015), the higher the concentration of *B. thuringiensis* which is applied to the mustard plant more also *P. xylostella* caterpillars are dying. The most effective concentration to reduce caterpillar populations of *P. xylostella* was 4 cc /l of water. Studies Don-Fronk (1971), found that application of *B. thuringiensis* microbial insecticide spraying at intervals of seven days is very effective in controlling pests of cabbage. According Sympathy (1985), that the deposit of *B. thuringiensis* for seven days after the application is still able to kill larvae of *P. xylostella* and *C. binotalis* . According Gazali *et al.* (1999) the higher dose of *B. thuringiensis* given on *P. xylostella* caterpillars increasingly *P. xylostella* infected and die. At the time of the research environment is highly favorable conditions for the development or proliferation of *B. thuringiensis*. This is in accordance with the opinion of Hilbert and Piggot (2004) *B. thuringiensis* ready to perform when the proliferation of environmental conditions such as temperature and nutrient availability support, while formation spore has proved triggered by internal factors and external including signals for hunger nutrition, cell density, and cell cycle progression. So that the environmental conditions that favor the process of infection of *B. thuringiensis* against of mustard leaf-eating pests sooner.

3.2 Agro-ecosystem stability

From the analysis of arthropod populations in cropping of mustard application by *B. thuringiensis* found that the arthropod diversity index, dominance index, index of species similarity and species richness index values obtained as shown in Table 2.
Table 2, indicating that the stability of agro-ecosystems are the highest found in of mustard crop treated with a concentration of *B. thuringiensis* spraying 4 cc/l is 1.867, while the lowest agro-ecosystem stability is the of mustard crop spraying treated by solution of *B. thuringiensis* concentration of 8 cc/l is 1.445. The higher stability of the of mustard crop agroecosystem treated 4 cc/l due to the planting of mustard treated 4 cc/l cause the index value of species richness higher that resulted in higher values of diversity index.

Table 2: Average value of arthropod diversity index, dominance index, index of species similarity and index of species richness on of mustard crop treated with various concentrations of a solution of *B. thuringiensis*

Treatments	Diversity index	dominance index	Index of species similarity	Species richness Index
Control (applied by water)	1,603	0,303	0,717	2,313
Application *B. thuringiensis* used a con-centration of 2 cc / l of water	1,744	0,217	0,778	2,247
Application *B. thuringiensis* used a con-centration of 4 cc / l of water	1,867	0,256	0,742	2,848
Application *B. thuringiensis* used a con-centration of 6 cc / l of water	1,748	0,074	0,742	2,526
Application *B. thuringiensis* used a con-centration of 8 cc / l of water	1,455	0,355	0,689	1,883

According to Odum (1993) states that diversity is determined by two things: the level of species richness and similarity. The more types teridentifikas then even higher species richness. While the degree of similarity is the distribution of all individuals that exist in a community. The higher the level of species richness and similarity, the higher the diversity index. Price (1997) states that an increasing number of food chain in an ecosystem will have an impact on improving the stability of the ecosystem. Dindal and Cllifford (1977) adds that the stability of the ecosystem can be formed by an organism steady condition and is shown by the balance against the interference of external factors.

Pielou (1975) states that the high environmental stability was preceded by a high diversity. A community of more complex the higher the stability of the community system, so that a high diversity will lead to a high stability of the community. Oka (1995) explains that if a predator is only dependent on a prey species, then the consequences will be very volatile both populations, especially when they are both in a simple environment, so it can lead to the destruction of both populations. Interactions will be more stable if there are two or more species of prey, because if the prey species that the number is very down, then the other prey species may be increased in number because it is not noticed by predators. So thus planting mustard is applied to *B. thuringiensis* with a concentration of 4 cc/l highest stability of the ecosystem compared mustard crop being treated in addition to 4 cc/l. Index of species diversity and level of species richness in this experiment is still relatively low, according to Suana and Haryanto (2007), The level of diversity is low when the value of the diversity index 1 <H <2, and richness levels are low if the richness index level of species has R <3,5. The lower the index value of diversity in cropping of mustard

treated with a concentration of *B. thuringiensis* 8 cc/ l, due to the dominance index value is higher.

According to Odum (1993), which controlled biological communities are affected by a single species or group of species that dominate the environment and the organism is dominant, resulted in a high dominance index. Diversity will tend to be low in the ecosystem who are physically restrained and regulated high in biological ecosystems. Croft (1990) states that the predators and parasitoids have large amounts on fauna communities in most agro-ecosystems. So the side effects of the application of *B. thuringiensis* directly or indirectly cause a significant change in the quantity of energy and the flow of nutrients in the ecosystem. Comparison of the effects between the treatment of the population variables can be seen in Figure 1.

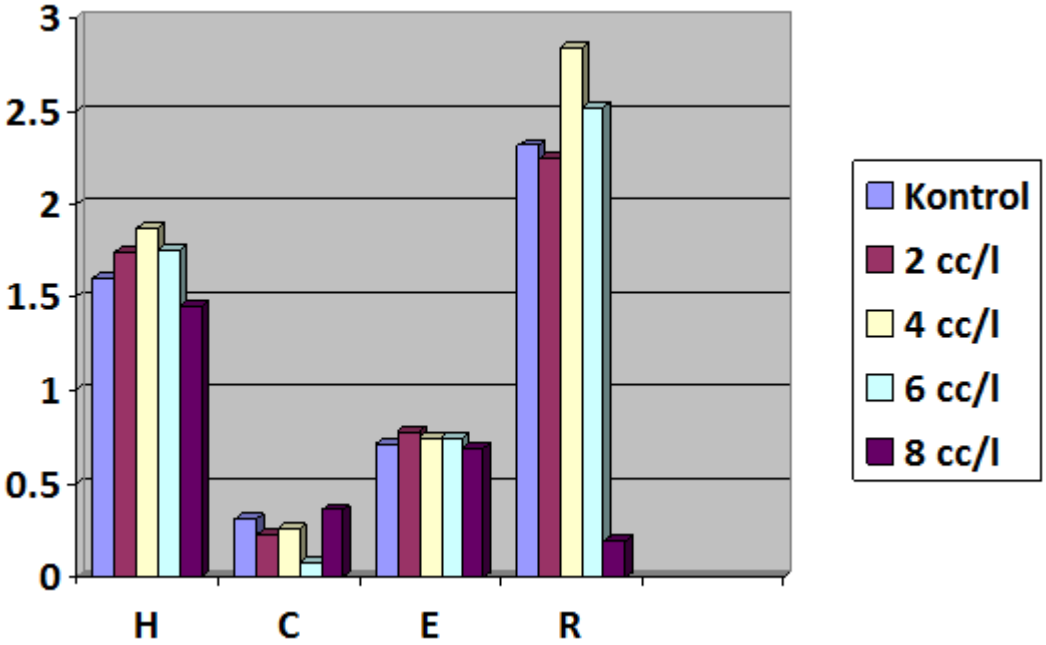

Figure 1: Graph of the comparison between the treatment of the population variable

Description: H = diversity index; C = Dominance Index; E = similarity Index of species; R = Richness Index of species.

4. Conclusions

From the research results can be summarized as follows:

1) Application *B. thuringiensis* can reduce the intensity of the damage caused by leaf-eating pests in the crop of mustard of mustard.
2) The concentration of *B. thuringiensis* most likely to decrease the intensity of damage is the concentration of 6 cc/l and 8 cc/l.
3) Cropping mustard has the highest stability of the ecosystem are planting of mustard treated *B. thuringiensis* application concentration of 4 cc/l lead diversity index 1.867.

5. Future Scope

The results of this study help to retain the quality and quantity of production of mustard, as well as health and environmental quality of mustard cropping ecosystems. Further research must be done on the field application of several different types of ecosystems crops that can be widely applied.

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