Effects of Bacillus thuringiensis-based bio-insecticides on the presence of Aphis gossypii and Coccinellid predators on intercropping cultivation

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Abstract. In the intercropping cultivation, pest problem becomes an important factor on reducing production. Control by using chemical insecticide should be done wisely otherwise there will be a negative effect to environment. An alternative strategy is by application of *Bacillus thuringiensis*-based bio-insecticide. The objective of this research was to investigate the effect of the B. thuringiensis based bioinsecticide on the presence of *Aphis gossypii* and Coccinellid predators on intercropping cultivation (cucumber and long bean). Research was conducted in experimental field in Sriwijaya University Indralaya Campus South Sumatra, from June to August 2018. The research was arranged by Completely Randomized Blocked Design, with three treatments and 6 replications. The treatments were application of B. thuringiensis based-bioinsecticide, chemical insecticide and non-application of both. Treatments were done once a week as well as observation of *A. gossypii* and Coccinellid predators. Sampling method used was visual control, hand picking, pitfall trap, and insect net. The results showed four species of Coccinelid were identified i.e. *Cheilomenes sexmaculata, Harmonia conformis, Verania discolor* and *Coccinella repanda*. Population of Coccinellid predators was the highest at application of *B. thuringiensis* based bioinsecticide eventhough A. gossypii was also high. B. thuringiensis based-bioinsecticide was an ecofriendly way to control insect.

1. Introduction

Intercropping is one system of the cropping and pest control systems. In integrated pest control, the method of farming is a recommended method before being carried out by other means of control [1]. Intercropping cropping systems provide benefits including more optimal land use, some types of crop production can be harvested and repeated, and economically beneficial as well [2.3]. As a system of control, intercropping can provide benefits including reducing insect pest populations, increasing beneficial insects, and weed suppression [4]. This planting system is also expected to reduce the level of synthetic chemical pesticides use, because it has been known the use of pesticides will cause negative impact such as decrease in the number of pollinator insects, and natural enemies are suspected to be killed during pesticide application. Therefore, the use of chemicals as insect poisons should be reduced and only be used when insect pests reach economic threshold [5]. A clean environment without contamination with chemicals for a long time will show a high level of biodiversity, in contrast to areas that are polluted with chemical pollutants, the level of diversity is decreasing [5,6]. The use of microorganism-based botanical ingredients has been used for several decades. Commercial use of Bacillus thuringiensis has also become a trend. In accordance with the mode of action, B. thuringiensis only kill target insects [7], therefore non-target insects, especially useful insects and their natural

enemies are not disturbed. *Aphis gossypii* (Homoptera: Aphididae) is an important pest in long bean and cucumber plants. Biological control can be done using Coccinellid predators [8]. The study aims to investigate the effect of the B. thuringiensis based bioinsecticide on the presence of A. gossypii and Coccinellid predators on intercropping cultivation (cucumber and long bean).

2. Material and Methods

The study was conducted in the experimental field Faculty of Agriculture, Sriwijaya University, Indralaya campus from June to August 2018. The intercropping of cucumber and long bean was intercropped with a size of 15 mx 8 m in 3 plots, including *B.thuringiensis* based-bioinsecticide treatment plots and chemical insecticide treatment plot, and one plot without treatment (control). Each treatment was made into 6 replications in a sub plot of 3 m x 6 m, there were 6 subplots. Distance between subplots was 2 m. On each subplot there were 3 mounds where each mound was planted as much as 7 cucumbers and 7 long bean plants respectively. The research was arranged in a Factorial Completely Randomized Design (FCRD) with 3 factors and 6 replications. These factors were addition *B.thuringiensis* -based bioinsecticides, chemical insecticides (Lamda sihalotrin active ingridient) and control (no application).

2.1. Preparation of B. thuringiensis-based bioinsecticides

The formulation of bio-insecticide was started with the preparation of seed culture [9] by placing one lope of B. thuringiensis Isolate SMR-04 in 10 ml of Nutrient Broth (NB) media and was shaken for 12 hours at 200 rpm. Then, 5 ml of this culture was taken and transferred to 10 ml of NB media and was shaken for 12 hours at 200 rpm. Seed culture was ready to be used to make bio-insecticide. Media growth of B. thuringiensis was total of 50 ml coconut water + 50 ml liquid waste of tofu industry + 13 g GSM (golden snail Meal) + mineral salts (50 mg CaCl2 + 50 mg MgSO4 + 50 mg K2HPO4 + 50 mg KH2PO4), in 250 ml Erlenmeyer flask (modification by Ref. 9). The growth of GSM in B. thuringiensis growth media was produced the highest spore density (unpublished data). The Erlenmeyer flasks were covered with aluminium foil and tighten with rubber band. The growth media were then sterilized using autoclave at 121 ° C and 1 atmosphere of pressure for 20 minutes. After the sterilized growth media were cool enough, 5 ml of seed culture was poured into each flask of treatment. The growth media were then agitated in the shaker at 200 rpm for 72 hours. Spores counting was conducted by using Haemocytometer at 400 x magnification before application leading to 6.05 x 107 spores / ml.

2.2. Bioinsecticide and chemical application in the field

B.thuringiensis based-bioinsecticide in the field has spore density of 5.6 x 108 spores / ml. Ten ml of Bt was dissolved in 1 liter of water and used for spraying in each subplot. Chemical insecticide spray dose used was according to the instructions listed on the label. Bioinsecticide and chemical applications were carried out once a week as much as 4 times.

2.3. The sampling method of Aphis gossypii and predator

Because A. gossypii behave low mobility, sampling was determined in one plant from each mound, so the observation was focused on 3 plants per sub-plot. The number of aphids that live on long bean and cucumber plants on the stems, petioles and leaves was calculated. Observations were carried out by calculating the number of seeds directly on the plant with a magnifying glass (loupe). Identification of A. gossypii was carried out in the Entomology Laboratory of Plant Protection Dept. Faculty of Agriculture Sriwijaya University. Because Coccinellidae were active flying insects, the calculation of Coccinellidae predator samples was done by direct observation and hand picking [10]. The coccinellidae insect caught was kept in a bottle (d=5cm h=10cm), then they were identified in the laboratory. Observations and sampling were carried out 4 times, ie once a week starting at 15 days after planting. Recorded population data of aphids and coccinellidae predators were done in each treatment and replication.

3. Results and Discussions

3.1. Population of Aphis gossypii

In long bean plants, the number of aphids was higher compared to population in cucumber plants. This occur in the bio-insecticide Bt treatment, chemical insecticide treatment and also in the control. However, in 4th week the number of aphids in long bean in the chemical treatment was the lowest. This showed that chemical insecticides was effective in controlling pest insects because they were broad spectrum and faster in killing pest insects [11]. Aphids in long bean plants in all treatments showed a higher number than the population in cucumber plants. This shows that long bean plants are preferred by aphids. This wass supported by the research of Saha et al.[12] who reported that A. gossypii is a cosmopolitic and polyphagous pest that attacks various types of plants including cotton and cucurbitaceae. Complete data on aphids populations are presented in Fig1, Fig 2 and Fig 3.

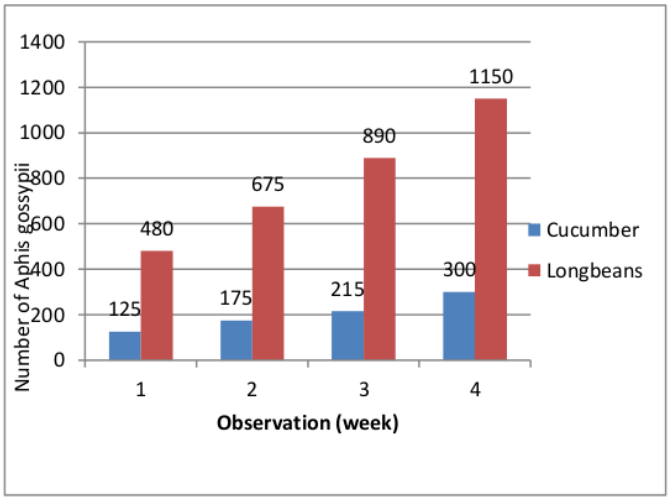

Figure 1. Number of Aphis gossypii observed in B. thuringiensis –based application in intercropping cucumber and long beans during 4 weeks observation

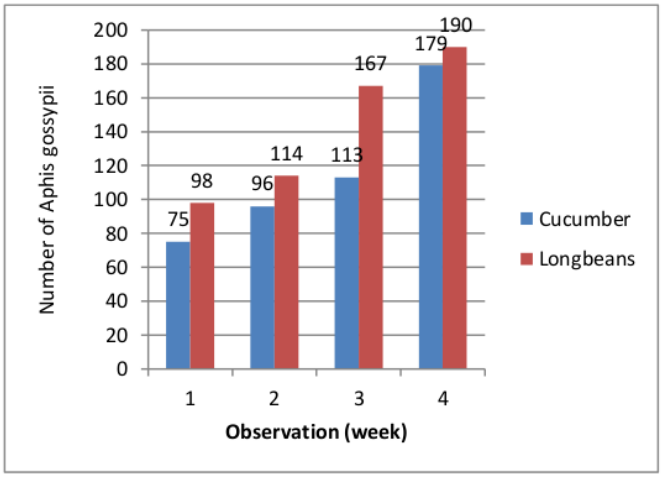

Figure 2. Number of *Aphis gossypii* observed in chemical insecticide application in intercropping cucumber and long beans during 4 weeks observation

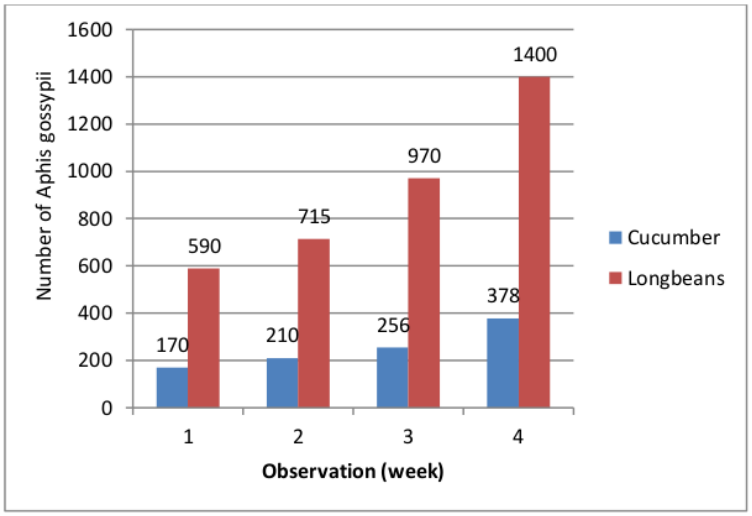

Figure 3. Number of *Aphis gossypii* observed in control (no- application) in intercropping cucumber and long beans during 4 weeks observation

3.2. Population of Coccinellid predators

The results of field observations on Coccinellid predators and identification of Coccinellids showed four species were obtained, namely Cheilomenes sexmaculata, Harmonia conformis, Verania discolor and Coccinella repanda. The highest number of predators in bioinsecticide treatment was V. discolor while control found was *C. sexmaculata* (Fig. 4.).

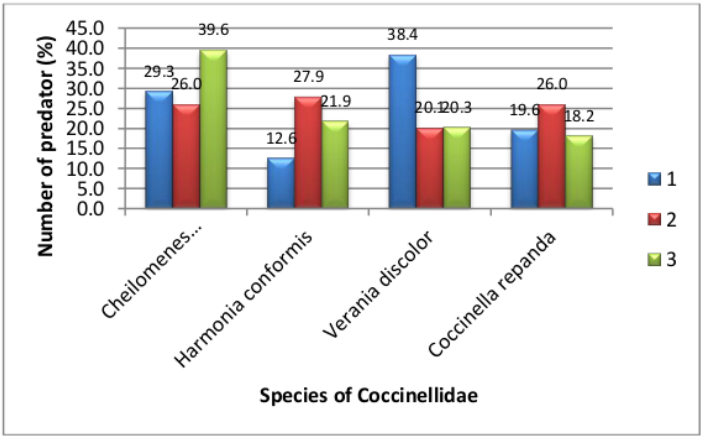

Figure 4. Number of spesies Coccinellid observed in intercropping cucumber and long beans during 4 weeks observation

Note : 1. B. thuringiensis-based bioinsecticide 2. Chemical insecticide 3. Control (no-application)

In B. thuringiensis treatment, it appears that the number of predators was dominated by V. discolor, followed by C.sexmaculata, C. repanda and H. conformis. In contrast to the treatment of insecticides, number of H. conformis, C. repanda, C. sexmaculata and V. discolor were found,

respectively. It was suspected the amount of Coccinellidae was related to the presence of aphids in the location. Observations made on Coccinellid predators were randomized to both cucumber and long bean plants. Coccinellid predators were polyphagous (attacking many prey) and have the ability to move quickly by running and flying [13].

3.3. The population of Aphis gossypii and Coccinellid predators.
In the application of bioinsecticide it was showed aphids population was higher than thus in the chemical insecticide treatment, but lower than thus in control. The highest percentage of predator population was in bioinsecticide treatment. The complete data can be seen in Fig. 5.

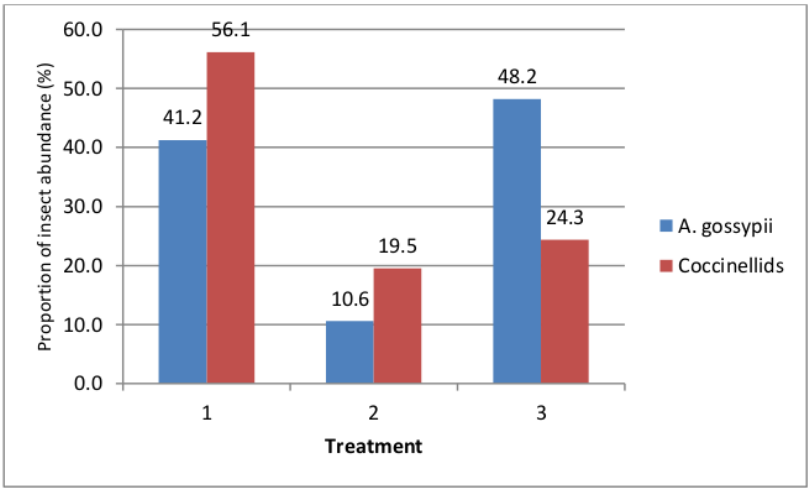

Fig. 5. Insect abundance (%) in intercropping cucumber and longbeans during 4 weeks observation
Note : 1. B. thuringiensis-based bioinsecticide 2. Chemical insecticide 3. Control (no-application)

Fig.5 showed that the aphis population of B. thuringiensis was higher than the population in chemical treatment, but the aphid population was still lower than the population in the control (without treatment). When compared to the presence of coccinellid predators, B. thuringiensis treatment was higher than thus in chemical treatment. It also appeared that by spraying chemicals the number of aphids became lower when compared with application of B. thuringiensis bioinsecticide. However, it also appeared that the Coccinellid population was higher in bioinsecticides treatment. This showed that chemical treatment will effectively kill or control aphid pests, but after many aphis died, the number of predators will also drop dramatically due to the lack of prey. In addition, chemical applications caused the decline number of predators can be caused by direct death of predators. This was supported by Hill et al. [14] that the use of chemicals in pest control will cause non-target insects die due to broad chemical pesticides. In contrast to the use of B. thuringiensis, the number of pests was indeed high but this was followed by a high number of predators. This was consistent with the statement of Saeed and Razaq [15] which stated a large number of prey will increase the number of predators in the environment.

4. Conclusion

Application of B. thuringiensis as a selective bioinsecticide against target insects was proposed. In intercropping of cucumber and long bean, population of aphids was quite high, but it was balanced with the number of Coccinellidae predators which were also high. In chemical insecticides application, the number of aphids was low but the number of predators was also low. B. thuringiensis based bioinsecticide was effective in controlling aphids and safe Coccinellidae predators.

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