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by Dr.ir.yulia Pujiastuti, Ms.

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Original Article

Evaluation of *Bacillus thuringiensis*-based bioinsecticide impacts on arthropod biodiversity in the intercropping system

Yulia Pujiastuti
Plant Protection Department, Faculty of Agriculture, Sriwijaya University

Abstract

The intercropping system is intended to increase vegetable production. However, The presence of pests reducing production levels is a challenging constraint and should be controlled. In this study, Control of insect pests will be evaluated by *Bacillus thuringiensis*-based bioinsecticide. The purpose of the research was to investigate the biodiversity of insect pests and their natural enemies in intercropping systems (cucumber and long beans), applied by *B. thuringiensis*-based bioinsecticide, chemical insecticide, and no-both application. Observation of existing insect-plant canopy was directly observed, and those on soil surface were observed by pitfall traps and yellow trays, as well. The research was performed in the experimental farm of the Sriwijaya University Faculty of Agriculture, Indralaya Campus, Ogan Ilir, South Sumatra, from July until October 2018. Results indicated three ecological roles of insects, namely predators/carnivores, pests/herbivores, and pollinators. Other insects and arthropods obtained from direct observation sampling method identified ten orders, but treatments of *B. thuringiensis*-based bioinsecticide resulted in 29 families of insects, chemical treatment was 17, and control (no treatment) was 32 families. *B. thuringiensis*-based bioinsecticides may be used in the intercropping system since it causes the highest biodiversity (H '= 2.96), high predator population (45%) and low pest population (41%) compared to other treatments.

Keywords: *Bacillus thuringiensis*, cucurbitaceae, insect pests, natural enemies, *Vigna sinensis*

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Introduction

The intercropping system, method of planting, trigger the increase in the quantity and quality of crop production, reduce crop damage due to attacks by pests, diseases, and weeds. (Mousavi and Eskandari, 2011). Moreover, it also increases the productivity of the land area. Compared to monoculture plants, the ecological system of overlays is more beneficial, especially in terms of insect spread. Insects have the opportunity to get their host plants (Rahimi et al., 2013). Therefore, insect-pests attacking should be controlled with the natural or non-chemical way in order to obtain chemical-free foods. Organic farming has invaluable influence, especially on birds, insects, weeds, wildlife, and soil flora and fauna. Compared to conventional agriculture, organic farming has cost-effective food products, free of synthetic fertilizers and pesticides (Siddique et al., 2014). Higher productivity of healthy food is required to meet society's consumption. Organic food is crop production without any chemical residues coming from fertilizer and pesticides. It is also free from contamination of pests and plant diseases. The application of chemical insecticides continuously for long periods of inappropriate dosage will lead to the residual occurrence. To reduce this impact, alternative chemical insecticides such as entomopathogenic bacteria *Bacillus thuringiensis* should be considered (Blanc et al., 2002; Sarker & Mahbub, 2011).

B. thuringiensis, Gram-positive bacterium producing spores and protein at the sporulation phase is entomopathogenic which will be consumed by *Insects*. At midgut with high pH conditions, the protein will be digested and converted to a toxin, absorbed in the intestine and lysed, and caused death in insect (Bravo et al., 2007). These bacteria can kill sensitive insects (Lacey et al., 2015). *B. thuringiensis* should be prepared as bio-insecticide for controlling insect-pests. In general, *B. thuringiensis* is very selective against target insects. The selectivity of *B. thuringiensis* is due to the pH condition of the insect's midgut.

B. thuringiensis application as a bio-insecticide in the intercropping system needs to be conducted. The application of chemical insecticides is mostly a broad-spectrum that potentially can kill both insect pests and natural enemies. Unlike broad-spectrum chemical pesticides, *B. thuringensis* toxins are selective, and negative environmental impacts are limited (Lacey et al., 2015; Sarker & Mahbub, 2011). The aim of this research is to study the biodiversity of insect pests and their natural enemies in the intercropping with the application of *B. thuringiensis*-based bioinsecticide.

* Corresponding Author:
Yulia Pujiastuti
Plant Protection Department, Faculty of Agriculture, Sriwijaya University
Phone : 0711-580663 Fax : 0711-580276
e-mail : ypujiastuti@unsri.ac.id

Method

The research was performed in the experimental farm of Faculty of Agriculture, Sriwijaya University, Ogan Ilir, South Sumatra (3°13'19,19"LS, 104 °38'46,12" E) from July-October 2018. The land was grown by long beans

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(*Vigna chinensis*) and cucumber (*Cucumis sativus*). Three locations of experimental farms were prepared. Applied treatments were 1) *B. thuringiensis*-based bio-insecticide; 2) chemical insecticide (active ingredient cyhalothrin) and no-application (as control).

Field Preparation

The designed location (50 x 15 m) was set in 3 different places with a distance of 20 meters each. Each locations was constructed with a mound of 1 meter wide and the distance between ridges was about 50 cm, resulting 10 ridges. The soil was prepared following to the farmer's practices. Every area was covered by plastic mulch. In each plastic mulch was made a hole with a distance of 20 x 20 cm. In each hole were filled with long bean seeds and cucumber seeds. The plant was assembled with a stake at the age of 2 weeks.

Preparation and application of *B. thuringensis*-bio insecticides

B. thuringiensis KJ3R5 (Pujiastuti et al. 2017) was characterized by ~~a~~ high toxicity of *Plutella xylostella* and armyworm *Spodoptera litura* (unpublished data). Briefly, The seed culture of *B. thuringensis* KJ3R5 was transferred into a 250 ml Erlenmeyer flask containing 50 ml of Nutrient Broth (NB) and shaken at 200 rpm, room temperature, for 12 h. 10 ml of culture cell transferred in 250 ml of Erlenmeyer flask containing 50 ml of NB and shaken at 200 rpm, room temperature, for 12 h. Resulting, seed culture was ready to be used for bio-insecticide production (Dulmage & Rhodes, 1971). Preparation of bio-insecticide was performed as the previous method with modification (Valicente et al., 2010). 150 ml of coconut water was supplemented with 50 mg of CaCl2, 50 mg MgSO4, 50 mg K2HPO4, and 50 mg KH_2PO_4 and transferred into a 250 ml flask as culture medium, covered with aluminium foil and sterilized in autoclave for 20 minutes at 120 ° C at 1 atm pressure. 5 ml of seed culture was transferred into culture medium and shaken at 200 rpm, room temperature, for 72 - 96 h. Spore density was performed before application. The calculation of spores was conducted by taking 1 ml of suspense and counted by haemocytometer under a binocular microscope. Suspension of *B. thuringensis* of 10^{11} spores/ml was used for Applications of *B. thuringiensis*-based bioinsecticide, and chemical insecticide in the intercropping longbeans and cucumber were conducted every week until harvesting. Application of chemical insecticide used a standard dose of cyhalothrin active ingredient.

Collecting Insect Pests and Arthropod Predators

The pitfall traps (36 pieces) and yellow tray traps (8 traps) were performed to collect insect and other arthropods in the farm of long beans. Briefly, the traps were supplemented with soapy water, mounted and left for 2 x 24 h. Besides, direct observation was conducted in every subplot of experimental subplots at 7-9 am. Arthropods were stored into plastic containers, then preserved with 70% alcohol and identified in Entomological Laboratory (Huseynov, 2006; Kalshoven, 1981; Triplehorn & Johnson, 2005).

Data analysis

Diversity Index was a formula to observe the diversity development based on the individual abundance of each species. The Shannon (H ') index is calculated using the following formula (Ludwig & Reynold, 1988; Magurran, 2009):

$$\mathbf{H'} = \sum_{i=1}^{s} \left(\frac{\mathbf{ni}}{\mathbf{N}}\right) \mathbf{In}(\frac{\mathbf{ni}}{\mathbf{N}}$$

Description: H: Index of Shannon Diversity, S: Number of species, number: number of individuals of all species. The criterion of species diversity based on Shanon-Wiener values index, as follows: H '<1: very low; H '> 1-2: low; H '> 2-3: medium (medium); H'3-4: high and H '> 4: very high.

Index of dominance: $D = \sum \left(\frac{ni}{N}\right)^2$

Description: D = dominance index, this is: number of individuals, N: number of individuals of all species.

Index of relative abundance: $KR = \frac{ni}{N} x\ 100\ \%$

Description: KR: index of relative abundance, this is: the number of individuals of species, N: number of individuals of all species.

Results

The total number of insects and arthropods collected by direct observation methods, pitfall traps, and yellow trays resulted in 10 orders and 35 families. Among this number, *B. thuringiensis*-based bioinsecticide treatment obtained 10 orders and 29 families, chemical insecticides-based treatment obtained 10 orders and 17 families, and no-application based-treatment obtained 10 orders and 32 families (Tab. 1).

The results of arthropod identification, illustrated the various roles of insects in cucumber and long bean intercropping. These roles are plant eaters (pests), predators, parasitoid and pollinator (insect pollinators). In each application-based treatment, a comparison of the insect role shown in figure 1.

To describe the wealth of organisms in a habitat, an indexing system is measured. Diversity index, dominance index and relative abundance index are the standards analysis to describe the wealth of these organisms. The greater the value of an index will illustrate the higher value of diversity, dominance and relative abundance. Results of observations and calculations of several indices in the study are presented in table 2 below.

Table 1. Insects and other arthropods found in intercropping of cucumber and long bean crops during 8 weeks observation

No	Orders	No	Family	Application			Ecological Roles
				B. thuringiensis	Chemical	No-application	
1	Araneida	1	Oxyopidae	v	v	v	predator
		2	Lycosidae	v	-	v	predator
2	Coleoptera	1	Carabidae	v	-	v	predator
		2	Coccinellidae	v	v	v	predator/pests
		3	Staphylinidae	-	-	v	predator
		4	Chrysomelidae	v	v	v	pests
		5	Elateridae	-	v	v	pests
3	Diptera	1	Asilidae	v	-	v	Predator
		2	Sarcophagidae	v	-	v	Predator
		3	Dolichopodidae	v	-	v	Parasitoid
		4	Syrphidae	-	v	v	Predator
		5	Tephritidae	v	v	v	Pests
4	Hemiptera	1	Miridae	v	v	-	Pests
		2	Reduviidae	v	-	v	Predator
		3	Pyrrhocoridae	-	-	v	Pests
		4	Coreidae	v	v	-	Pests
		5	Pentatomidae	v	-	v	Pests
		6	Aphididae	v	v	v	Pests
5	Homoptera	1	Cicadellidae	v	-	v	Pests
		2	Aleyrodidae	v	v	v	Pests
		3	Pseudococcidae	v	v	v	Pests
6	Hymenoptera	1	Formicidae	v	v	-	Predator
		2	Ichneumonidae	v	-	v	Parasitoid
		3	Vespidae	v	-	v	Pollinator
		4	Apidae	v	v	v	Pollinator
7	Lepidoptera	1	Noctuidae	-	v	v	pests
		2	Tortricidae	v	-	v	pests
		3	Pyralidae	v	-	v	pests
8	Mantodea	1	Mantidae	v	v	v	predator
9	Odonata	1	Aeshnidae	v	-	v	predator
		2	Libellulidae	v	-	v	predator
		3	Coenagrionidae	v	-	v	predator
10	Orthoptera	1	Grylidae	v	v	v	predator
		2	Acrididae	-	v	v	pests
		3	Gryllotalpidae	v	-	v	pests
			Total	29	17	32	

Note: v: presence; - : absence

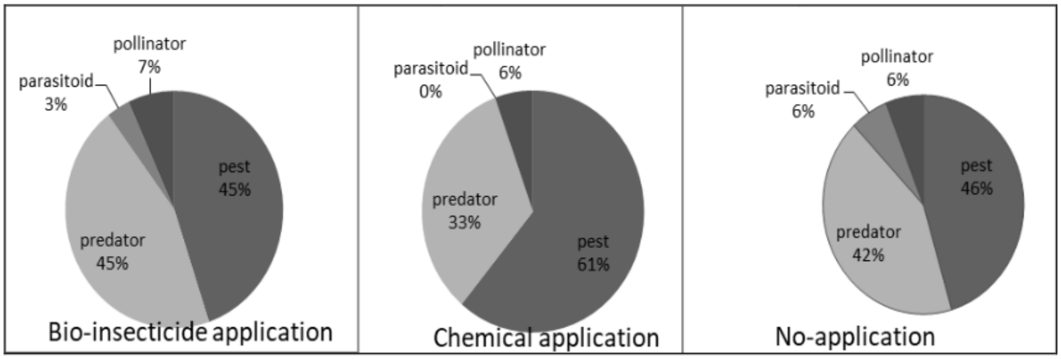

Figure 1. Percentage of arthropods found in intercropping cucumber-long beans according to their ecological roles

Table 2. Biodiversity, dominancy and relative abundance indexes of arthropods found in intercropping cucumber and long beans

Application	H' index	D index	KR index
Bt-Bioinsecticide	2.96	0.08	1.1 – 22.1
Chemical insecticide	2.26	0.16	1.41 – 31.1
No-application	2.75	0.11	0.21 – 23.3

Discussion

Based on arthropod identification, it is known that there are various roles for insects in cucumber and long bean intercropping. These roles are plant eaters (pests), predators, parasitoid and pollinator (insect pollinators) as shown in figure 1.

Insects and other arthropods found in application-based treatment were similar in several orders, but it was different in a number of families. Although there were 10 orders, in chemical insecticides-based treatment, possessed 17 families. Compared with bioinsecticide treatment (29 families) and without treatment (32 families), it is supposed that many other insects and arthropods died caused the chemical insecticides. In general, chemical insecticides have broad-spectrum, can kill various species of insects. This is supported by the Chowański et al. (2014) and Aktar et al. (2009) who stated chemical insecticides contain the toxic ingredient. Because it was widely applied, target insect or non-target insect will be affected, leading to death. Moreover, the use of chemical insecticides which is not appropriate with the standard will cause mortality of significant insects, especially parasitoid and predator (Bueno et al., 2017). It was also reported that application of insecticides would cause the decreasing number of pollinating insects (Brittain et al., 2010; Kumar et al., 2018). In this observation, it was noticed that chemical insecticides-based treatment, number of pollinator insects was only one family (Apidae) while in bioinsecticides and non-treatments, it was found two families (Vespidae and Apidae).

Predatory arthropods were found in all treatments, from family Oxyopidae (order Aranida), family Mantidae (order Mantodea) and family Gryllidae (order Orthoptera). Suggesting, arthropods ability to survive in all habitats. As it was known, the family of Oxyopidae is an active spider in moving, jumping and climbing between stems and leaves (Aviles, 1994; Huseynov, 2006). Spiders commonly hide to get their prey. The Mantidae family, the praying mantid, is a polyphagous predator. It can move actively, but at the time of waiting for prey, this insect dwells motionless, so it is not known by its prey. The Gryllidae family, known as mole crickets, is active at night, can move and jump actively (Gawalek et al., 2014), and commonly hides in its nest inside the soil. The three families were found in all three habitats (with various treatments) since they have the ability to avoid chemical insecticides at the time of application.

In one family of insect, generally, they play a role as pests and predators, such as the order Coleoptera, family Coccinellidae. For example, *Epilachna admirabilis* acts as a pest and *Menochilus sexmaculatus* acts as a predator (Weber & Lundgren, 2009), however, there was one order which all members play as pests. For example, Lepidopteran order acts as a pest. The Odonata order, three families, were found and all played as predators, namely Aeshnidae, Libellulidae and Coenagrionidae families. The last family was a small dragonfly commonly called a damselfly. The three families were not found in intercropping plants treated with chemical insecticides. There are allegations by application of chemical insecticides, insect pest species as prey to the Odonata were decreasing, and Odonata does not visit the plant. This statement was supported by the Sih (1979), who stated predators would find a place where insects (prey) were in a high population condition and can meet the life needs of these predators.

In bioinsecticide-based treatment containing active ingredients of *B. thuringiensis*, there were 45% of insects played as pests and 55% of insects played as non-pests (Predator/parasitoid/pollinator). Compared to chemical insecticides-based treatment, 61% of insect played as pests, and 39% played as non-pests. This phenomenon showed that application of chemical insecticides-based treatment would harm insects (other arthropods) that played as non-pests. The impact can be in the form of non-pest insect deaths or also moving to other places. With its broad-spectrum, chemical insecticides can kill non-target insects (Bueno et al 2017; Chowański et al., 2014). In the application of *B. thuringiensis* based bioinsecticides, there are specificities in how to kill pest insects. *B. thuringiensis* has a selective target in insects, so it does not cause death in non-target insects. This specificity can be observed from the protein content of *B. thuringiensis* (Bravo et al., 2007).

The existence of many predators will suppress pest populations. As known, predators are predatory animals which actively looking for and hunting prey and will feed some or all of their prey's body (Evans, 1982). it is worth to notice that bioinsecticides based-treatment where predators amounted to 45% and pest populations amounted to 45%. In the chemical insecticides-based treatment, although the number of insect pests was high (61%) and predator population was relatively half of its prey (33%). Therefore the ability of feeding seems to be sufficient for the needs of these predators. The reduction of insecticide application, the ability to prey on predators will increase (Fernandes et al., 2010).

Biodiversity index in all treatments ranged from 2.26 - 2.96, resulting that diversity was moderate. This result showed that habitat managed by humans would have limitations, especially in abiotic factors. Other insects and arthropods were inseparable from biotic and abiotic influences in their lives (Savopoulou-Soultani et al., 2012; Vidya et al., 2015). No individual dominates. However, the H' index in *B. thuringiensis*-based-bioinsecticide treatment was higher than other treatments. Indicating that insect diversity in the treatment of B. *thuringiensis* is higher than that in the treatment of chemical insecticides. This can be seen in the number of families found in Bt bioinsecticides. Although the number of orders was similar, the number of families differs quite far, namely 29 families in *B. thuringiensis* based bioinsecticides and 17 families in chemical insecticide treatments. Suggesting that biodiversity in crops was associated with a lower chemical insecticide treatment.

The dominance index showed low value (0.08-0.16) in each location treated with *B. thuringiensis*-based bioinsecticide, chemical insecticide based-treatment and without treatment. Suggesting, there was no family of insects dominated in the community. In general, the observation area was closed to each other, which was caused by insect pests and natural enemies to spread quickly. Moreover, insect behaviour also affected their existence elsewhere (Schowalter et al., 2005).

The relative abundance index in bioinsecticide based-treatments was 1.1 - 22.1%, in the chemical insecticides based-treatment was 1.41 - 31.1% and the treatment without applications was 0.21 - 23.3%. In each treatment, the relative abundance index value was found in Aphididae family (Hemiptera order). These insects act as polyphagous pests in both cucumber and long bean plants and have a life cycle of 11.87 - 12.57 days (Singh & Singh, 2015).

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