Comparing The Toxicity of Some Formulate Synthetic and Organic Insecticides to Black Soldier Fly (*Hermetia illucens*) Larvae

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Abstract. Agricultural wastes had been considered as one of the major sources of environmental as most of the waste consist of organic wastes which produces a significant amount of greenhouse gases when decompose. Some studies showed the benefit of these wastes as a material for insect farming, such as larva of black soldier flies (*Hermetia illucens*), to produce a protein and lipid-rich biomass for various types of bioindustries. However, other studies showed that most of the organic agricultural wastes originated from local farms, contaminated with insecticides. This condition caused a major concern on the health of the larvae during farming as the information on the effect of insecticide on black soldier fly larvae is considered rare. In this study, we tested the toxicity of some common synthetic and organic insecticides, applied in the local farming system, to black soldier fly larvae. Two types of synthetic insecticides (active ingredient endosulfan and profenofos) and one type of organic insecticide (a microbial insecticide with active ingredient δ-endotoxin) were mixed with commercial chicken feed which used as feeding material for the 10 days-old larvae. A total number of 120 larvae were used in this study and the mortality rate was observed for 72 hours. The result of this study showed LC₅₀ of endosulfan was recorded at 236.25 ppm, profenofos at 380.62 ppm, and δ-endotoxin at more than 7600 ppm. All of this value significantly higher than insecticide residual limit regulation and reports on the level of insecticide residual level of common local agricultural products. Based on this study, it can be concluded that common organic agricultural wastes are applicable to be used as feeding material of black soldier fly larvae directly although further studies are needed regarding the safety of the final product of insect farming.

Keywords: black soldier fly, *Hermetia illucens*, insecticides, toxicity

1. Introduction

Black soldier fly (*Hermetia illucens* (Diptera: Stratomidae)) is one of the saprophages which convert organic wastes into biomass rich in protein and lipid [1]. This species known as a general feeder that able to consume various types of organic wastes included decaying fruits and vegetables. However, studies showed that pesticide residues were found in fruits and vegetables produced in various regions in Indonesia [2,3]. As a beneficial insect,
black soldier fly never expose to insecticide during their life. This condition may be made them susceptible to insecticide residues in their feed.

This study will determine the level of insecticide susceptibility of black soldier fly to common insecticides applied in local agriculture. The insecticide susceptibility was expressed in lethal concentration, doses, and time [4-6]. This information will provide better fruits and vegetables based wastes bioconversion practices to maintain the health of the agent (e.g. black soldier fly) and final product produces by this method.

2. Research methods

2.1 Hermetia illucens

*Hermetia illucens* larvae were originated from the egg produced by *H. illucens* population that was kept, since 2016, in the Entomology laboratory of School of Life Sciences and Technology, Institut Teknologi Bandung.

2.2 Insecticide bioassay

Insecticide tested in this study consisted of synthetic insecticides with varied active ingredients such as endosulfan (organochlorine), profenofos, and organic insecticides δ-endotoxin (microbial insecticide). The level of susceptibility was tested in 3 concentrations, created by mixing an insecticide with distilled water, for each insecticide (Table 1).

| Insecticide     | Concentration (ppm) |
|-----------------|---------------------|
| Endosulfan      | 34.5 172.5 345      |
| Profenofos      | 75  375   750       |
| δ-endotoxin     | 760 3800 7600       |

Ten (10) black soldier fly larvae aged 14 days was applied as bioassay subject. The bioassay was conducted three times for each concentration. Larvae were kept inside the plastic cup (3 cm diameter and 3 cm height) and fed with commercial chicken feed (100 mg/larvae/day, water content 70% (w/w)) [7,8] mixed with insecticide accordingly. On the other hand, the control group was fed with commercial chicken feed mixed with distilled water only.

2.3 Mortality

The mortality rate of black soldier fly was observed at 1, 4, 8, 12, 24, 48, and 72 hours after treated with synthetic insecticides and every 24 hours for 7 days after treated with organic insecticides. Death larvae were tested by touching larvae with a pincher and nonresponsive larvae considered as death [9].

2.4 Data Analysis

The mortality rate was applied as the subject for probit analysis to obtain lethal concentration 50% (LC50), lethal doses 50% (LD50), and lethal time 50% (LT50). All tests were conducted by a macro program developed in Microsoft Office Excel 365.

3. Result and Discussion

3.1 Mortality rate of black soldier fly larvae for each type of insecticide

On average, a high mortality rate was recorded in the application of insecticide at middle and high concentrations. There was no synthetic insecticide that produced a 100% mortality rate (Table 2). Almost all synthetic insecticide produced rapid mortality at a level of 50-90% (after 24 hours).
The rapid mortality of *H. illucens* larvae in this study could be caused by the mode of action of insecticide applied. All insecticides used in this study were neurotoxin and considered as a fast-acting insecticide [10]. Profenofos has a mode of action as acetilkolinesterase inhibitor while endosulfan act as GABA-Gated sodium channel blocker [11]. The result of this study showed a genetic diversity of the larvae as there is part of the larvae population that has a natural resistance to specific insecticide, but this hypothesis needs to be tested by further study. In general, compared with the data of the synthetic insecticide group, the mortality rate of *H. illucens* larvae was lower (26.7%) (Table 3).

### Table 2 Average mortality of *H. illucens* larvae treated with synthetic insecticides

| Insecticide | Concentration (ppm) | Observation period (hours) | 1 | 4 | 8 | 12 | 24 | 48 | 72 |
|-------------|---------------------|---------------------------|---|---|---|----|----|----|----|
| Endosulfan  | 34.5                |                           | 0 ± 0 | 10 ± 0 | 20 ± 0 | 30 ± 0 | 33 ± 4.7 | 33 ± 4.7 | 33 ± 4.7 |
|             | 172.5               |                           | 20 ± 8.2 | 33.3 ± 18.9 | 40 ± 14.1 | 40 ± 14.1 | 53.3 ± 4.7 | 53.3 ± 4.7 | 53.3 ± 4.7 |
|             | 345                 |                           | 23.3 ± 9.4 | 33.3 ± 17.0 | 40 ± 1.41 | 46.6 ± 18.9 | 53.3 ± 24.9 | 53.3 ± 24.9 | 53.3 ± 24.9 |
| Profenofos  | 75                  |                           | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.33 ± 0.47 |
|             | 375                 |                           | 23.3 ± 4.7 | 26.7 ± 4.7 | 26.7 ± 4.7 | 36.7 ± 4.7 | 40 ± 8.2 | 43.3 ± 9.2 | 60 ± 8.2 |
|             | 750                 |                           | 3.3 ± 0.47 | 16.7 ± 4.7 | 23.3 ± 4.7 | 30 ± 8.2 | 83.3 ± 12.4 | 96.7 ± 4.7 | 96.7 ± 4.7 |

δ-endotoxin attacks the digestive tract of insects destroyed mid-gut epithelial [12]. This toxic is a protein produces by *Bacillus thuringiensis* and specific to particular species that match the chemical structure of the protein [13,14]. In this study, δ-endotoxin applied is designed to cause great mortality in Tobacco cutworm (*Spodoptera litura*: Lepidoptera) and Diamondback moth (*Plutella xylostella*: Lepidoptera). However, this protein able to caused mortality of a part of the population which may indicate the similarity of the midgut structure to Lepidoptera larvae. On the other hand, there is possibility that low mortality at organic insecticide application caused by the natural resistance of black soldier fly larvae to δ-endotoxin as the larvae consumed a decaying organic material full of microbes. However, further study needs to test this hypothesis.

### 3.2 Lethal Concentration (LC) and Lethal Time (LT)

Based on probit analysis, LC$_{50}$ and LC$_{90}$ of endosulfan were lower than profenofos. This result showed that to kill 90% of tested insects, only required a contration of 997.98 ppm of endosulfan, while profenofos required 1,139.0 ppm. Edosulfan is a chlorinated insecticide that is chemically similar to DDT, which was used nearly 40 years ago. Endosulfan has a long persistence in the soil and it often pollutes the environment, so tat in several countries it has been has been banned. On the other hand, there were no LC$_{50}$ and LC$_{90}$ on organic insecticide (Azadirachtin) as the number was above the testing range (Table 4).

### Table 4 LC$_{50}$ and LC$_{90}$ of insecticide tested in this study

| Insecticide | LC$_{50}$ (ppm) | LC$_{90}$ (ppm) |
|-------------|----------------|----------------|

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Profenofos is an organophosphate that inhibits acetylcholinesterase which seem more tolerable for black soldier fly larvae than GABA blocker of endosulfan. On the other hand, δ-endotoxin is considered a slow-acting insecticide and required a longer time for microbes to grow in the insect gut. The possibility of natural resistance of black soldier fly to δ-endotoxin should be explored in the future.

Data on lethal time also showed that profenofos need a longer period to kill 90% of black soldier fly larvae population than endosulfan when applied in similar concentration. On the other hand, the time is considered unlimited for δ-endotoxin (Table 5). The slower action of the profenofos due to its mode of action as acetylcholinesterase inhibitor at muscarinic receptor [15].

Table 5 LT₉₀ of insecticide tested in this study

| Insecticide     | Concentration (ppm) | LT₉₀ (hours) |
|-----------------|---------------------|--------------|
| Endosulfan      | 172,5               | 9278,57      |
|                 | 345                 | 15089,18     |
| Profenofos      | 375                 | 31891,11     |
|                 | 750                 | 44,53        |
| δ-endotoxin     | 3800                | -            |
|                 | 7600                | -            |

LC₅₀ and LC₉₀ of all insecticides tested in this study were higher than the maximum limit of insecticide residues allowed (Table 6) and the common level of insecticide residue in agriculture products [3]. Under this condition, most agricultural waste could be considered safe to apply as feedstock for black soldier fly larvae.

Table 6 Comparison of the maximum allowed pesticide residue [16] to LC₅₀ and LC₉₀ of insecticide in this study

| Insecticide     | BMR       | LC₅₀ [ppm] | LC₉₀ [ppm] |
|-----------------|-----------|------------|------------|
| Endosulfan      | 0,001 - 5 | 172,5      | 6.108      | 40.019     |
|                 | 345       |            |            |
| Profenofos      | 2         | 375        | 294,13     | 889.59     |
|                 | 750       |            |            |
| δ-endotoxin     | -         | 3800       | -          | -          |
|                 | 7600      |            |            |

4. Conclusion

There was a lethal effect of both endosulfan and profenofos to black soldier fly larvae while no significant lethal effect for δ-endotoxin. This study found that the lethal concentration of black soldier fly larvae much higher than possible insecticide residue at fruit and vegetables. This result indicates the safety of direct application of typical agriculture waste of local agriculture as feedstock for black soldier fly larvae

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