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Screening and Evaluation of Biopesticide Compounds from *Mirabilis jalapa* L. (Caryophyllales: Nyctaginaceae) and Its Combination with *Bacillus thuringiensis* against *Spodoptera litura* F. (Lepidoptera: Noctudiae)

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ABSTRACT

Spodoptera litura is classified as a plant-disturbing organism. Efforts to control *S. litura* by using chemical insecticides have a detrimental impact on the environment and the potential to harm non-target organisms. Bioinsecticides provide a safe alternative for reducing the agricultural pest problem. The purpose of this study was to investigate the specific amino acid from *Mirabilis jalapa* extract using high performance-liquid chromatography (HP-LC) analysis and to identify their potency as a biopesticide. *Mirabilis jalapa* extract in combination with *Bacillus thuringiensis* influences the weakening of the *S. litura* immune system to explain the cause of *S. litura* death. The results showed that the *M. jalapa* extract had seven sequences of the highest amino acid compounds from *M. jalapa*, namely: Glu, Asp, Lys, Val, Leu, Arg, and Ala. Alanine compound has the potential as a biopesticide that breaks down the muscle and nervous system and blocks its receptors. The combination of *B. thuringiensis* in LC_{50} concentration also caused the death of *S. litura*. Finally, the combination of

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0.2% concentration of *M. jalapa* and *B. thuringiensis* at a sublethal concentration (1.07%) applied in 12-hour intervals within 24 hours showed the optimum mortality of *S. litura* (p<0.05): the death of larvae was characterized by damage to the midgut organs in the digestive tract observed by the histological microanatomy.

Keywords: *Bacillus thuringiensis*, biopesticides, *Mirabilis jalapa*, mortality, *Spodoptera litura*

INTRODUCTION

The ever-increasing human demand for agricultural produce has an impact on the polluted environment. One of the causes of environmental damage is the use of chemical insecticides in agriculture. Agricultural pests are an obstacle to food production worldwide, and they have become increasingly resistant to a variety of insecticides. Insecticide synthesis has been widely used to kill various insect pests in agriculture farming, plantation, and even in residential areas because the pest is harmful and even acts as a vector in the spread of diseases (Isman, 2006). The accumulative impact of excessive use of insecticides has given rise to the resistance of insect pests. Insects' resistance as a result of the increasing use of insecticidal doses has caused an urgent problem. Pest control becomes the world's attention to obtain agricultural produce in good quantity and quality. Because of this problem, it is considered necessary to find solutions for the problem of pest resistance and the use of synthetic insecticide (Romeis et al., 2008). Integrated pest management (IPM) offers a solution in controlling insect pests using physical, biological, and chemical combination techniques (Metcalf, 1989; Pedigo, 1999). The success of the IPM program is indispensable to the reduction of the insecticides' purchasing funds and the protection of the environment from further pollution. Additionally, the sustainable nature-based IPM principles support the continuity of the food network that results in a lasting balance because no species are dominant in numbers. In other words, IPM is a program that increases agricultural production and environmentally friendly plantation (Yusof & Kueh, 2013).

The use of botanical insecticides is an offer as well as an effort to control pests (Kumar & Singh, 2015). Botanical insecticides are bioinsecticides made from plants that are known to be environmentally friendly to control insects (Gokce et al., 2010; Kumar et al., 2010). *Mirabilis jalapa* extract is one of the bioinsecticides that can be used to kill pests (Maulina et al., 2018b). The previous studies indicated that *Mirabilis jalapa* L. (Caryophyllales: Nyctaginaceae) as a botanical insecticide inflicted physiological damage on the insects by reducing the number of first and second offspring and refusing to lay eggs on plants that were being banned by botanical insecticides. *Mirabilis jalapa* can produce secondary metabolites in the form of specific compounds indicated as a biopesticide and antifeedant for *Spodoptera litura* F. (Lepidoptera: Noctuidae) (Maulina et al., 2018a, 2018c). *Mirabilis jalapa*

extracts do not kill the insect directly but can influence its physiology. *Spodoptera litura* is the most dangerous pest in crops. *Spodoptera litura* is a polyphagous insect pest that can defoliate up to 80% of the crops' leaves. The resistance of *S. litura* to various chemical compounds needs to be the concern for controlling. Their distribution is very wide across Asia and the South Pacific (Schreiner, 2000; Sparks & Nauen, 2015). Therefore, there is an urgent reason for controlling *Spodoptera* pests with alternative efforts using biopesticides (Kandagal & Khetagoudar, 2013).

The previous study referring to the resistance cases reveals that the molecular mechanisms and physiology are important in preventing the occurrence of resistance in the use of bioinsecticides (Zhu et al., 2016). The mechanism of immune weakening as a physiologic response can be determined by testing the target pest precisely. Immune response indicators are seen in humoral and cellular activity. The use of sublethal biopesticide *M. jalapa* extract causes a low impact on the mortality of *S. litura* (Maulina & Anggraeni, 2014; Suryani & Anggraeni, 2014). Therefore, to improve the work of biopesticides in pest control it is necessary to combine the two types of biological agents in low dosage. Biopesticide *M. jalapa* extracts are applied in combination with the *Bacillus thuringiensis* as an entomopathogenic microbe. *Bacillus thuringiensis* is a bacterium producing delta-endotoxin compounds that damage the digestive system of the pests. The endotoxin crystalline proteins produced by *B. thuringiensis* will work on specific target pests without affecting mortality on non-target organisms. Delta-endotoxins in *B. thuringiensis* are easily biodegradable resulting in no build-up of toxins that pollute the environment (Hansberger, 2000). The preventive response to the occurrence of resistance to larvae of *S. litura* larvae is by applying the combination of the sub-lethal concentration of *M. jalapa* extract with LC_{50} of *B. thuringiensis*.

Sub-lethal concentrations of *M. jalapa* and *B. thuringiensis* were selected as the best optimum concentration to be applied as an appropriate form of pest control. This information is the basis for the development of environmentally friendly IPM that involves a combination of physical, biological, and chemical controls that have never been done before. Thus, pest control that is carried out by measuring the mortality rate of *S. litura* pests is safe for the environment. The purpose of this study was to obtain an optimal formulation in attenuating the immune system of *S. litura* pest after exposure to the combination of insecticides from *M. jalapa* extract with sublethal concentration (0.1%, 0.2%, 0.4%, 0.8% (w/v) and *B. thuringiensis*.

MATERIALS AND METHODS

Insect Larvae Culture

Sample of *Spodoptera litura* larvae was obtained from the Indonesia Sweetener and Fiber Crop Research Institute (ISFCRI/ BALITTAS), Malang, East Java, Indonesia. The fourth instar larvae of *S. litura* were used as a sample in this study. *Spodoptera*

litura larvae were reared at 23°C in the rearing jars, with each jar containing 50 *S. litura* larvae. During the rearing process, the larvae were given a feed of green mustard leave.

Extract of *Mirabilis jalapa*

Mirabilis jalapa leaves were obtained from the field in the Lampung Province. *M. jalapa* leaves were dried (without being exposed to light) and dampened using 96% ethanol. The maceration process was carried out for three days until a crude extract was obtained from the whole *M. jalapa* leaf. The extract was concentrated using an evaporation process to obtain a paste (assumed to have 100% concentration).

The acquired sub-lethal concentration was obtained through multilevel dilution. The used of sub-lethal concentrations were 0.1%, 0.2%, 0.4%, 0.8% (w/v), and the control. In this study, % (w/v) is defined as the percent of the weight of *M. jalapa* extract (in gram) in the total volume of solution (100 ml ethanol). *Mirabilis jalapa* extract having a certain concentration was sprayed throughout the surface of the green mustard feed.

Analysis of Leaf Extract using High Performance-Liquid Chromatography (HP-LC)

The sample in the HP-LC analysis was *M. jalapa* leaf extract. The analysis has obtained a sequence of specific amino acid compounds from *M. jalapa*. This process was performed with test equipment with reverse-phase liquid chromatography **(**LC) system, with a motion phase of trisodium citrate pH 3.25. The technique of splitting the LC pump gradient system (LC-20 AT) and vacuum pump flow rate was 1 ml/min. The detector was a RF 20-A Fluorescence detector, λ 450, OPA, and with wavelength detector at 450 nm. The LC was equipped with ASVP CTO 10 column volume of Shimadzu shim-pack VP ODS 5µm in column dimensions: 150 mm × 4.6 mm with column temperature 40°C. The injection volume was 100 µl of test material using a nitrogen generator at a gas temperature of 250°C. Mass spectrometry detector (SCL -10 AVP) with a positive ionization electro-spray ionization (ESI) technique with a period of 30 minutes. HP-LC analysis was conducted at the Natural Chemistry Laboratory, University of Muhammadiyah Malang.

Dose-response Bioassay of *Bacillus thuringiensis*

Bioinsecticide used in this study was delta-endotoxin of *B. thuringiensis* var. Aizawai strain GC-91: 3.8%. Endospores of *Bacillus thuringiensis* were diluted using distilled water. The fourth instar of *S. litura* larvae was used in these bioassays. The concentration of 0.2% was used as the highest concentration of 100% mortality (sense). Testing to find sublethal dose was conducted using 5 different concentrations; 0.2%, 0.15%, 0.1%, 0.05%, and 0% (w/v). The acquired sub-lethal concentration was obtained through multilevel dilution. Mortality was observed every 24 hours.

Treatments

This study employed a complete randomized design with five concentrations of *M. jalapa* extract: 0.2%, 0.15%, 0.1%, 0.05%, and 0% (w/v). The *S. litura* fourth instar larvas were placed in a petri dish, each containing five larvae. A test plot consisted of five Petri dishes. Every concentration condition hereinafter referred to as the test plot, consists of five plots. The experiments (exposure and measurement) were replicated on five different larvae for each plot condition. The treatment was implemented individually on each larva and was replicated on five different larvae for each condition.

The Combinations of the Two Biological Control Agents Analysis

Spodoptera litura fourth instar larvae were exposed to *Mirabilis jalapa* with different concentrations, and then after 3 and 12 hours (according to the plot experiment), the larvae food was replaced with food containing *B. thuringiensis*. After 24 hours, the number of larval mortalities was recorded. In this assay, bioinsecticides concentrations used were LC_{50} 0.107%.

The combinations of the two biological control agent's data analyses were performed with analysis of variance (ANOVA). Arcsine transformation was used to normalize the cumulative mortality percentage and subjected it to ANOVA using SPSS 17.0 software. Means were separated using the Tukey-Duncan significant difference test at $p<0.05$.

Histological Preparations of Digestive *Spodoptera litura*

Histological preparations were made with the paraffin method. This method began with the process of fixing the *S. litura* sample which had been given combination treatment of *M. jalapa* and *B. thuringiensis* with a different time interval. The sample was immersed in Bouin's solution for 24-hour, then washed twice with 50% alcohol, and 70% ethanol soaked for two days. The next step processing was made with 85%, 95% absolute, and xylene glow alcohols each for two hours. The sample went into the clearing process using xylene absolute then xylene saturated with paraffin cuts by heating at a temperature of 60°C.

The samples were immersed in paraffin-xylene and replaced with pure paraffin. The embedding process used liquid paraffin and cooled to solidify. The trimming stage was done by putting the sample into a paraffin mold, then the sample was sliced using a microtome into an incision band. The result of the incision tape was glued on the glass of the object, to be further dyed using 0.5% eosin dye in 95% alcohol.

RESULTS

The results of HP-LC analysis of *M. jalapa* extract showed that there were 20 types of amino acid content shown in Figure 1. There were seven highest compounds of *M. jalapa*, namely: Glu, Asp, Lys, Val, Leu, Arg, and Ala. The representation of the content of amino acid compounds is shown in Table 1. Based on the magnitude of the content

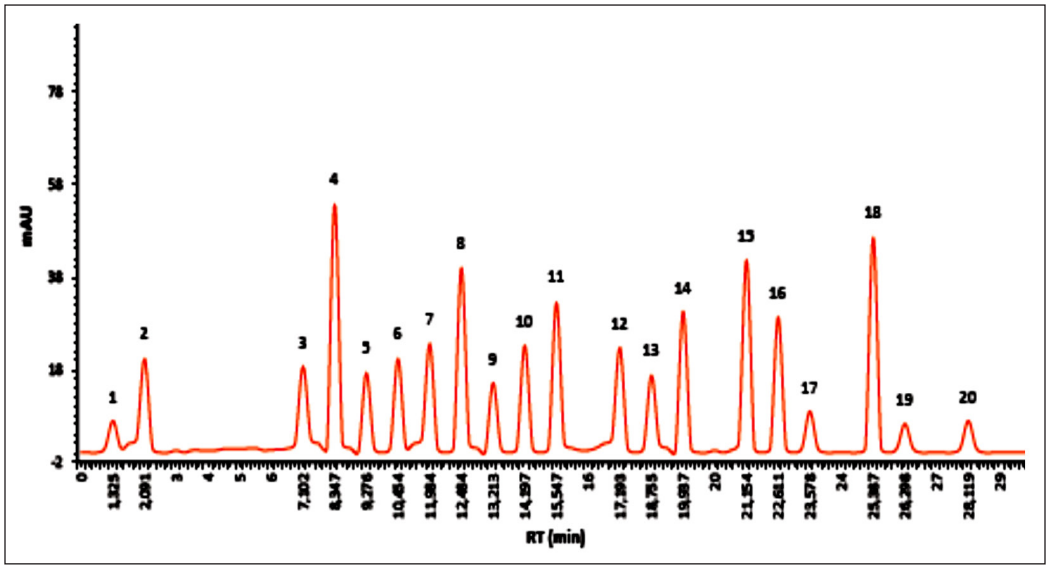

Figure 1. Chromatogram of amino acid compounds from *Mirabilis jalapa* extract

Table 1
Amino acid compounds of Mirabilis jalapa *extract*

Curve peak number	Real-time (min)	Area curve	Result (mg/g)	Result of compound analysis
1	1.33	6.90	0.38	Asn
2	2.10	20.36	1.99	Thr
3	7.10	18.73	1.80	Ser
4	8.35	53.75	6.00	Glu
5	9.27	17.32	1.59	Pro
6	10.45	20.46	1.99	Gly
7	11.98	23.62	2.37	Ala
8	12.48	40.29	4.27	Val
9	13.21	15.07	1.34	Met
10	14.20	23.18	2.28	Ile
11	15.55	32.48	3.31	Leu
12	17.19	22.82	2.24	Tyr
13	18.75	16.76	1.52	Phe
14	19.94	30.62	3.15	His
15	21.15	41.72	4.47	Lys
16	22.61	29.31	2.98	Arg
17	23.58	8.92	0.63	Trp
18	25.38	46.59	4.89	Asp
19	26.30	6.26	0.31	Gln
20	28.12	6.8	0.40	Cys

Note. The grey columns show alanine compounds that identified as biopesticides

of the amino acid compounds, these seven compounds were selected and matched based on the high larvicidal properties. In line with the results of *in silico* analysis which showed that the alanine compound had the highest potential for larvicidal properties, it could be inferred that it had the potential as a biopesticide (Maulina, 2018a).

Alanine is a phenol compound group that has an important role in controlling herbivorous pest insects because these bioactive compounds from alanine peptides act as natural toxins. The compounds produced by secondary metabolites provide good benefits of acting as natural pesticides (Daniel et al., 1999).

Spodoptera litura Mortality with a Combination of Two Biological Agents *Mirabilis jalapa* and *Bacillus thuringiensis*

Both combinations of biological agents were used at sub-lethal concentrations and LC_{50}. The results showed that there was an increase in mortality for a combination of biological agents with a different application of different agendas. Table 2 is the result of mortality test analysis with 3 hours and 12 hours interval of a biological agent after treatment of extract of *M. jalapa* then combined with delta-endotoxin from *B. thuringiensis*.

Table 2 shows that the combination of biological agents with an interval of 3 hours resulted in significantly higher mortality of *S. litura* compared with control (p<0.05). However, an increase in each sub-lethal concentration of *M. jalapa* was not accompanied by an increase in larval mortality. This suggests that an interval of 3 hours of biological agent application causes the performance of both toxins in the larval body not to run synergistically.

Mortality of *Spodoptera litura* Larvae Based on Histological Analysis

The combination of biological agents *M. jalapa* and *B. thuringiensis* causes the death of larvae identified by characteristic changes in cadaver: the larvae's body structure becomes soft, size becomes small, body condition becomes brittle

Table 2
The effect of a combination of Mirabilis jalapa *extract and* Bacillus thuringiensis on *mortality of* Spodoptera litura

Concentration (%)	Mortality	
	3 hours after application of *Mirabilis jalapa*	12 hours after application of *Mirabilis jalapa*
Control	3 + 0.55[a]	1 + 0.45[a]
0.1 Mj + LC_{50}Bt	10 + 2.71[b]	12 + 3.89[b]
0.2 Mj + LC_{50}Bt	10 + 4.23[b]	15 + 3.71[bc]
0.4 Mj + LC_{50}Bt	10 + 2.71[b]	19 + 4.84[c]
0.8 Mj + LC_{50}Bt	11 + 3.84[b]	21 + 5.84[c]

Note. The numbers followed by different alphabet in the same column show a significant difference p<0.05 (Mj: *Mirabilis jalapa*; Bt: *Bacillus thuringiensis*)

accompanied by changes in body color that turns blackish and smelly. The results showed that the cross-sections of the midgut *S. litura* were composed of a composite columnar epithelial layer. Hemocoel was the cavity of a midgut pad where the food circulation was coated by the peritrophic membrane. *Bacillus thuringiensis* plays a role in destroying the epithelial midgut of *S. litura* epithelium. Delta-endotoxins can perforate the epithelial cell membrane of the midgut epithelial cells resulting in lysis due to osmotic events (Figure 2). The results showed that there was a difference in midgut *S. litura* treated with a combination of *M. jalapa* with *B. thuringiensis* at a 3-hour interval after being infected with *M. jalapa* (Figure 2). The concentration of 0.1% has been able to damage epithelial plot to make a hole in the midgut of *S. litura*. The application of 0.2% concentration of *M. jalapa* extract causes epithelial cells in the midgut lysis and detaches from the basement membrane. Midgut damage is getting worse, this condition leads to the death of the organism. Concentrations of 0.4% and 0.8% result in acute tissue damage. Midgut organs become destroyed and organs functionally cannot work anymore.

DISCUSSIONS

The application of *M. jalapa* biopesticide did not not kill *S. litura* directly. However, it was able to induce an immune system reaction by decreasing physiological function. Sub-lethal concentration was aimed at preventing ongoing resistance to target pests (Leng et al., 2011). Resistance prevention

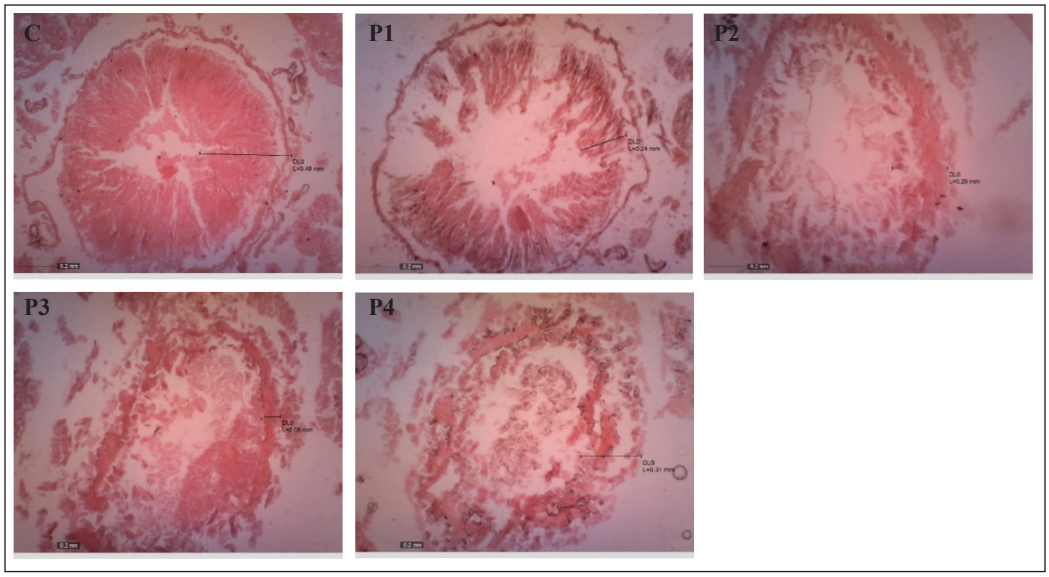

Figure 2. The microanatomy of *Spodoptera litura* midgut treated by the combination of *Mirabilis jalapa* with *Bacillus thuringiensis* at 12 hours interval time

Note. Each microanatomy image has a same size with DL_0; L = 0.48mm and DL_0; L = 0.26mm

C : Control; P1: 0.1% concentration of *M. jalapa* and *B. thuringiensis*; P2: 0.2% concentration of *M. jalapa* and *B. thuringiensis*; P3: 0.4% concentration of *M. jalapa* and *B. thuringiensis*; P4: 0.8% concentration of *M. jalapa* and *B. thuringiensis*

is necessary for easy control of these pests through the application of botanical insecticides (Zibaee, 2011). In the event of resistance, a resurgence is confirmed by the multiplication of insecticidal dosage (Dutcher, 2007; Sparks & Nauen, 2015). Therefore, the use of biopesticide *M. jalapa* prevents these two things from happening following the principle of integrated pest management (IPM).

Alanine compound was detected in *M. jalapa* extract as a biopesticide that could act as an inhibitor of glutamate in the insect's body by changing the conformation of the structure of glutamate in the neurotransmitter signaling (Maulina, 2018a). Glutamate blocking on the original receptors in the insect's body results in a disturbance in response to the stimulating pathways of muscles and smell in insects (Kolodziejczyk et al., 2008; Missbach et al., 2014). Disruption of signaling from the pathway mechanism in neuromuscular has resulted in disruption of the mechanism of muscle movement throughout the insect's body. It does not make body movements dysfunctional but there is a disruption in the muscle mechanism in the respiratory, digestive, and circulatory systems. Coordination of nerves and muscle disruption results in a decrease in motion activity that leads to insect paralysis.

The antenna is an important organ for insects. It is the center of the olfactory. The olfactory nerve cell disruption disrupts the recognition response to the environment. Besides, it fails potential membrane transduction which results in failed action potentials (Stengl et al., 1999). The effect of this series is the failure to receive and continue stimuli originating in the olfactory regulation center, which leads to metabolic disorders.

The *M. jalapa* extract causes disorders of the olfactory and muscle nerve response (neuromuscular). The weakening of various responses to the entry of foreign substances is an indication of the weakening of the insect defense system. The biopesticide toxin of *M. jalapa* causes the defense mechanism in the insect body to become weak. Exposure of *M. jalapa* to *S. litura* causes an increasingly weakened state of immunity. The application of natural compounds from *M. jalapa* as biopesticide compounds to control the amount of *S. litura* could be used safely in nature. Efforts to control *S. litura* pests by measuring mortality rates were performed by combining *M. jalapa* extract and *B. thuringiensis* endotoxin. The two types of biological agents were combined to enhance the action of natural compounds from *M. jalapa* since the subaudible concentrations of *M. jalapa* and *B. thuringiensis* are used to prevent the possibility of resistance to the larvae. The results showed that there was an increase in mortality for the combination of biological agents with a different application of different agendas. Table 2 is the result of mortality analysis with 3 hours and 12 hours' time interval of a biological agent after treatment of extract of *M. jalapa* then combined with delta-endotoxin from *B. thuringiensis*.

The combination of biological agents with an interval of 3 hours resulted in significantly increased mortality of *S. litura* compared with control ($p<0.05$). However,

an increase in each sub-lethal concentration of *M. jalapa* was not accompanied by an increase in larval mortality. This suggests that an interval of 3 hours of biological agent application causes the performance of both toxins in the larval body not to run synergistically. Hillyer stated that the response of *Aedes aegypti* phagocytosis ran 5 minutes after being induced by bacteria (Hillyer et al., 2003). This suggests that an interval of 3 hours of biological agent application causes the performance of both toxins in the larval body not to run synergistically. When the larvae are re-infected with *B. thuringiensis* the larvae can eliminate the endotoxin that enters the body so that the mortality of the larvae becomes low. The average mortality at the time of application of larvae test of *S. litura* with a combination of the concentration of *M. jalapa* equal to 0.8% and delta-endotoxin with an application of time interval of 3 hours was increased by 20%.

The data showed that there was a difference when giving a combination of biological agents with an interval of 12 hours. It is shown in Table 2 that the mortality of *S. litura* differed significantly from controls in the exposure of the four sub-lethal concentrations of *M. jalapa* (p<0.05). The highest mortality occurred in the combination of 0.8% concentration of *M. jalapa* extract with *B. thuringiensis* sub-lethal concentration. The 12-hour physiological impairment performed by toxic compounds in *M. jalapa* can significantly weaken the *S. litura* larvae and the ability of the immune system decreases significantly. The additional combination with delta-endotoxin *B. thuringiensis* results in *S. litura* larvae being unable to activate the immune defense mechanism and death. The *M. jalapa* extract infections given to *S. litura* larvae will affect the immunity characterized by enzyme PO and decrease in average. Giving a concentration of sub-lethal infections of *M. jalapa* is not lethal to *S. litura* pests. It only effectively weakens the larvae's physiology. Therefore, to improve its pest control efficiency biological agents *M. jalapa* and *B. thuringiensis* need to be used (Maulina et al., 2018b).

A toxic substance produced by *M. Jalapa* weakens the insect immunity, while delta-endotoxins from *B. thuringiensis* invades *S. litura* by damaging intestinal epithelial cells along the gastrointestinal tract. When the insect's body's defenses weaken due to *M. jalapa*, the combination of delta-endotoxin *B. thuringiensis* entering the midgut will easily damage the entire tissue and organs. In an unstable condition due to *M. jalapa*'s invasion into the body, the insects will become very weak to repair their damaged ones. Damage to *S. litura* bodies occurs very easily when the immune defenses of *S. litura* larvae have been weakened, tissue and organ damage will occur thoroughly in insect bodies resulting in insect mortality.

The provision of toxins with a 12-hour time interval in the application of *M. jalapa* exposure with a concentration of 0.8% has an impact on increasing the mortality of *S. litura*. *Bacillus thuringiensis* application within 12 hours resulted in a 42% increase in

total mortality compared to controls. Thus, the time interval of combined exposure of biological agents in pest control applications such as combining the exposure of toxic substances greatly affects the increase in total lactational mortality of *S. litura*.

Bacillus thuringiensis has become a biocontrol of insects in many agricultural countries (Weinzierl et al., 1997). The results of the study using the combination of *M. jalapa* and *B. thuringiensis* were focused on the mortality of *S. litura*. The cause of death was found to be the damage to the gastrointestinal tract. Therefore, the results of this study in histopathology form in the damage of the tissue are caused by a combination of biological insecticides used. The combination was performed with a time interval, i.e. 24 hours given *M. jalapa* afterward 3 hours and 6 hours given *B. thuringiensis*. Both biological insecticides are administered simultaneously with oral feed with a synergistic mechanism of action (Agrebi et al., 2010).

The digestive tract of *S. litura* consists of foregut, midgut, and hindgut. Midgut plays a role in the process of food absorption which is a major part of the digestive tract and is an important organ for an insect. The midgut is a hemocoel consisting of a layer of epithelium. Functionally this section holds control of the nutrient traffic (Chapman, 2009). This organ plays an important role in other physiological regulations such as metabolism, immune response, electrolyte homeostasis, osmotic pressure, circulation, and more. Therefore, impaired function and tissue damage in midgut can result in the mortality of insects.

The results showed that the cross-section of the midgut *S. litura* was composed of a compact columnar epithelial layer (Figure 2). This research proved that *M. jalapa* played a role in weakening the immune system and *B. thuringiensis* played a role in damaging the digestive tissue in the body of *S. litura* (Castro et al., 2019). The level of tissue damage that occurred in the midgut depended on the level of concentration of *M. jalapa* and *B. thuringiensis* extracts. The higher treatment concentration of *M. jalapa* and *B. thuringiensis* caused the worse and more acute the tissue damage in the midgut of *S. litura* is. Hemocoel is the cavity of a midgut pad where food is circulated by the peritrophic membrane (Pigott et al., 2007). *Bacillus thuringiensis* plays a role in destroying the epithelial midgut of *S. litura* epithelium. Delta endotoxin can perforate the epithelial membrane of the midgut epithelium that causes the cell to become less because of the osmosis event (Pandey et al., 2009). The concentration of 0.1% had been able to damage epithelial plot to make a hole in the midgut of *S. litura*. The application of 0.2% concentration of *M. jalapa* extract caused epithelial cells to lyse and became detached from one another. Midgut damage got worse, this condition leading to death of the organism. Concentrations of 0.4% and 0.8% resulted in acute tissue damage. The midgut organs become destroyed and organs can not functionally work anymore. The midgut of *S. litura* treated with a combination of *M. jalapa* and *B. thuringiensis* at 6-hour time combination interval suffered severe organ

damage. Each concentration resulted in severe tissue damage. The damage occurred throughout the midgut to the epithelial tissue and even the midgut organs were destroyed.

In the early stages of bacterial infection, insects exhibit a decrease in feeding activity and tend to seek shelter in a hidden place (under the leaves). Furthermore, the larvae experience diarrhea, secrete a fluid from their mouth, paralyzed on the food channel; resulting in decreased movement activity, and ending with death. Bacteria infect through the mouth and gastrointestinal tract, very little through eggs, integuments, and trachea. Bacteria enter as parasitoids and predators. By infecting through the gastrointestinal tract, the bacteria will produce enzymes (lecithinase, proteinase, and chitinase) that will attack the intestines before entering the hemocoel. The toxins will then damage the walls of the intestinal tract. When the intestinal tissue is damaged, the bacteria enter the insect hemocoel (Vega & Kaya, 2012). Here the poison of the bacteria will decompose (hydrolysis). These toxic substances will be released from their endotoxins, which will poison the epithelial cells of the food duct until they are destroyed.

CONCLUSION

Mirabilis jalapa extract had seven sequences of the highest amino acid compounds from *M. jalapa* namely: Glu, Asp, Lys, Val, Leu, Arg, and Ala. Alanine compound has the highest potential for larvicidal properties that have the potential as a biopesticide. The combination of 0.2% concentration of *M. jalapa* and *Bacillus thuringiensis* at the sublethal concentration in 12-hour intervals within 24 hours showed the optimum mortality of *Spodoptera litura* as much as $15+3.7^{bc}$ ($p<0.05$). The death of larvae cadaver was characterized by damage to the midgut organs in the digestive tract observed by the histological microanatomy. Therefore, the combination of *M. jalapa* and *B.thuringiensis* with low concentrations could be considered as integrated pest management.

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