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Lectin Protein Activity of *Spodoptera litura* After Exposured by Potential Biopesticide from *Mirabilis jalapa*

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Abstract

This research aims to analyze the existence of lectin protein as an indicator of immunity reaction's activation *Spodoptera litura* after exposing biopesticide *M. jalapa*. Lectin test was conducted by using spot-test hemagglutination assay (HA) then was seen its speed forming of titer. The result of research shows that lectin was on the part of hemolymph *S. litura* supernatant. The result test shows that on the concentration 0.2% binding of lectin, carbohydrate and erythrocyte cells of vertebrate formed faster. The speed of titer forming was influenced by the number of hemocyte. This is caused by immulectin receptors were on the cell's surface. Therefore, exposing *M. jalapa* can induce lectin activation which functioned as the recognizing receptor of strange object which directly bound with carbohydrate related to the reaction of body immunity.

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Keyword
Biopesticide, *Mirabilis jalapa,* lectin, Immunity system, *Spodoptera litura.*

Introduction

The best quality and quantity of agricultural yield to fulfill the need of human being is in priority scale until this recent day. However, the pests become limitation factor of the condition. The pests become resistance toward various pesticides, which affect the population boom. It is seen on the increasing resistance of agricultural pests toward various insecticides (Bai *et al.*, 2011; Leng, 2011; Romeis, J, 2008; Shapiro, 2000). This condition is expected as the impact of using chemical (synthetic) insecticide excessively which is very dangerous and is not recommended by environment observer because can endanger the non-target organisms (Kumar, 2012). To overcome that problem, botanical insecticide becomes one of alternatives in conserving the non-target organism in order to keep the natural balance.

The use of natural biopesticide of plant's extraction gives the secure effect (Kandagal, 2011; Nathan, 2004; Leng, 2011). Its application of use is not leave behind the chemical residue which endangers the non-target organism, human being and environment (Horne & Page, 2008; Tanada & Kaya, 1993). Botanical insecticide which aims as biopesticide for the

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insect's pests, one of them is the use of plant extraction *Mirabiis jalapa* (Maulina, 2018[a]; Maulina, 2018[b]; Maulina, 2018[c]). This plant contains *antiviral and antiviroid activity compounds* in form of *Ribosome Inactivating Protein* (RIP) which is known as *Mirabilis Antiviral Protein* (MAP) (Vivanco, 1999). Testing of effectiveness and determining concentration of that compound are needed to prevent the resistance of target pests. The result of laboratory test which had been done by *M. jalapa* as biopesticide has LD_{50} on concentration 0.8% toward Spodoptera (Maulina, 2018[a]). The use of sub lethal concentration of *M. jalapa* is conducted in order the pest target is not become resistance with it. The main aim on application of biopesticide *M. jalapa* is to weaken its body immunity.

Spodoptera litura is one of dangerous pests of agricultural plants. Its folifagus characteristic can destruct the plants of yield agriculture thus makes 100% defoliation (Suharsono, 2011). Resistance of *S. litura* toward various chemical compound needs to be anticipated because the spreading covers South Pacific and Asia region (Sparck, T.C, 2014; Mei, Z.X, 2012; Scheiner, 2000). This becomes urgent reason to restrain spodoptera pest by using alternative effort of botanical biopesticide. Focusing on its restraint, immune system becomes the main thing that needs to be known of its whole mechanisms which occurs in its body. Therefore, biopesticide *M. jalapa* gives chance toward weakening immune system of *S. litura.*

Immune system becomes the indicator toward the death of pest. This case occurs because defense mechanism becomes the barrier of its body toward the strange object and its response when its body is getting the attack of strange object. The main task of *M. jalapa* as biopesticide is to break the defense, thus spodoptera is weakened. Generally, insect has cellular and humoral defense. Mechanism of humoral and cellular response cannot be separated one another. Both of them stimulate each other to do their role (Gillot, 2005; Chapman, 2009). Humoral response becomes the most crucial part because has the role to activate the enzyme and stimulation of strange object's recognition. In its humoral mechanism, it will stimulate immune system of running cellular. Therefore, humoral response becomes one of parameters in immunity sequence which is one of the factors that cause of its death.

This research aims to analyze how does the influence of giving leaves extract of *M. jalapa* toward the defense of humoral immune *S. litura*. The response of humoral immune which was observed is the expression of lectin protein. Lectin is protein, not enzyme or glikoprotein which binds or reacts with carbohydrate of various strange objects (Yu, QX, 2000; Yu QX, 2005; Marmaras, 2009). Lectin has very important role in defense mechanism of insect's body. Through the observation with sub-lethal concentration of *M. jalapa* will be seen how does the response of its humoral run. The final result of this research will determine the potential of *M. jalapa* as biopesticide toward *S. litura* pest is observed by defense mechanism of its body.

Materials and Methods

Sample of Research

This research used larva *S. litura* the fourth instar as sample of research. Larva *S. litura* was obtained by Balai Penelitian Tanaman Pemanis dan Serat (BALITTAS) Malang, East Java. The process of multiplication and treatment of larva was conducted at laboratory of plant pest with the condition of temperature 25-26˚C and humidity 50-55%. During the process of larva multiplication was given woof diet of darker colored mustard greens and was

treated inside of plastic topless (volume 5 liter, diameter 12 cm and height 11 cm). Each topless contains 50 larva of *S. litura* and the topless of treatment was cleaned every 12 hours. Furthermore, hemolimf of research sample was used as the subject of research for the content of lectin protein.

Extract of *M. jalapa* Biopesticide

The crude extract of *M. jalapa* was obtained through maceration process which conducted at UPT Materia Medica Batu-Government Health Service of East Java Province. The leaves of *M. jalapa* were obtained from field searching at Lampung province. The leaves of *M. jalapa* was dried without sunlight. Meseration was conducted by using ethanol 96% during 3 days. The result of this maseration collection was done further on evaporation process until it formed the paste. The paste was in concentration 100%.

This research was used sub-lethal concentration 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 weight of *M. jalapa* extract (in gram) in the total volume of solution (100 ml ethanol). *M. jalapa* extract having certain concetration was sprayed throughout the surface of green mustard feed. After 24 h of exposure, hemolymph of the larvae were taken for the immune system measurements. The treatment was implemented individually on each larva, and was replicated on five different larvae for each condition.

Lectin Analysis

Hemolimf *S. litura* was collected in tube eppendorftube which had been filled by crystal phenylthiourea (PTU). The solution was centrifuged during 5 minutes with temperature 4^{o}C; 800g then pellet and supernatant were separated on different epperndorftube. Supernatant was used for Hemagglutinin-Assay (HA) test, pellet was washed by using TBS pH 7.4. Then, 50 ul TBS and pellet were suspended with speed 12.000 g during 15 minutes, the result of centrifugal deposits was used for HA. Hemolimf 2 ml was homogenized in the pressure 400kp/cm^2 during 5 minutes, was continued by centrifuging in the speed 12.000 g during 15 minutes, this supernatant was used as lectin source.

Hemagglutination assay test was conducted by preparing blood of vertebrate animals with its anticoagulant. Wash 3 times TBS pH 7 concentration 2%, prepare test container (titertek) “v”, put in test sample as much 24 µm (centrifugal deposits) in TBS pH 7,4, drip 25µm erythrocyte suspense and was incubated with room temperature during 60 minutes (Suryani, 2014).

Results and Discussion

Immune system of insect is the result sequence of defense body toward specific and non-specific response toward detected strange object of its body. The defense mechanism of insect covers cellular and humoral defense. Humoral response holds the main role in the sequence of immune system through various enzymatic and non-enzimatic reaction which has the role in recognizing the body toward strange object and its resistance effort. The mechanism depends on recognition effort of strange object through its receptor. There are 8 receptors which involved in mechanism of humoral immune, namely: immulectins, Thioester-continuing proteins (TEPs), LPS-binding protein, Peptidoglican recognitions proteins (PGRPs), Gram-negative bacteria binding protein (GNBP), β 1,3-glukan recognition protein (βGRP), Haemolin (immunoglobulin superfamily) and B. mori multibinding protein (BmMBP). Induction of strange object which is received by these receptors will give the impact to the

action, such as: (1) induction and secretion of anti microbial peptides (AMPs); (2) mechanism of melanization (Marmaras, 2009).

Receptor becomes the important part in defense mechanism. Lectin is protein which acts as recognizing receptor of strange object which binds directly with carbohydrate (Yu QX, 2005). Its existence becomes the main key to activate phenoloxidation on hemolimf plasma (Yu, QX, 2000; Yu QX, 2005; Marmaras, 2009). Immulectin lies in granular and eonocytoid cell also has function in increasing encapsulation (Yu QX, 2004). Its ability in recognizing strange object as its non-self in form of glycoprotein and glycolipid makes it as the main receptor in mechanism of immune system. Lectin that able to induce the sequence of cellular and humoral in defense system becomes the indicator for recognizing strange object and for further signal transduction.

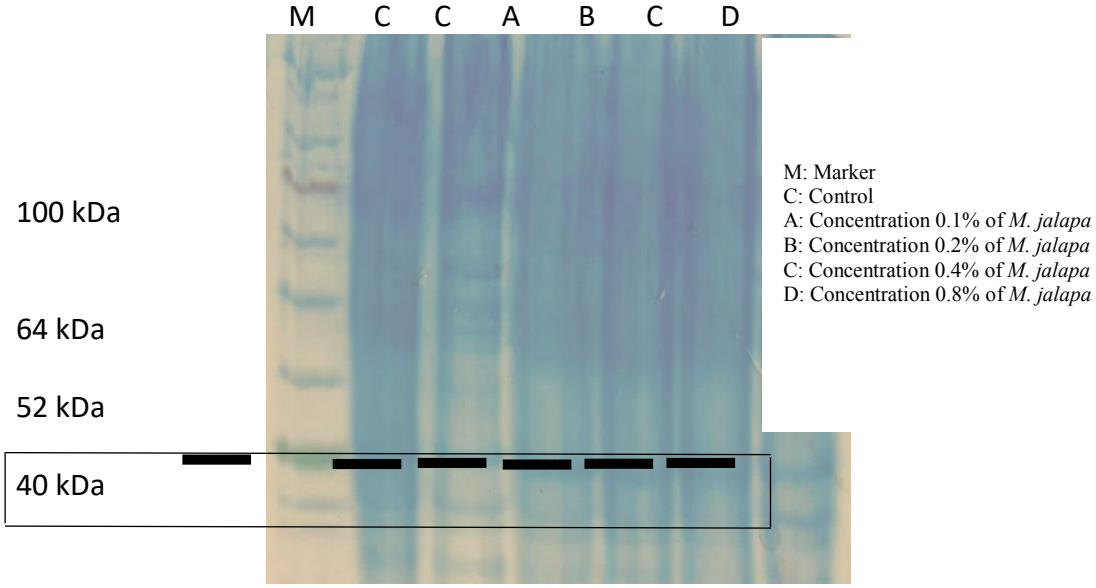

Figure 1. The profile of *Spodoptera litura* lectin protein

Result of research showed that lectin protein existed in *S. litura* when was exposing biopesticide *M. jalapa*. This is shown by the profile of lectin protein which has molecule weight in 40kDa. Figure 1 is the result of electrophoresis test of hemolimf *S. litura* in part of supernatant. The speed of lectin response was measured through the form of hemaglutination assay (HA) measurement ability (titer) which showed different result on each given concentration. Test of HA which was conducted observes the lectin response toward its binding with membrane cell of blood of vertebrate animals (carbohydrate). There are 4 concentrations of sub-lethal *M. jalapa* which were exposed in larva S.litura, namely: 0.8%, 0.4%, 0.2% and 0.1%. Test result shows that on concentration 0.2% lectin binding with carbohydrate and erythrocyte cell of vertebrate were formed faster than the control (figure 2).

Forming of HA measurement ability (titer) on the control occurred in the eightieth minutes while concentration 0.1% and 0,2% occurred faster in the sixtieth minutes (figure 1). The speed of measurement ability (titer) forming was influenced by many hemocyte, because the receptor of immulectin was on the surface of its cell, thus the more number of hemocyte, then the number of lectin receptor will more increase and the activity of lectin binding with strange object will be faster to be recognized. Concentration 0.2% is the optimum concentration of immune system's mechanism which was shown by the increasing number of hemocyte ($P<0.05$) (Maulina, 2014; Suryani, 2014).

Giving of *M. jalapa* on the concentration 0.4% and 0.8% gave longer time response than in the control, they were in the ninetieth minutes and in the hundredth five minutes. This was expected because of more decreasing number of hemocyte. On the higher concentration, hemocyte cells cannot proliferate anymore. Toxicity of high concentration causes enzymatic and coordination system disturbance thus cells which induced the cell mitosis are obstructed (Maulina, 2013).

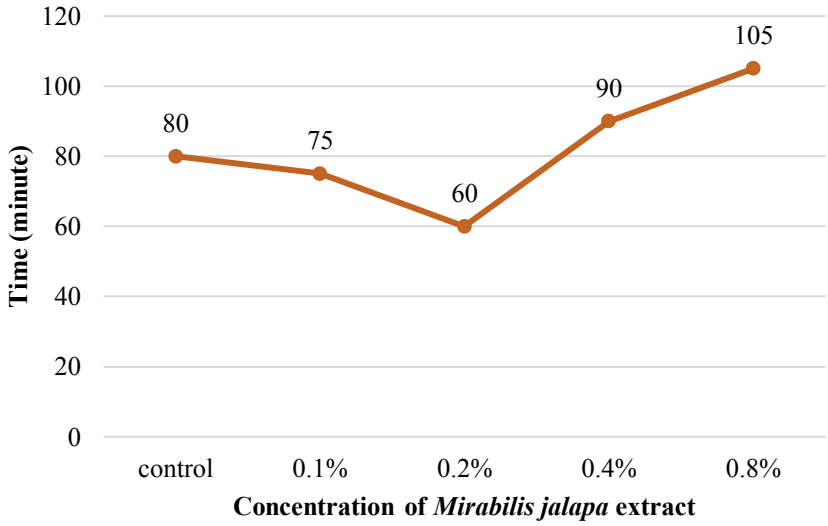

Figure 2. Speed of Forming Hemaglutination

In principle, the application of biopesticide *M. jalapa* is not kill the target pest *S. litura* totally but its use can induce the reaction of immune system by lowering the physiological function at the whole (Maulina, 2014). This condition is intended on the preventive efforts of sustainable resistance of target pest. The prevention of resistance is needed in order to be done easier for the control of this pest. If resistance occurs, resurgence will occur certainly because there is a multiplying use of insecticide doses (Spark, 2015; Dutcher, 2007). Therefore, the use of biopesticide *M. jalapa* prevents both of cases occur based on the principle recommendation of Integrated Pest Management (IPM) (Kumar, 2015; Kather, 2012; Kumar, 2012; Kandagal, 2011; Leng, 2011; Nathan, 2004) . The result of this research can be used as basic reference in using biopesticide *M. jalapa* to apply widely in agricultural land. Setting the amount of spodoptera pest needs the consideration in the proper concentration in applying on the actual agricultural land. The aim of weakening immune system which was conducted in this research ends on the mortality of target pest thus can be used to control the population of pest.

Conclusions

Mirabilis jalapa has the potential as biopesticide, the indicator was seen through mechanism of humoral immune system of larva *Spodoptera litura* body. The result of analysis is seen that the given leaves' extract of *M. jalapa* can induce the activity of lectin protein. Giving the concentration 0.2% of *M. jalapa* extract able to induce the speed of lectin forming reaction, this condition stimulates the occurrence of weakening sequence of immunity process of *S. litura.*

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