THE PROFILE OF MIDDLE DIGESTIVE TRACT (MIDGUT) TISSUE DAMAGE ON *Spodoptera litura* FABRICIUS LARVAE DUE TO THE INFECTION OF *Helicoverpa armigera* NUCLEAR POLYHEDROSIS VIRUS (HaNPV)

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ABSTRACT

The research to determine what can larva *Spodoptera litura* be used as an alternate host for *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV) production has been conducted. The research has been done with laboratory experiments approach, using complete randomized design (10 treatments / infection time) and three repetitions, with concentration of PIB was 1.13 x 10^5 PIB/ml. The result was observed through creating histologic slide of midgut tissue damage of larva *S. litura* of fourth instar through Mallory-Azan staining method. The weight of larva was measured in observation time, and the present of Polyhedral Inclusion Body (PIB) in infected tissue was also monitored. Observation was conducted every 8 hours from the 1st until the 72nd hour after infection. Results showed that time treatments after the larva was infected by virus has significant impact ($\alpha < 0.05$) on the middle section digestive tract (midgut) tissue damage and the presence of PIB. Damage has begun since the 8th hour after infection. It was characterized by the enlargement of columnar nucleus with PIB within columnar cell and the loss of peritrofic membrane. Observation at the 24th hour showed that almost all columnar cells had lisis, but at the 32nd hour the columnar cells have been regenerated. After the 64th up to 72nd hour after infection, all columnar cells lisis and PIB form into granule (polyhedral granules). Therefore larva *S. litura* can be used as an alternate host for *Ha*NPV production.

Keywords : Helicoverpa armigera Nuclear Polyhedrosis Virus (HaNPV), Spodoptera litura, Histologic Structure, Mallory-Azan, Polyhedral Inclusion Body (PIB)

SUMMARY

The principle of plant disease control, nowadays much applied, in the whole world, is the common system of plant disease control involving economic loss prevention and life environment damage simultaneously. One of the methods that can be applied is by using life agent that can originate from parasitoid organism or pathogenic larva itself.

Most of the ordo of Lepidoptera, Diptera, and Coleoptera are plant disease insects on agriculture plants. Due of the species which is polifagus (whole eater) is *Helicoverpa armigera*. This species likes fiber plants such us beans, onions, cabbage, cotton, Tomato and tobacco. About 50% of agriculture production cost is spent only for insect plant disease control in question.

Nuclear Polyhedrosis Virus (NPV) is a virus that can infect insect larva of the ordo of Lepidoptera, Diptera and Coleoptera, so that this virus has a great potention to be used as life agent. Specifically, NPV attacking larva of insect *H. armigera* is *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV).

The production of insect virus to be used commercially as life agent can be done via the method *in vivo*, namely using natural host as virus multiplication media. Up to the the present, the method in question is still the most economical way of production but the *Ha*NPV production through this method is difficult to carry out. That is because *H. armigera* larva as host can not be cultivated in group. This larva is cannibalistic and small sized, so that virus production is less maximal. Individual larva cultivation can increase production cost and manpower.

Larva *Spodoptera litura* proves to be a plant disease for a number of agriculture plants. This larva is big, is not cannibalistic and vulnerable of *Ha*NPV infection. This is marked by the formation of Polyhedra Inclusion Body (PIB) in the body of the larva in question. It is known that the highest production of PIB happens on larva instar four. Thus, this larva can be used as substitute host, but until now, it is not known yet, how big the capability of PIB *Ha*NPV formation in the larva *S. litura* is.

The midgut is the first place where virus infection takes place in the body of insect larva. This can happen because this place has PH base atmosphere or alkali (9.5 –

11.5), so that it can cause polihedrin, virion cover to dissolve and to free virion. Through larva digestive cell movement, free virion will penetrate peritrofic membrane and moves towards brush border of midgut epithelium tissue. In this way, change will take place, even damage on peritrofic membrane and brush border of midgut epithelium tissue.

Up to now, the research discussing the profile of peritrofic membrane damage and epithelium tissue of midgut *S. litura* as initial indicator of the presence of *Ha*NPV infection has not been much reported. Based on this, the research aims at observing the profile of midgut epithelium tissue damage of larva and the presence of PIB so that we know the potention of substitute host *S. litura* as *Ha*NPV production medium.

MATERIALS AND METHOD

The materials used are specimen bottle, sweet corn, 10% honey solution and other chemical substances for the production of *Ha*NPV preparation and histologic equipment used in paraphin method with Mallory Azan coloring.

The test animals used are larva *H. armigera* and *S. litura* obtained from Lembang Vegetable Plant Research, as long as both kinds of larva are cultivated in the laboratory in zalp pot of 75 g wide woof of sweet corn. Adult insects are given woof of 10% honey solution. Larva *H. armigera* is cultivated as material of *Ha*NPV virus preparation. The preparation of virus is prepaired using the method of Indrayani *et al.* (1993) which has been modified.

Larva *S. litura* instar 4 is used as test animal. The larva *S. litura* instar 4 infected orally with *Ha*NPV polihedra of 1.13×10^5 PIB/mL concentration. Observation is done 10 times, starting one hour afer infection. Larva *S. litura* treatment control and experiment is weight and later put into a solution of FAA fixation for the necessary the histologic equipment of midgut track. The next observation is done at the 8^{th}, 16^{th}, 24^{th}, 32^{nd}, 40^{th}, 48^{th}, 56^{th}, 64^{th}, and 72^{nd} hour after infection with the same procedure.

Weight measuring during observation is important because it shows the capacity of cells as PIB *Ha*NPV production medium. The histologic cut of Midgut larva *S. litura*

is done longitudinally. Equipments are observed microscopic ligt to see the damage of midgut epithelium tissue compared to the control according to the criteria as follows.

Table 1. Criteria of Histologic Damage of Midgut Larva S. litura Based on Scores

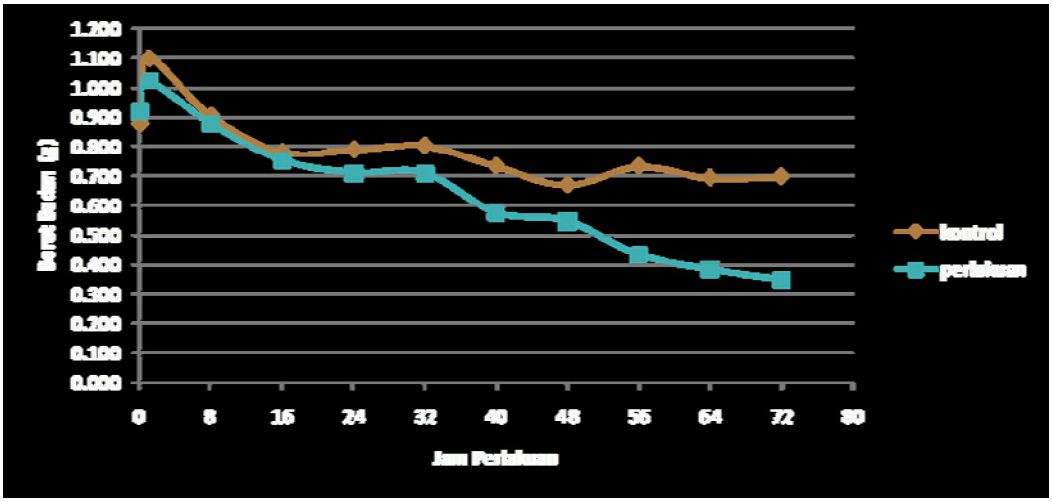
Scoring Level	Criteria of Damage
0	Normal
1	Peritrofic membrane damage
2	PIB is available in or outside midgut epithelium cell nucleus and lumen
3	Brush border cells, partly lisis and regenerative cells are still to be found
4	Brush border cells, all of them lisis and no regenerative cells are formed

Data of larva weight change is later analyzed by using *independent t-test* while the level of histologic damage of middle larva is analyzed using *Kruskal Wallis Tes*t and *Wilcoxson Test*.

RESULT AND DISCUSSION

The Measuring of Larva *S. litura* Weight During Observation

The result of measuring the overage weight of larva *S. litura* during observation which is repeated 3 times can be seen on Picture 1. below.



Picture 1. The Change of Larva S. litura's Weight

Based on picture 1, it can be seen that on the whole the weight of larva *S. litura* which is infected each hour of observation compared to control shows a smaller weight. The 48th hour of observation, the weight of larva either under control or infected shows a decrease. This is because the larva begins to enter instar 5 (prepupa). The larva at this stadium has entered the period of rest. According to Rajak and Rochimat (2007), a decrease of appetite takes place on larva instar five as preparation for the formation of pupa. Ben-Shaked and Harapaz (1996 in Miranti, 2008) also state that larva instar five undergoes the mechanism of resistance ripening due to change of hormonal level needed for pupa formation.

It is different from the larva of control group which still shows higher eating activities compared to the group of infected larva. This is because the appetite of infected larva group has been influenced by virus entering peritrofic membrane and damages cell on midgut epithelium tissue. As we know, in the tissue membrane, there are also gland cells such as goblet cells containing digestive enzymes which help digestive process (Leeson & Paparo, 1990). The damage of these gland cells can cause intake of food unable to be digested completely, so that food piling takes place. This condition makes the larva feed satisfied so that its appetite also decrease.

The test result with *independent t-test* shows that the infection of *Ha*NPV virus has no obvious influence on the weight of larva *S. litura*. This can be explained because

of the distance of observation hour is too close so that the significance between treatment is not visible.

Histologic Structure of Midgut Larva *S. litura* and The Presence of PIB *Ha*NPV

Histologic structure of Midgut larva *S. litura* control shows the same condition at each hour of observation. Peritrofic membrane and undamaged brush border form, midgut epithelium cell are clearly seen without the presence of cell nucleus swelling and PIB is not to be found either inside or outside midgut epithelium cell.

Observation at the first hour after *Ha*NPV infection shows the condition of peritrofic membrane and midgut epithelium cell which is not yet changed. This can be explained by O'reilly and Miller research (1989), which states that one hour after virus infection process, the active virus (virogenic stroma) will be released from nucleocapsid and is ready to carry out replication by using host cell replication system. The virogenic form can not seen by microscope light, so that it can not be found, but this virus has entered peritrofic membrane. This can be explained at the next hour of observation result.

Starting from the 8^{th} hour of observation up to the 72^{nd} hour after *Ha*NPV infection the peritrofic membrane had lisis, sweling of cell nucleus of midgut epithelium with PIB inside but hemosit cell still seen. The case coincides with the result of research by Hawtin *et al.* (1992) starting that progenic virus in the form of PIB can already be seen in epithelium cell nucleus of larva insect starting from the 8^{th} hour after infection.

Observation at the 24^{th} hour after *Ha*NPV infection shows damage of midgut epithelium tissue becoming more serious. The form of brush border is not undamaged, a great part of epithelium is lisis and fills lumen, PIB is found either inside cell nucleus or outside midgut epithelium cell but hemosit cell still seen. This is because epithelium cell which is lisis causes PIB to be free and is ready to infect cells inside the tissue of the larva body. The speared of virus at this level is sporadic. The virus will infect other cells such as hemosit, fat body, muscle tissue of endodermis cells and ganglion nerves. According to Hawtin *et al.* (1992), cellular virus spread has been started when virus

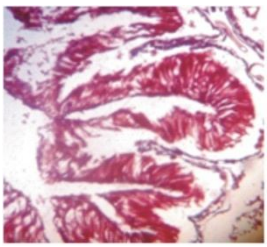
makes budded virus (BVs) in midgut of epithelium cells and then budding will get out of the cell. The virus is active in infecting other tissues in the body of the larva. Flipsen (1995) and Kawamoto *et al.* (1977) say that at furher infection stadium, the number of PIB in epithelium cell nucleus is abundant so that it causes cell disintegration. Larva *H. armigera* which undergoes further infection will experience death, its whole body will lisis so that PIB came out of its body.

Observation at the 32nd hour after *Ha*NPV infection shows regenerative cells on midgut epithelium tissue which start to form new columnar epithelium cells. The hemosit cell can seen. Columnar epithelium cells which are formed already contain PIB. Epithelium cells which are lisis together with PIB in lumen are seen wider apart. That is because both part have passed midgut track and are thrown away together with feces. In this way, PIB can be collected from the feces of *S. litura* larva 24th hours after infection.

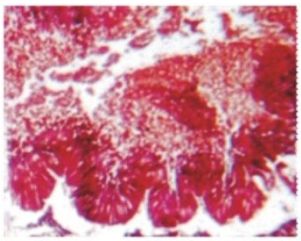
Observation at the 64th hour and the 72nd hour after *Ha*NPV infection show midgut epithelium cells which are freed from the connective tissue. There is cell disintegration and brush border form which is not undamaged. There are no regenerative cell on midgut epithelium tissue and hemosit cell. PIB is already in the form of granules (polyhedral granules) namely looking like a chain connected to one another. According Flipsen (1995) and Kawamoto *et.al.* (1977) infection at further stadium describes the number of PIB in nucleus which is abundant so that it causes cell disintegration. Based on *Kruskal-Wallis Test*, it shows that *Ha*NPV infection has obvious influence on midgut damage of larva *S. litura*. Further testing with *Wilcoxson test* shows that damage condition of larva at the first hour is not obviously different from control, but damage has happened starting from the 8th hour up to the 72nd hour after *Ha*NPV infection.

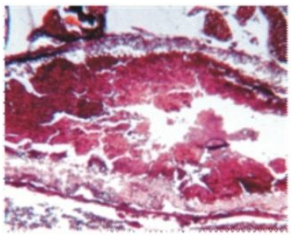
Based on result of observation on the whole, conclusion can be drawn that as long as regenerative cells in midgut epithelium tissue and hemosit cell can be formed, larva can still stay alive. This can be seen at the 32nd hour of observation result. The regenerative cells and hemosit cell are still formed well, although at the 40th hour of observation this cell undergoes destruction, but the larva still stags alive until the 72nd hour, because the regenerative cells and hemosit cell still formed again at the 48th hour after infection. The case is different at the 64th hour up to the 72nd hour after *Ha*NPV

infection. The regenerative cells and hemosit cell are no longer found and larva *S. litura* can not stay alive. Miranti (2008) say that larva *H. armigera* which are infected by *Ha*NPV will die in about 14 days, while with the same dose larva *S. litura* die in 3-5 days. This is because *Ha*NPV is a foreign virus for larva *S. litura* so that virus infection in question can not be controlled as perfectly as on is original host.

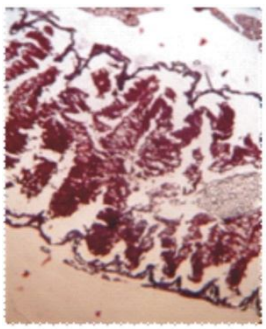


(A) (B)





(C) (D) (E)



(F) (G)

Picture 2. Midgut Longitudinal Cut of Larva S. litura, Control and Infected by HaNPV, 40 times Enlargement. (A) control, (B) 1st hour after infected by HaNPV, (C) 8th hour after infected by HaNPV, (D) 24th hour after infected by HaNPV, (E) 32nd hour after infected by HaNPV, (F) 64th hour after infected by HaNPV, (G) 72nd hour after infected by HaNPV.

CONCLUSION

There is damage on midgut track of larva *S. litura* instar four which is infected by *Ha*NPV, starting at the 8th hour. This is identified by the lisis of its peritrofic membrane, swelling of midgut epithelium cell nucleus with PIB inside and ends with epithelium cell disintegration, and the form of brush border which is not undamaged. Thus, larva *S. litura* can be used as substitute host for the production of *Ha*NPV, because *Ha*NPV can replicate perfectly with crop time more rapidly compared to its original host.

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