In Vivo Production of *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV) in *Spodoptera litura* : The Effect of Viral Serial Passages in *S. litura* on production and Pathogenicity of the Virus to *H. armigera*.

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ABSTRACT

Previous studies on our Indonesian isolate of *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV) isolated from cadaver of *Helicoverpa armigera*, indicates that the virus exhibits a great potential to be used as bioinsecticide to control population of *Helicoverpa armigera.* The use of insect virus as biological control agent is still limited, partly due to problems related to the difficulties in virus production. For *Ha*NPV in particular, invitro production is hinderd by the high production cost, while invivo production techniques is difficult to perform, since *Helicoverpa armigera* larvae used for production host exhibits canibal behaviour, thus have to be reared individually. Previous studies shows that *Spodoptera litura* is also pathogenic *Ha*NPV, that it can be used as a host for *Ha*NPV invivo production. This research study the effect of *Ha*NPV serial passages in *S. litura* on production and pathogenicity of the virus to *H. armigera* to determine the effect of *Ha*NPV serial passage in *Spodotera litura* on the production of *Ha*NPV and pathogenicity of the resulting *Ha*NPV to *Helicoverpa armigera*. The result indicates that *Ha*NPV subcultured in *Spodoptera litura* exhibited a similar patogenicity to *Spodoptera litura* compared to that subcultured in *Helicoverpa armigera*. Third instar of *Spodoptera litura* infected with *Ha*NPV_0, *Ha*NPV_1, *Ha*NPV_{10} and *Ha*NPV_{20} exhibit an average larval death of 12,6 days, 9.33 days, 8.66 days and 10.83 days respectively. Furthermore, the level of viral production was not influenced by the level of *Ha*NPV subcultures in *Spodoptera litura*. Third instar of *Spodoptera litura* infected with *Ha*NPV_0, *Ha*NPV_1, *Ha*NPV_{10} and *Ha*NPV_{20} exhibit *Ha*NPV production of 0.126 X 10^{10} PIB/ind, 2.65 X 10^{10} PIB/ind, 2.02 X 10^{10} PIB/ind, and 2,97 X 10^{10} PIB/ind. The result indicates that *Ha*NPV repeated subcultures in *Spodoptera litura* do not change the pathogenicity of the virus to *Spodotera litura* and level of viral replication capacity of the virus. However further observation on the morphology of the polyhedra indicates that there are chages, possibly mutation, on *Ha*NPV subcultured in *Spodoptera litura*. The polyhedra tend to deformed as the level of subculture increased. Further investigation showed that the polyhedral deformation does not alter the pathogenicity of *Ha*NPV to *Helicoverpa armigera*. Mortality data shows that infection *Ha*NPV_0, *Ha*NPV_1, *Ha*NPV_{10} and *Ha*NPV_{20} to third instar of *Helicoverpa armigera* larvae led to a similar mortality rate ie : 100%, 93.33%, 100% and 93.33% respectively.

Key word : *Ha*NPV, *Helicoverpa armigera, Spodoptera litura*
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Background

Previous studies on our Indonesian isolate of *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV) isolated from cadaver of *Helicoverpa armigera*, indicates that the virus exhibits a great potential to be used as bioinsecticide to control population of *Helicoverpa armigera* (Sanjaya, 2000 ; Miranti, 2001). Despite of this promising potential, the use of insect virus as biological control agent is still limited, partly due to problems related to the difficulties in virus production. For *Ha*NPV in particular, invitro production is hinderd by the high production cost, while invivo production techniques is difficult to perform, since *Helicoverpa armigera* larvae used for production host exhibits canibal behaviour, thus have to be reared individually (Miranti, *et al*., 2007; Wahyuni, 2007). Previous studies shows that *Spodoptera litura* is also pathogenic *Ha*NPV, that it can be used as a host for *Ha*NPV invivo production (Miranti, 2008). However, a number of studies indicates that viral serial passages led to mutation and reduction of pathogenicity (Chakraborty and Reid, 1999 : Moscardi, 1999). This research study the effect of *Ha*NPV serial passages in *S. litura* on production and pathogenicity of the virus to *H. armigera*

Objective

The main objective of this research is to determine the effect of *Ha*NPV serial passages in *Spodotera litura* on the production of *Ha*NPV and pathogenicity of the resulting *Ha*NPV to *Helicoverpa armigera*.

Method

The *Ha*NPV was kindly provided by The Centre of Life Sciences, Institut Teknologi Bandung (ITB). It is a wild type nucleopolyhedrosis virus isolated from cadaver of *Helicoverpa armigera* collected from tomato farm in Lembang, West Java, Indonesia. The *Ha*NPV used for this study was propagated in *Helicoverpa armigera* and *Spodoptera litura*. *Ha*NPV_0 was prepared by propagating the *Ha*NPV in *Helicoverpa armigera*, the *Ha*NPV_1 was prepared by propagating the *Ha*NPV in *Spodoptera litura* and isolated after only 1 passage, the *Ha*NPV_{10} was prepared by propagating the *Ha*NPV in *Spodoptera litura* and isolated after 10 passages and the *Ha*NPV_{20} was prepared by

propagating the *Ha*NPV in *Spodoptera litura* and isolated after 20 passages. The *Ha*NPV were isolated acording to O'reilly, *et al.*, (1994) and the *Ha*NPV stock solutions were prepared to contain 4 X 10^7 polyhedral inclusion body/ml. Larval infection were carried out orally through feed contamination method, by mixing 30 gram of sweet corn kernel (larval feed) with 1 ml of various *Ha*NPV stocks.

Third instars larval of *S. litura* and the parents of *H. armigera* are result from rearing in a laboratorium obtained from Vegetables Research Center (Balai Penelitian Sayuran) in Lembang.

Result

The result indicates that the serial passages of *Ha*NPV subcultured in *Spodoptera litura* exhibited a similar patogenicity to *Spodoptera litura* compared to that subcultured in *Helicoverpa armigera* (Fig. 1).

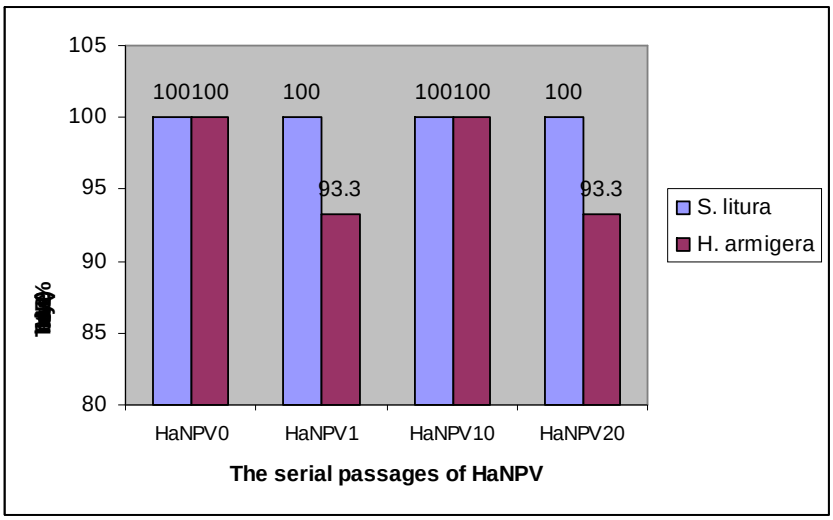

Figure. 1. The effect of serial passages of *Ha*NPV against the mortality rate of *S.litura* and *H. armigera* larvae

The mortality rate of *S. litura* which infected by *Ha*NPV from serial passages in *S. litura* is average 100 %. It shows that serial passages of *Ha*NPV was effective to kill *S. litura* which used as a host for virus production. The level of patogenicity of *Ha*NPV from serial passages in S. litura tends stability against the mortality rate of *S. litura*.

Thiem, (1997), said that the virus could be replicated in the cells from the alternate host for virus production. The level of mortality rate (up to 100%), shows that the alternate host is sensitive against viral infection. Stairs, (1991), found that the serial passages of virus in alternate host will increase the virus virulent against alternate host itself.

Third instar of *H. armigera* infected with *Ha*NPV$_0$, *Ha*NPV$_1$, *Ha*NPV$_{10}$ and *Ha*NPV$_{20}$ exhibit an average larval death of 12,6 days, 9.33 days, 8.66 days and 10.83 days respectively (Fig. 2). The serial passages of *Ha*NPV in *S. litura* tends to reduce the lethal time of *H. armigera* infected larval. Harrison dan Summer, (1995), said that the effect of serial passages of virus led the faster of the lethal time of non main host, because the virus produce the most infected mutagenic virion.

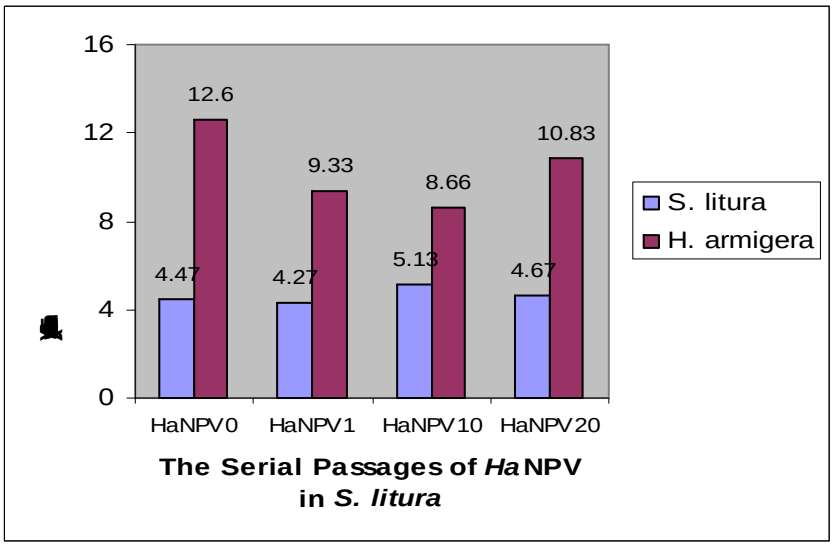

Figure 2. The effect of serial passages of *Ha*NPV in *S. litura* against to the lethal time of *S. litura* and *H. armigera*.

The figure 2, shows that the virus infection tends more effective to reduce lethal time against *S. litura* than *H. armigera*. The short lethal time against *S. litura* means that the production of *Ha*NPV in *S. litura* more effectively to harvest the production of virus.

Stairs, (1991), found that third times serial passages of *Bombyx mori* NPV (*Bm*NPV) in *Galleria mellonella* will reduce the lethal time of *G. mellonella*.

Furthermore, the level of viral production was not influenced by the level of *Ha*NPV subcultures in *Spodoptera litura*. The production of virus is the count of the polyhedral of virus which produced in cadaver of larval which infected by virus. The level of polyhedral will increase if the larval died. The production of polyhedral from cadaver of larval a day shows in Table 1.

Table 1. The effect of serial passages of *Ha*NPV in *S. litura* against *Ha*NPV polyhedral production.

The Serial Passages of *Ha*NPV in *S. litura*	Polihedral Inclusion Bodies (PIB) Production (PIB/individual)	Lethal Time (average) (days)
*Ha*NPV$_0$	0.126 X 10^{10}	5
*Ha*NPV$_1$	2.65 X 10^{10}	5
*Ha*NPV$_{10}$	2.02 X 10^{10}	5
*Ha*NPV $_{20}$	2.97 X 10^{10}	5

Table 1, shows that the serial passages of *Ha*NPV is increase the production of the virus. Third instar of *Spodoptera litura* infected with *Ha*NPV$_0$, *Ha*NPV$_1$, *Ha*NPV$_{10}$ and *Ha*NPV$_{20}$ exhibit *Ha*NPV production of 0.126 X 10^{10} PIB/ind, 2.65 X 10^{10} PIB/ind, 2.02 X 10^{10} PIB/ind, and 2,97 X 10^{10} PIB/ind. The result of the research shows that the serial passages of *Ha*NPV in *S. litura* up to 20th times tends the stability of virus production.

Then, the researchers found that the sustainable serial passages of virus in alternate host led the mutation of virus and reduced the level of patogenicity of the virus to the main host and reduced the level of virus production (Chakraborty and Reid, 1999 : Moscardi, 1999 ; Mulock and Faulkner, 1997 ; Narayanan, *et al*., 2002).

The result indicates that *Ha*NPV repeated subcultures in *Spodoptera litura* do not change the pathogenicity of the virus to *Spodotera litura* and level of viral replication capacity of the virus.

However further observation on the morphology of the polyhedra indicates that there are changes, possibly mutation, on *Ha*NPV subcultured in *Spodoptera litura*. The polyhedra tend to deformed as the level of subculture increased. Further investigation showed that the polyhedral deformation does not alter the pathogenicity of *Ha*NPV to

Helicoverpa armigera. Mortality data (Fig. 1), shows that infection *Ha*NPV$_0$, *Ha*NPV$_1$, *Ha*NPV$_{10}$ and *Ha*NPV$_{20}$ to third instar of *Helicoverpa armigera* larvae lead to a similar mortality rate ie : 100%, 93.33%, 100% and 93.33% respectively.

Evans, (1981), and Stairs, (1991) said that the subculture virus had high proliferation, and leds the high mortality level to the larvae, before pupation.

Conclusion :

1. *Ha*NPV subculture in *Spodoptera litura* led to deformation of viral polyhedra. However this does not lead to the reduction of *Ha*NPV pathogenicty to *Spodoptera litura* and viral production in *Spodoptera litura*.
2. The resulting *Ha*NPV shows a similar pathogenicity to *Helicoverpa armigera*.

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