The Potential of Subculture *Helicoverpa armigera* Nuclear Polyhedrosis Virus as an Alternate Synthetic Insecticides to Control Insect Pests in Cabbages Plantation (*Brassica oleracea* var. *capitata* L.)

Mia Miranti, Wawan Hermawan, Melanie, Rahman Perdana Hadi, Dicky Budi Sugiarto and Desy Efriyani Anggraeny Nasution

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Jl. Raya Bandung, Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia

Abstract: Subculture *Helicoverpa armigera* nuclear polyhedrosis virus (HaNPV) is an entomopathogenic virus isolated from cadaver of *Helicoverpa armigera* larvae, and is successfully produced in *Spodoptera litura* larvae as an alternate host. These viral agents have been used to control the population of three species insect pests *Spodoptera litura*, *Crociodolomia pavonana* and *Plutella xylostella*, which were infested in cabbages plantation. The concentration of virus of 4×10^7 polyhedra/mL was sprayed in cabbage leaves 24 h before infested of larva and every four days after. The research used randomized design which consisted of one factor (three species of second instar larvae, *S. litura*, *C. pavonana*, *P. xylostella*) and eight replications. The result showed that the three species of larvae were sensitive against subculture HaNPV infection. The mortality of each larval species (*S. litura*, *C. pavonana* and *P. xylostella*) was 100%, 97.5% and 98.7%, respectively. Subculture HaNPV can be utilized to control the population of three species of insect pests in cabbage plantation.

Key words: Subculture HaNPV (HaNPV1), polyhedra, cadaver, alternate host.

1. Introduction

Helicoverpa armigera nuclear polyhedrosis virus (HaNPV) is a specific entomopathogenic virus isolated from cadaver of *Helicoverpa armigera* larvae [1]. HaNPV should only be pathogenic to the target insect pests [2, 3]. *Autographa californica* nuclear polyhedrosis virus (AcNPV) does not persist in mass culture of mamalian cells, including human kidney's cells [4]. HaNPV infected several species of insect pests from Lepidopteran as *Helicoverpa zea*, *Spodoptera litura*, *Spodoptera exigua* and *Crociodolomia pavonana*. These insect pests are found in vegetable plants [5]. HaNPV has relatively wide host range and potential to be used as a microbial

agent for controlling a population of insect pests on vegetable cultivation.

HaNPV is effective to control the population of insect pests in vegetable plantation and is environmentally friendly, as it has a specific host target (Lepidopteran as *Helicoverpa armigera*, *Spodoptera litura* and *Crociodolomia pavonana*) [5] and does not infect non-target host as *Helicoverpa virescens* endoparasitoid [6]. HaNPV used as a microbial control agent of insect pest population does not leave harmful residues in human food.

A mere problem of utilization of HaNPV as a microbial agent is a limited capacity of its production for commercial uses. In Indonesia, providing medium and building a specific laboratory for *in vitro* production of HaNPV in cell cultures are very expensive. Alternatively, *in vivo* production can be

Corresponding author: Mia Miranti, Ph.D., research field: microbiology. E-mail: miantariksa@gmail.com.

conducted because it is easier and cheaper means of production. However, the main host *HaNPV* of *in vivo* production (*H. armigera* larvae) faces a problem of strong cannibalism behavior of *H. armigera* larvae itself.

To solve the problem of *HaNPV* production for commercial use, *in vivo* production of *HaNPV* has been conducted in an alternate host (*S. litura* larvae). *S. litura* is used as the alternate host because it is sensitive to *HaNPV* infection, bigger (the average weight of each *S. litura* larvae is 2.000 mg but *H. armigera* is less than 1.000 mg) than *H. armigera* larvae [7] and produce more of polyhedral virus [8].

Several researches showed that an appropriate alternate host for specific virus production must be able to provide materials in order to enable a perfect virus replication. Research done by Miranti [7] indicated that *HaNPV* hosted in *S. litura* larvae can produce polyhedral virus. Sudhakar and Mathavan [9] viewed that polyhedral virus in an alternate host means that such virus is able to perfectly replicate.

Miranti [7] has found *HaNPV*₁ (subculture *HaNPV*) is the virus from *in vivo* production in the alternate host *S. litura* larvae. *HaNPV*₁ has been successfully passed a laboratory test to infect *S. litura* and *H. armigera* larvae. In this research, *HaNPV*₁ will be used as a microbial agent to control the population of *S. litura*, *C. pavonana* and *P. xylostella* larvae, which are infested in cabbage plantation of limited area. These larvae were chosen because they frequently damage cabbages and are sensitive to *HaNPV*.

2. Materials and Methods

The method used in this research is randomized block design with one factor (species of tested second instar larvae (S)) consisting of:

$S_1 = S. litura$ larvae, $S_2 = C. pavonana$ larvae, $S_3 = P. xylostella$ larvae.

Each treatment was taken eight replications, so total amounts of sample were 24. The second instar larvae from each species (10 larval) infested on 10-week-old

cabbage plants. The total amount of each larval species used for the experiment was 80 individuals, respectively. The concentration of virus suspension was 4×10^7 polyhedra/mL. The mortality of larvae in 20 days' observation was a parameter of the experiment.

Data were processed with ANOVA, but when it is significant, continued with Duncan test (at 5%).

2.1 Virus Stock Preparation

The *HaNPV*₁ was produced by propagating the *HaNPV* in *S. litura* and isolated after only one passage. *S. litura* third instar was infected with 4×10^5 polyhedra/mL virus suspension concentration. The cadaver of infected larva was collected in glass container and stored at 4 °C. Then, the cadavers of 40 larvae were crushed by mortar and mixed with 20 mL triss buffer (1 mM, pH 7.6) solution and 20 mL 0.1% sodium dodecyl sulphat (SDS) solution. This concentrate was stored at 4 °C for 24 h.

After storage, the concentrate of virus was filtered with two layer of muslin cloth. The suspension of virus was centrifuged at 3,500 rpm for 15 min at 4 °C. The first supernatant fraction was discarded, because polyhedra was collected in the bottom of the tube as pellet. The pellet was resuspended in 5 mL triss buffer (1 mM, pH 7.6) solution and 5 mL 0.1% SDS solution and it continued to be centrifuged at 3,500 rpm for 15 min at 4 °C. The step of washing for purifying virus replicated three times. The last pellet of the washing step was resuspended with mixed triss buffer (1 mM, pH 7.6) solution and 0.1% SDS solution by adding 0.2% natrium azida.

To count the polyhedral numbers of virus, 0.1 mL of resuspended virus pellet was mixed by adding 0.9 mL mixed solution of triss buffer (1 mM, pH 7.6) and 0.1% SDS with 1:1 ratio. The polyhedral numbers in stock virus suspension were counted with a Neubauer haemocytometer by a light microscope (magnification 400×). The presence of polyhedra in the suspension was cuboidal shape and green colour.

The concentration of virus suspension sprayed in the cabbage plants was 4×10^7 polyhedra/mL solution.

2.2 The Larvae

The larvae have been taken from Vegetables Research Center (Balai Penelitian Sayuran) in Lembang, West Java. Every species of larvae are kept in plastic containers (1,000 mL volume), covered with tulle on top container. The larvae are fed with free synthetic insecticide cabbage leaves. The second instar larva was used for the experiment.

2.3 The Cabbage Plant

The 6-week-old cabbage (*Brassica oleracea* var. *capitata* L.) plants have been taken from Lembang. The cabbages planted in soil from Lembang were mixed with manure fertilizer in 5 kg volume of polybag. Each cabbage was covered by tulle to protect it from other insect pests and cultivated in arboretum area in University of Padjadjaran, Jatinangor Sumedang, West Java.

After 10-week-old cabbage plant, the virus suspension was sprayed on the leaves of cabbage at 17:00-18:00. After 24 h, the second instar of each larval species was infested (10 individuals/each plant) on cabbage leaves. Then, the cabbage plant was covered by tulle to prevent larvae run off from the plant. The observation of larval mortality was conducted in 20 d or until larval was not found in the cabbage plant.

The suspension of virus with concentration of 4×10^7 polyhedra/mL was sprayed with hand sprayer (500 mL volume) every four days in 20 days' observation.

3. Results and Discussion

The data analysis of the effectivity of *HaNPV*₁ suspension sprayed to *S. litura*, *C. pavonana* and *P. xylostella* which were infested in cabbage plant, are shown in Table 1.

In Table 1, the analysis of ANOVA shows that the mortality of each larval species is not significant. The mortality of larval species indicated that the larvae were sensitive to virus infection. The virus virulence of *HaNPV*₁ (subculture *HaNPV*) which produced in alternate host tends to be stable. The virus is still effective to infect *S. litura*, *C. pavonana* and *P. xylostella* larvae as *HaNPV* (subculture only in main host *H. armigera* larval). It means that *HaNPV*₁ is effective to protect cabbage plant cultivation in land from limited insect pests (only *S. litura*, *C. pavonana* and *P. xylostella*).

The numbers of larval mortality for each species of larval in this experiment are shown in Fig. 1.

Fig. 1 showed that effectivity of *HaNPV*₁ to infect each larval species and cause mortality to *S. litura*, *C. pavonana* and *P. xylostella* with the values of 100%, 97.5% and 98.7%. This experiment indicated that *HaNPV*₁ can be applied directly to control the population of larvae which infest the cabbage plants.

In this research, the data were taken based on the mortality rate of larvae. The imago of each species of insect did not found. It means that the larvae infected by virus could grow to imago stadium. The level of mortality rate (up to 100%) showed that the insect pest larva was sensitive against viral infection.

The higher value of mortality indicates that *HaNPV*₁ is effective to be used as a microbial agent to

Tabel 1 The data analysis of mortality of larval species which were infested in cabbage plant and sprayed with virus suspension in 4×10^7 polyhedra/mL concentration.

Source diversity	Degree of freedom (df)	Sum of squares	Central squares	F count	F table (5%)
Treatment	2	0.250	0.125	1.105ns	3.47
Error	21	2.375	0.1131		
General	23	2.625			

ns = not significant at 5%.

Fig. 1 The mortality numbers of the larvae infested in cabbage plants and sprayed with *HaNPV*₁ suspension with 4×10^7 polyhedra/mL concentration in 20 days' observation.

control the population of several insect pests especially in cabbage cultivation. From the high values of mortality, Christian [10] indicated that the virus can replace syntetic insecticide in plant cultivation. The use of viruses to control insect pest population is eventually helpful for environment conservation and human health [3]. *HaNPV* is a member of baculovirus hat has polyhedra. The polyhedra is resistant to environment effect [11]. This makes baculoviruses very suitable for the selective control of pest insects [12].

The experiment showed that *HaNPV* subculture in alternate host did not lead to the reduction of *HaNPV* pathogenicity to *S. litura*, *C. pavonana* and *P. xylostella*. *HaNPV*₁ is still effectively used to protect the cabbage plant from several insect pests. *HaNPV*₁ tends to increase the mortality of larvae that were infested in cabbage plantation. However, *HaNPV*₁ can be recommended as a microbial agent to replace synthetic insecticides on cabbage plantation.

4. Conclusions

*HaNPV*₁ used as a microbial agent is more effective to control some species of insect pests, such as *S. litura*, *C. pavonana* and *P. xylostella*. *HaNPV*₁ can replace synthetic insecticides for plant protection from insect pests on cabbage plantation.

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