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THE POTENTIAL OF SUBCULTURE *Helicoverpa armigera* NUCLEAR POLYHEDROSIS VIRUS (*Ha*NPV) TO BE UTILIZED AS AN ALTERNATE SYNTHETIC INSECTICIDES TO CONTROL INSECTS PESTS IN CABBAGES PLANTATION (*Brassica oleracea* var. capitata L)

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ABSTRACT

Subculture *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV) is an entomopathogenic viruses isolated from cadaver of *Helicoverpa armigera* larvae, and was succesfully produced in *Spodoptera litura* larvae as an alternate host. These viral agent have been applied to control the population of three species insects pests *Spodoptera litura*, *Crocidolomia pavonana* and *Plutella xyllostella* which were infested in Cabbages Plantation. The concentration of virus of 4 x 10^7 Polyhedral/ml was sprayed in cabbage leaves 24 hours before infested of larval and every four days after. The research used randomized design which consisted of factor (three species of second instar larvae, *S. litura*, *C. pavonana*, *P. xyllostella*) and eight replications. The result showed that the three species of larval were sensitive against subculture *Ha*NPV infection. The mortality of each larval species (*S. litura, C. pavonana* and *P. xyllostella*) were 100%, 97.5% and 98.7%. Subculture *Ha*NPV can be utilized to control the population of three spesies of insects pests in Cabbage Plantation.

Key words : Subculture *Ha*NPV, Polyhedral

INTRODUCTION

Helicoverpa armigera nuclear polihedrosis virus (*Ha*NPV) is a specific entomophatogenic virus isolated from cadaver of *Helicoverpa armigera* larvae (Teakle, 1994). *Ha*NPV infected several species of insect pests from lepidopteran as *Helicoverpa zea, Spodoptera litura, Spodoptera exigua*, and *Crocidolomia pavonana*. These insect pests are found in vegetable plants (Miranti, *et. al*., 2007). The result of this research showed that *Ha*NPV had a relative wide host range and had a potential to be used as a microbial agent to control the population of insect pests in vegetable cultivation.

*Ha*NPV is effective to control the population of insect pests in vegetable plantation and environtment-friendly for it has a specific host target and has not infected non target host. The most important advantage of using *Ha*NPV as a microbial agent to control the population of insect pests is that it does not leave a dangerous residues in vegetables for human consumption.

A mere problem of utilisation of *Ha*NPV as a microbial agent is a limited capacity of its production for commercial uses. In Indonesia, providing medium and building a specific laboratorium for in vitro production of hanpv in cell cultures is very expensive. Alternatively, in vivo production can be conducted because it is easier and cheaper means of production. However, in vivo production in the main host of *Ha*NPV (*H. armigera* larvae) faces a problem of strong cannibalism behavior of *H. armigera* larvae itself.

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To solve the problem of *Ha*NPV production for commercial use, in vivo production of *Ha*NPV has been conducted in an alternate host (*S. litura* larvae). *S. litura* is used as the alternate host because it is sensitive to *Ha*NPV infection, bigger than *H. armigera* larval (Miranti, 2008) and results in a higher amount of production of polyhedral virus (Miranti and Wardono, 2009).

Several researches showed that an apropriate alternate host for specific virus production must be able to provide materials in order to enable a perfect virus replication. Research done by Miranti (2008) indicated that *Ha*NPV hosted in *S. litura* larvae can produce virus polyhedral. Sudhakar and Mathavan (1999) viewed that polyhedral virus which can form polyhedral in an alternate host means that such virus is able to perfectly replicated.

Miranti (2008) has found *Ha*NPV_1 (subculture *Ha*NPV), an in vivo production *Ha*NPV in the alternate host. *Ha*NPV_1 has been successfully passed a laboratory test to infect *S. litura* and *H. armigera* larvae. In this research, *Ha*NPV_1 will be used as an microbial agent to control the population of *S. litura, C. pavonana* and *P. xyllostella* larvae which are infested in cabbage plantation of limited area. These larval were choosen because they frequently damage cabbages and sensitive to *Ha*NPV.

This research suggests that *Ha*NPV_1 can be utilised as a environmental-friendly microbial agent to controll population of several insect pests, particularly in vegetable crop.

MATERIALS AND METHODS

The methods used in this research is experimental method with randomized block design with one factor (spesies of tested second instars larval (S)) consisted of :

s_1 = *S. litura* larvae

s_2 = *C. pavonana larvae*

s_3 = *P. xyllostella* larvae

Each treatment was taken 8 replication with total amounts of sample were 24. The second instar larval from each species (10 larval) infested on 10 weeks old cabbage crop. The total amount of each larval species used for the experiment were 80 individual, respectively. The concentration of virus suspension was 4 x 10^7 polyhedra ml^{-1}. The mortality of larval in 20 days observation was a parameter of the experiment.

Data processing with ANOVA, when it is significants that continue by Duncan test (in 5 %).

Virus stock preparation

The *Ha*NPV_1 was prepared by propagating the *Ha*NPV in *Spodoptera litura* and isolated after only 1 passage. *S. litura* third instars infected with 4 X 10^5 polyhedral ml^{-1} virus suspension concentration. The cadaver of infected larval collected in glass container was stored at 4^{o}C. Then, the cadaver of 40 larval was crush 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^{o}C for 24 hours.

After storage, the concentrate of virus filtered with two layer of muslin. The suspension of virus was centrifuged (3500rpm, 15 min) at 4^{o}C. the first supernatant fraction was threw. The pellet was resuspended in 5 ml triss buffer (1 mM, pH 7.6) solution and 5 ml 0.1% sodium dodecyl sulphat (SDS) solution and it was continued centrifuged (3500rpm, 15 min) at 4^{o}C.

The washing step for purifying virus treatment were three times replication. The last pellet of the washing step was resuspended with mixed triss buffer (1 mM, pH 7.6) solution and 0.1% sodium dodecyl sulphat (SDS) solution which adding 0.2 % Natrium Azida.

To count the number of polyhedra 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% sodium dodecyl sulphat (SDS) with 1 : 1 ratio. The polyhedral number in stock virus suspension were counted using a Neubauer haemocytometer, using a light microscope (magnification 400x). The presence of polyhedra in the suspension was cuboidal shape and green colour dispersed.

The concentration of virus suspension sprayed in the cabbage plants was 4 X 10^7 polyhedral ml^{-1} solution.

The larval insect

The larva insects have been taken from result from rearing in a laboratorium obtained from Vegetables Research Center (Balai Penelitian Sayuran) in Lembang, West Java. Each species of larval storage in plastic container (1000 ml volume), covering with tulle on top container. The larval is feeding with free synthetic insecticide cabbage leaves. The species of larval used for the experiment were second instar larval.

The cabbage plant

The six weeks cabbage (*Brassica oleracea* var. capitata L) plant have been taken from Lembang. The cabbage planted in soil from Lembang mixed with manure fertilizer in 5 kg volume of polibag. Each cabbace covered by tulle to protect it from another insect pests and cultivation in arboretum area in University of Padjadjaran.

After 10 weeks old cabbage plant, the virus suspension sprayed on the leaves of cabbage at 17.00 – 18.00. In 24 hour after, second instars of each larval species infested (10 individual/ each plant) on cabbage leaves. Then, the cabbage plant covered by tulle to prevent run off larvae from the plant. Mortality of larval observation conducted in 20 days or until larval not found in the cabbage plant.

Suspension of virus with concentration 4 X 10^7 polihedra ml^{-1} sprayed with hand sprayer (500 ml volume) every four days in 20 days observation.

RESULT AND DISCUSSION

The data analysis of effectivity of the *Ha*NPV_1 suspension sprayed to *S. litura, C. pavonana* and *P. xyllostella* were infested in cabbage plant shows in Table 1.

Tabel 1. The data analysis of mortality of each larval species with ANOVA which infested in cabbage plant and sprayed with virus suspension in 4 X 10^7 polyhedra ml^{-1} concentration.

Sumber keragaman	Derajat bebas (db)	Jumlah kuadrat (jk)	Kuadrat tengah (kt)	F hitung	F table(5%)
Perlakuan	2	0,250	0,125	1,105ns	3,47
Galat	21	2,375	0,1131		
Umum	23	2,625			

ns = not significants in 5%

In table 1 the analisys of anova shows that mortality of each species of larval is not significant. The species of larval indicated that the larval sensitive to virus infection. *Ha*NPV$_1$ (subculture *Ha*NPV) which produced in alternate host tends the stability of virus virulence. The virus still effective to infect *S. litura, C. pavonana* dan *P. xylostella* larval as *Ha*NPV (subcultur only in main host *H. armigera* larval). It means that *Ha*NPV$_1$ effective to protect cabbage plant cultivation in land from limited insect pests (only *S. litura, C. pavonana* and *P. xylostella*).

The number of larval mortality for each spesies of larval in this experiment are shown in Figure 1.

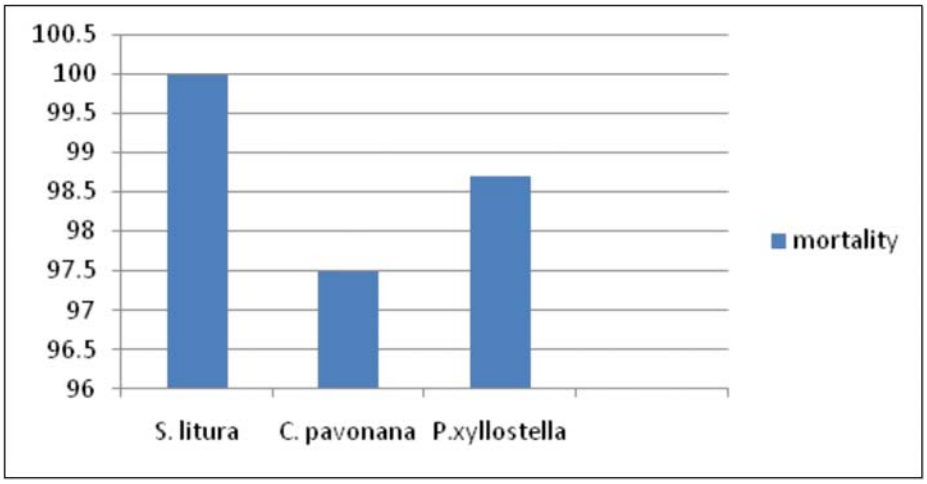

Figure 1. The number of the mortality of each larval species infested in cabbage plants and it sprayed by *Ha*NPV$_1$ suspension with 4 X 10^7 polihedra ml^{-1} concentration in 20 days observation.

In figure 1 shows that effectivity of *Ha*NPV$_1$ to infect each larval species caused mortality to *S. litura, C. pavonana* and *P. xyllostella* with the values of 100%, 97,5% and 98,7%. This experiment indicated that *Ha*NPV$_1$ can be aplicated directly to control the population of larval which it infested in cabbage plants.

In this research, the values of the mortality was in larval stadium. The imago of each spesies of insect did not found. It means that the application of *Ha*NPV$_1$ to control the population of insect pest larval. The larval infected by virus could be growth to imago stadium. The level of mortality rate (up to 100%), shows that the insect pest larval is sensitive against viral infection.

The higher values of mortality tends that *Ha*NPV$_1$ effective used as a microbial agent to control the population of several insect pest especially in cabbage cultivation. Christian, (1994), shows that the high values of mortality indicated that the virus can be replaced syntetic insecticide in plant cultivation. *Ha*NPV is a member of Baculovirus which a group of virus exclusively occluded in proteinaceous crystals. The polyhedra is resistant to environtment effect (Maramorosch and Sherman, 1985). This makes baculoviruses very suitable for the selective control of pest insects (Groner, 1986 in van Lier, 1991).

The experiment shows that *Ha*NPV subculture in alternate host does not lead to the reduction of *Ha*NPV pathogenicity to *Spodoptera litura, Crocidolomia pavonana* and *Plutella xyllostella*. *Ha*NPV$_1$ still effective to use for protect the cabbage plant from several insect

pests. *Ha*NPV$_1$ tends to increase the mortality of larval infested in cabbage plant cultivation. However, *Ha*NPV$_1$ can be recommended as a microbial agent to replace synthetic insecticide in cabbage cultivation.

CONCLUSIONS

The use of *Ha*NPV$_1$ as a microbial agent to control the population of several spesies of insect pest as *S. litura, C. pavonana* and *P. xyllostella* was effective. *Ha*NPV can be replaced a syntetic insecticide to protect the cabbage plant in agriculture area.

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