1. The effectivenes of stored SpltMPV 2015

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THE EFFECTIVENESS of STORED *Splt*MNPV on MORTALITY and NORMALITY of *Spodoptera litura*

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Abstract

The storage of *Spodoptera litura* multiple nucleopolyhedrosis virus biopesticide is an important stage and often performed before the virus was applied, to maintain the purity of the virus. This study aims to determine *Splt*MNPV in vitro effectiveness which have been stored for 20 months at -80 ° C on mortality and normality *S. litura*. *Splt*MNPV in vitro at doses of A = 5.95 x 10^8 PIBs / ml, B = 5.95 x 10^7 PIBs / ml. C = 5.95 x 10^6 PIBs / ml and D = Control was infected to third instar Larvae of *S. litura*. This research was conducted in the laboratory with repeatedly for fifth times and each replication used 10 animals caterpillar. Infected Larvae has been incubated for 17 days and had been given an artificial feed.The parameters observed in the form of mortality and normality *S. litura*. Research result shows that in *S. litura* infected with *Splt*MNPV in vitro dose of 5.95 x 108 PIBs / ml caused high mortality of 44%, *S. litura* abnormal approximatly 14% and the rest managed to become a moth normal life. Lowest mortality at doses C = 5.95 x 10^6 PIBs / ml; the mortality of *S. litura* is 38% and abnormalities *S. litura* is about 12%. *S.litura* abnormal morphology was observed in the Larvael stage, PrePupae and Pupae.

Keywords: Effectiveness, *Spodoptera litura* Multiple Nucleopolyhedrosis virus (*Splt*MNPV), mortality, abnormalities, *Spodoptera litura*

Introduction

Spodoptera litura multiple nucleopolyhedrosis virus (SpltMNPV) is biopesticide made from the virus. The effort hasbeen made to propagate the virus in vitro by culturing cells midgut epithelial cells or ovarian cells of various insect Larvae such as *S. litura*, *S. frugiperda*, *Pseudoletia unipuncta*, *M. sexta* and *Heliothis virescens*. According to a preliminary test by [1], cultured midgut epithelial cells of of S. Litura Larvae grow very fast growing / divide rapidly, reaching 975 times / 24 hours.The culture can be used to produce a wide range of bioproducts such as biopesticide virus production virus (baculovirus) and certain protein product that is typically use for genetic engineering.

The Pathogenicity of SpltMNPV biopesticide a result of in vitro propagation in midgut epithelial cell culture that was recultured second time and formulated with photo-protectant and infected to *S. litura* second instar Larvae, resulting 100 % of S. litura Larvae mortality at the fifth day [1] According to [2], latent virus in the shape of polyhedral or prism in epithelial cells cultured *S. litura* Larvae that have been saved (in the freezer in Grace's medium temperature ± 20°C) for at least 2 years then pathogenecities were 10 percent. However when the virus were infected repeadly to the original host Larvae of *S. litura* their pathogenecitas increased to 14% after the first infection, and 63% in the second infection, and 90% in the third infection.

The factors that determine the level of effectiveness of the virus, among others, are the nature, concentration, application mode, storage, and persistence level of the virus. NPV inoculum are resistant when they are stored in a refrigerator in the form of dry polyhedra.

Mixture polyhedrosis usually last for four to five years, granulosis last for two to three years, and cytoplasmic polyhedrosis last for one year [3] Once applied on the field, the virus will survive for along time if it is not exposed to direct sunlight. If it is directly exposed constantly to the sunlight, then its virulence will reduce by 50 percent each day. According to Pramono (2000), the NPV pathogenicity remains unchanged after 1 year of storage. This is supported by Balitsa Lembang's study. He has packed NPV mixed with talc and then placed it in a plastic bottle. The result showed that the NPV can be stored for more than one year. In addition, based on a study conducted by Klaten research center, NPV was also quite effective in maintaining the stability and effectiveness up to 6 months when placed in a dry room temperature.

Based on the background this study will examine the effectiveness of in vitro propagated SpltMNPV on epithelial cells of *S. litura* Larvae that had been stored for 20 months in Ex-cell medium at -80 ° C, on the mortality and normality of *Spodoptera litura*.

Materials And Methods

A.Equipment and Materials Research

The tools used in this study include the main equipment and ancillary equipment. The main equipment consists of incubator, light microscope, Laminar Air Flow (LAF), incubator, centrifuge, magnetic stirrer, and blender. The support equipment consists of capsule bottles, brushes, gauze box, centrifuge tubes, syringe, aluminum foil, knife, and label.

The materials needed in the study are SpltMNPV Wonosobo, Central Java isolates that are propagated in vitro, third instar Larvae of *Spodoptera litura*, artificial feed for Larvae of *S. litura*, Bayclin, Alcohol 70% as a sterilizing agent, and distilled water.

A. Research Design

This research is an experimental study using a complete randomized design, one factor is the dose SpltMNPV propagated in vitro in the midgut epithelial cells of Larvae of *S. litura* which has been stored at a temperature of - 80 ° C for 20 months, infected Larvae fed by using artificial feed (Balitas , Malang).

This study aimed to test the pathogenicity of in vitro SpltMNPV that has been stored for 20 months on the mortality and normality of *S. Litura*. The study was repeated 5 times and used 10 Larvae in each repetition. Larvae of S. litura were given SpltMNPV in vitro treatment with a dose: A = 5.95 x 108 PIBs / ml; B = 5.95 x 107 PIBs / ml; C = 5.95 x 106 PIBs / ml; D = Control (akuadest). The results of research in the form of mortality and infected normality S. litura were analyzed descriptively.

B. Research Procedure

1. Preparation: The calculation of the concentration of SpltMNPV

SpltMNPV which are newly stored in the refrigerator in the temperature of 80oC adapted beforehand to the same temperature as the room.Virus concentration is calculated using haemocytometer. SpltMNPV concentration used is A. 5.95 x 108PIBs / ml; B. 5.95 x 107PIBs / ml and C 5.95 x 106 PIBs / ml.

2.Propagation test insect (*Spodoptera litura*)

F0 S. litura Larvae captured in the area around the jatropha and tobacco plantation in Malang, and subsequently maintained in the Laboratory of Microbiology UNESA up to the stage of imago and feeded using jatropha leaves as natural food.Imago were moved into screen box with the male and female ratio of 1: 3. Imago were allowed to marry and fed using liquid honey 10%. Eggs produced in this step is an F1 generation. Eggs obtained were subsequently maintained until they hatch into Larvae F1.The Larvae then immediately transferred into bottle maintenance/ bottle capsules with a volume of 50 ml.Feed given to the 1st and 2nd instar caterpillar is the natural feed in the form of jatropha leaves, and artificial feed used to feed instar 3 to 5 use. The composition of 1 liter of artificial feed includes: soybean flour (42 g), agar-agar powder (14 g), yeast (42 g), Wheat germ 36 grams), salt Wesson (10 g), vitamin mix (9.6 gr), parabernsoat (1 g), sorbic acid (1 g), aureomycin (1 g), streptomycin (0.2 g), Benlate (1 g) and formaldehyde (2 ml) that were cooked and printed as gelatin to size 1 x 1 cm square. S. litura Larvae used in this study was the third instar Larvae of *S. litura*.

C. Test of SpltMNPV pathogenicity in vitro which have been kept for 20 months on the Larvae of S. litura

Larvae of *S. litura* were infected orally by dipping Larvae feed on virus suspension. The infected Larvae were incubated in the capsule bottles individually for 17 days (Larvae become moth). Furthermore, the infected Larvae were observed in terms of their mortalities and morphological changes to see normality. Morphological changes of the Larvae were observed every day starting from initial infection until caterpillars' death.

Results And Discussion

A. The mortality and normality of *S. litura* infected with SpltMNPV

The result of this study is the effectiveness of SpltMNPV which were reproduced on midgut epithelial cells of *S. litura* Larvae on the mortality and normality of*S. litura*, with different SpltMNPV oncentrations and were infected with food dipping method. The treatment was repeated three times and there were 10 Larvae in each repetition. The percentage of mortalities, abnormalities and normalities of *S. litura* infected by SpltMNPV presented in Table 1, as follows:

Table 1. The percentage of mortalities, abnormalities and normalities of *Spodoptera litura* by SpltMNPV in vitro after 20 months of storage (observed at day 17)

Treatment	Imago	Replay					Average
		1	2	3	4	5	
A	Dead	40	50	50	40	40	44
	Abnormal	0	10	20	20	20	14
	Normal	60	40	30	40	40	42
B	Dead	50	20	40	30	60	40
	Abnormal	0	10	10	20	20	12
	Normal	50	70	50	50	20	48
C	Dead	10	50	50	50	30	38
	Abnormal	20	10	20	10	0	12
	Normal	70	40	30	40	70	50
D	Dead	20	10	20	30	10	18
	Abnormal	10	10	20	0	0	8
	Normal	70	80	60	70	90	74

Descriptions :

A = 5.95 x 108 PIBs / ml; B = 5.95 x 107 PIBs / ml; C = 5.95 x 106 PIBs / ml; D = Control

Data desribed as the percentage of mortalities, abnormalities and normalities of *S. litura* that died because of being infected by SpltMNPV (Table 1) can be seen at the seventeenth post infection**.** Based on the data in Table 1 above, it appears that the highest mortality achieved in SpltMNPV in vitro with a dose of 5, 95 x 108 PIBs / ml were 44%, the next row at a dose of 5.95 x 107 PIBs / ml and 5.95 x 106 PIBs / ml with a mortality of 40% and 38%. Compare to the research conducted by [1] the same, but still new cultured, virus which were infected on second instar Larvae of *S. Litura* achieved 100% mortality on the fifth day.The mortality obtained by this study is lower than that was resulted by the new cultured virus. This is caused by the fact that the virus in this study has undergone sufficiently long time storage, that was 20 months.

According to Mangundihardjo et al (1992), the weakness of NPV stored for a long time is the virus does not get live cells so that its activity stops and crystallizes and it needs some time to be active when it find the host. Evidence that the virus which has been stored for a longer period shapes crystal/like a prism has been successfully photographed by [2] using scanning electron microscope, while the NPV which is still isolated from the new host shapes polyhedra (hexagon like dice).In addition, strains of SpltMPNV used were less virulent because the virus has undergone propagation in different environments i.e. in vitro cell culture conditions are not the same when SpltMNPV attack in vivo. In the in vitro conditions, the host's immune system does not exist so that the virus is not adaptive when inserted into the original host body.

According To Pramono, at the virus storage period for 1 year, the pathogenicity of the virus applied in the field reaches the mortality of *S. litura* as much as 49.33% on the tenth day. Thus, the virus storage for 20 months caused mortality of *S. litura* was slightly lower (44%) than that of 1 year virus storage. %. According to Suwandi (2007), NPV stored in a dark room for 24 hours and infected on caterpillar H. Talaka (inch caterpillar on tea leaves), its pathogenicity decreased by 26.66%.In the in vitro conditions, the host's immune system does not exist so that the virus is not adaptive when inserted into the original host body.

Besides, the relatively low mortality was also obtained at post infection on the seventeenth day. In the long period of death, according to Granados and Federici (1986, in Suwandi, 2007), NPV is said to be virulent if it can kill S. litura within 2-5 days, and is said to be less virulent if the NPV can only kill it within 2 to 3 weeks. So, based on the opinion, SpltMNPV in vitro which have been stored at -80 ° C for 20 months will be classified as less virulent.The use of SpltMNPV resulted from in vitro propagation causes the growth of *S. litura* at various abnormal stages (Figure 1,2 and 3).

It can be seen from the evidence that *S. litura* virus were dying in the stage of PrePupae, Pupae and imago. In the Larvael stage, virus rarely died. This shows that in the Larvael stage, the Larvae are able to make high defense against infection of SpltMNPV. Only in the intermediate stage of the Larvae become PrePupae, PrePupae into a Pupae and imago stages.

At this stag**e,** *S. litura* are less able to form a high defense against viral infection. PrePupae is a critical stage because the Larvae feeding activity has stopped. The energy stored when the Larvae are widely used to convert the organs in the body of the Larvae into other organs whose functions change.

For example, the digestive organs was in the form of mouth /chewing food in the form of solids (leaf) will turn into fluid/ honey-sucking mouth. This applies to other organs such as the formation of the wings to fly which did not occur in the Larvae l stage.

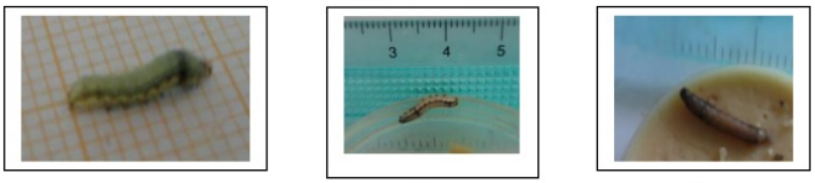
B. The Morphology of SpltMPV *S. litura* infected in vitro

The morphology of *Spodoptera litura* infected with SpltMNPV at various stages was observed to see the abnormalities produced.

Observations on abnormalities done at the Larvae l, PrePupae, Pupae and imago stages.

Table 2. Characteristics of Larvae, PrePupae, Pupae and imago of *S. litura* infected with SpltMNPV in vitro.

No	Stages *S. litura*	The characteristics of *S. litura* abnormal	The characteristics of *S. litura* normal
1.	Larvae	The infected Larvae tend to has bright and shiny colors, especially in the lower abdomen, the mandible when it was touched its response is slow, the Larvae tend to rise to the top (closed bottle), his movements is slow, lazy to feed, time needed by instar to turnover tends to be longer, her body swelled and when died the virus body are easily broken when we touch, it discharged white milk containing viral polyhedra (Figure 1 A).	Color matches the color of the Larvae feed, on the day of the beginning of each instar Larvae tend to be bright colors (beige / brown) but on days 2-3 ahead instar change color becomes darker. The texture /line on the Larvae is clear. When being touched Mandible Larvae will respond quickly. Larvae at Instar 5 tend to be darker. The Larvae eat actively (Figure 1.B, C)
2.	PrePupae	PrePupae color is darker / blackish brown, sometimes the color is uneven (half brown and half dark brown), its body segments are irregularly, if we touch its response was slow, the formation of candidates for the head imago is not perfect, some PrePupae failed to shrink his body, even enlarged/swelling, diabdomen leg is still visible, diabdomen segment of the middle part shrink, soft body and when we touched it discharge milk chocolate which contains many polyhedra (Figure 2A)	PrePupae color is still like instar 5 Larvae that is dark brown, the length and width of the Larvae shrink nearly half the normal size, PrePupae eat inactively, make a nest by collecting leftover food and feces are made such that the whole body covered. Food remains and excrement held together with a kind of yarn produced by saliva. When we touch, PrePupae response is slow, and the body tends to curved (Figure 2B)
3.	Pupae	The size of the Pupae is smaller than the normal Pupae (<2 cm), Pupae color is uneven (there are several bulkhead colored younger), head of the Pupae enlarged, abdominal shrinking, the legs are still seen, bulkhead abdomen retracts but not hardened, its color is dark brown tends to black, head arched, some abdomen / head is soft and easily broken when we touch and issuing white liquid filled with polyhedra. (Fig 3 A)	Pupae normal size (± 1.5 to 2 cm), the color of the Pupae from young to old Pupae terraced ranging from light brown to dark brown. When touched, its response is fast (Figure 3B).
4.	Imago	Imago wing curling, sometimes broken and small, when we touch the response is slow, passive and not actively foraging.	The shape and size of a normal imago (± of 1 - 1.5 cm), grayish-brown color with distinctive spots. When attached to the substrate it resembles an isosceles triangle. Fast response when touched. Tend to be nocturnal, actively approaching the feed in the form of liquid honey.



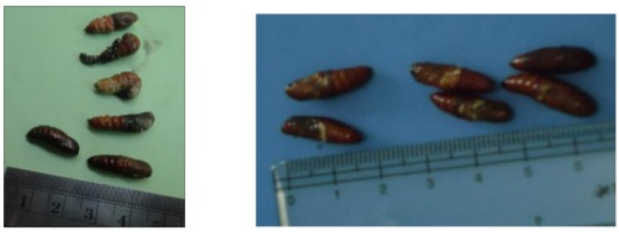
A B C

Figure 1. A third instar Larvae of *S. litura* normal, and B, C third instar Larvae of *S. litura* infected SpltMNPV (abnormal)

Based on Table 2, the changes in morphology and behavior of (healthy) normal *S. litura* Larvae and S. litura Larvae infected with SpltMNPV in vitro (abnormal) can be observed. Healthy *S. litura* Larvae movement is still active, feeds actively, the color of caterpillar is normal, that is cream- brown, dark brown (accordance with the color of the feed)., the color is clear, when it is touched mandible is still moving. Colors on the body of S. litura Larvae is in accordance with the color of the food he ate.

A B

Figure 2. A. PrePupae abnormal because infected SpltMNPV and B. The development of normal uninfected PrePupae SpltMNPV



A B

Figure 3. A. B. Pupae normal abnormal

In this study, the feed used is in the form of artificial feeding (formula of Balitas Malang), cream / yellow colored, so that most of the Larvae color is also cream. The older the instar Larvae, the darker the color of their bodies. Their skin color then fade when the change their skin as a marker of instar stages of change. The color change is due at the time of the skin change, cuticle that has melanin pigment was released. The cuticle layer will be replaced with a new cuticle that has little melanin.

The *S. litura* larvae infected with SpltMNPV in vitro showed changes in morphology and behavior.The changes can be seen in the Larvael body at the beginning of the infection caused by SpltMNPV begin damaging cells it attacked. The initial infection usually begins in the digestive tract. This causes the larvae will not eat, because many cells in the gastrointestinal tract begins to rupture / lysis. This resulted in the production of digestive enzymes are inhibited (eg proteases), metabolism becomes impaired, the energy derived from the metabolism decreases as a result of passive larvae (slow motion).Larvae look began to stop eating, and tends to rise to the end of the bottle as in their natural habitat, that is in the plant, which tend to rise to the top of the plant.The infected Larvae look swollen, especially on the back / dorsal, while it looks redness at the abdominal / ventral.The swelling is caused by the many larvae were infected by lysis so that the cells secrete fluid mixes with hemolimfa. In the abnormal Larvae that died, the color of reddish brown caterpillar can bee seen in the abdomen, and at the back has white colored cream milk or destroyed at the end of the bottle with the liquid that comes out milky white. Soft and when it was touched, it broke and secrete a milky white liquid. Mandible did not move. Caterpillar size is smaller.One characteristic of *S. litura* larvae infected with the virus is dead in a dependent position as the letter "V" inverted with abdominal leg as a footstool. The fluids which broke from the body of dead caterpillars infected SpltMNPV SpltMNPV containing polyhedra which, if left in a few moments, will cause a bad smell. This odor is due to the fermentation of the remnants of larvae metabolites that were out of the lysis cell or because of the degradation of the remnants of lysis cells / tissues / organs.

At normal PrePupae, PrePupae color is still like the 5 instar Larvae, that is dark brown, the length and width of the Larvae shrink nearly half the normal size, PrePupae eat inactively, make a nest by collecting leftover food and feces are made such that the whole body covered.Food and excrement remnants held together with a kind of yarn produced by saliva. When PrePupae is touched, its response is slow, the body tends to curve.

Meanwhile, abnormal PrePupae because of being infected by darker / blackish brown virus, sometimes the color is uneven (half brown and half dark brown), its body segments are irregularly, if it was touched its response was slow, the formation of candidates for the head of imago is not perfect. Some PrePupae failed to shrink his body even enlarged / swollen, diabdomen legs are still visible, diabdomen segments of the middle part shrink, the body is soft and when it was touched it discharged milk chocolate contains a lot of polyhedral.

The normal growth Pupae showed the characteristics of normal size (± 1.5 to 2 cm), the color of the Pupae from young to old Pupae terraced ranging from light brown to dark brown. When it was touched, the response was fast (Figure 3B). Meanwhile, abnormal Pupae has specific characteristics, namely size of the Pupae is smaller than the normal Pupae (<2 cm), Pupae color is uneven (there are several bulkhead colored younger), head of the Pupae enlarged, abdominal shrinking, legs are still visible, abdomen bulkhead retracts but not hardened, dark brown color tends to black, curved head, some of its abdomen / head was soft and easily broken when it was touched and issued white liquid filled with polyhedra. (Figure 3 A).

In the imago stage formed after 8 days old Pupae, morphology imago. The shape and size of imago is normal (± of 1 - 1.5 cm), its color is grayish-brown with distinctive spots. When attached to the substrate, it resembles an isosceles triangle. It has fast response when was touched. Tend to be nocturnal, actively approaching the feed in the form of liquid honey. Conversely, abnormal imago has characteristics of curly wings, sometimes broken and small, when it is touch the response is slow, passive and not actively foraging.

Conclusion

Based on the data above it can be seen that SpltMNPV propagated on epithelial cells of *S. litura* Larvae, and had been maintaining in the storage at -80 ° C for 20 months caused the mortality of third instar S. litura Larvae highest achieved at doses SpltMNPV 5.95 x 108 PIBs / ml by 44 %, the next row at a dose of 5.95 x 107 PIBs / ml and 5.95 x 106 PIBs / ml with a mortality of 40% and 38%. The mortality was observed for 17 days after treatment Mortalitas tersebut diamati 17 hari setelah perlakuan. Stadia *S. litura* in the forms of Larvae, PrePupae, and Pupae or imago which were infected with SpltMNPV showed the characteristics of specific abnormalities that are

different from normal.The percentage of abnormal imago decreased is equivalent with the decrease of SpltMNPV concentrations given.

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Refferences

[1] Asri, M.T, 2013" Mechanism of infection and the effectiveness of *Spodoptera litura* Nuclear Polyhedrosis Virus (SpltMNPV) In Vitro In-protectant Photo Formula Against Mortality of Larvae of Spodoptera litura," unpublished.

[2] Filayani. M.I. 2013, "The increase in pathogenicity Latent Virus Isolation Results From Cell Culture *Spodopera litura* Larvae midgut against Larvae of *Spodoptera litura* Through Recurring penginfeksian," unpublished.

[3] Huffaker and Messenger, 1989 "Theory and Practice of Biological Control," Jakarta: Publisher University of Indonesia,pp 352.

[4] Pramono, 2000 "Effect Against Storage Polyhedrosis Virus Virulence Applied Nuclear Plant Soybean In Esophageal armyworm (*Spodoptera litura),"* Fabr. J. Plant Pests and Diseases of Tropical Vol. 1, No. 1: 29-32 (2000) ISSN 1411-7525

[5] Mangoendihardjo, S., Wagiman, and Wijonarko, Arman, 1992 "Use of Nuclear Polyhedrosis Virus (NPV) in Control of armyworm (*Spodoptera litura* Fabr.) And eaters Flowers and pods (Helicoverpa armigera HBN.) In Soybean", Final Report of the IPM Research Support in the Framework of Implementation of the National IPM Program,Cooperation projects between the Faculty of Agriculture UGM with Bappenas,p. 28

[6] Suwandi. I, 2007 "Effect of Sunlight and Virulence Against Nuclear Storage Time Polyhedrosis Virus (NPV) In Hiposidra Talaka (Walk) (Lepidoptera: Geometridae).unpublished.

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