THE EFFECT OF HaNPV FREQUENCY APPLICATION TOWARD PROTECTION OF TOMATOES (SOLANUM LYCOPERSICUM) FROM INSECTS LARVA ORDO LEPIDOPTERA ATTACK

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ABSTRACT

Insects of the order Lepidoptera pests are extremely destructive and cause substantial losses to the agricultural business. A common method used to control insect pests is to use synthetic insecticides. But they can cause harmful effects on the environment, such as the emergence of resistance and resurgence of insect pests, killing of non-target organisms, environmental pollution and human health problems due to residues of synthetic insecticides on the crop. Through his research, ITB has developed a virus-based pesticide for controlling pest insects of the order Lepidoptera. Various studies in the laboratory showed that isolates HaNPV ITB effectively control various species of insects of the order Lepidoptera and non pathogenic to insect species from other orders. This research studied the effectiveness of pesticides in the field to determine the effect of the applied frequency HaNPV tomato plant protection from insect larvae of the order Lepidoptera. The experiment was carried out with one factor, namely the frequency of applications of HaNPV with 5 experimental. The experiment was carried out by applying a completely Randomized design (CRD) and 50 repititions. Parameters observed included insect infestation pests of the order Lepidoptera dan insect infestations of pests other than order Lepidoptera. The results of this study indicate that HaNPV used as an effective insecticide to control insect pests of the order Lepidoptera. Tomato plants treated with synthetic insecticides showed infestation of insects of the order Lepidoptera with 0.0000 level individuals who are not significantly different from infestations of pests on tomato plants are treated HaNPV, with pest infestations its value each individual is 0.0025, 0.0012 individuals and 0.0018 individuals. The results also showed that the use of HaNPV was not effective in protecting tomatoes planst from insect pests other than *Lepidoptera*, with the level of insect infestation in the treatment $p^2 = 8.6$ individual while the plants applied to HaNPV on plants p3 = 11.6 individual, p4=12,14 individual and p5 = 12,16 individual, these results indicate that tomato plants with the application of synthetic insecticides are more effective in controlling insect infestations ordo than Lepidoptera.

Keywords: HaNPV, Bioinsektisida, Lepidoptera, PHT

INTRODUCTION

The purpose of study was to determine the effect of application frequency on the ability of HaNPV to protect tomato plants from pests of the ordo Lepidoptera. Many studies have been carried out on a laboratory scale and the result show that HaNPV is effective in controlling Lepidoptera. However, the effectiveness of HaNPV in controlling insect pests from the Lepidoptera has not been tested in the field, for this reason researchers want to test the effectiveness of this HaNPV on a field scale. Insects of the order *Lepidoptera* are very destructive pests. The world spent more than 40% of insecticides to control *Helicoverpa armigera* which is an insect of the order *Lepidoptera* (Christian, 1994). These insects attack horticultural and industrial crops such as tomatoes, onions, corn, soybeans, potatoes, spinach, cabbage, tobacco and cotton. These insects damage plants by attacking all parts of the plant (leaves, flowers and fruit). The population develops very widely and lives as larvae for quite a long time, followed by a fairly long life cycle (30 days). The long larval stage causes this insect to be uncontrollable, causing enormous damage in line with

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enormous losses for farmers (Kogan et al, 1978; Christian, 1994). To protect crops from insect pests, farmers generally use synthetic insecticides. Observations of farmers in Lembang, West Java showed that tomato farmers sprayed synthetic insecticides once or twice a week from planting to harvest. The use of insecticides has resulted in the very intensive components of synthetic insecticides becoming dominant in agricultural activities. Sastrosiswojo (1993) stated that the cost of purchasing synthetic insecticides has had a negative impact, namely causing resistance and resurgence of insect pests, environmental pollution, killing non-target organisms and human health problems due to residues on plants. To reduce the negative impact of using synthetic insecticides, an integrated pest control (IPM) method was developed, using various methods and agents to control insect pest populations. One component of IPM is the use of biological insecticides and biological insecticides that have high potential to be developed as insect pest control are Nuclear polyhedrosis virus (NVP).

NPV Nuclear Polyhedrosis Viruses are rod-shaped and contained in inclusion bodies called polyhedra. Polyhedra are multi-faceted crystals and are found in the nucleus of susceptible cells of the insect host, such as hemolymph, fat bodies, hypodermis, and tracheal matrix. Polyhedra are 0.5-15 m in size and contain viral particles called virions. Virions are rod-shaped, measuring 40-70 nm X 250-400 nm, and contain a nucleocapsid containing a deoxy-ribonucleic acid (DNA) molecule. Virions containing one nucleocapsid are called singly-enveloped NPVs, while those containing multiple nucleocapsids are called multiply-enveloped NPVs (Payne and Kelly 1981). The morphology of polyhedra and virions can be seen under the electron microscope by negative staining or by the technique of slices of NPV-infected tissue.

NPV infects many insects and each species has a specific species. NPV infects more than 500 species, *Lepidoptera* is an important host of NPV. The infective particles of this virus or virion can be enveloped by a single SNPV or multiple MNPVs. The polyhedra of the NPV contain several to many virions. After being ingested by the host and will reproduce in the midgut cells, or other tissues and organs of the insect become infected, especially the fat body, epidermis and blood cells. Infected insects will generally die after 5-12 days after infection depending on the virus dose, temperature and larval instar stage at the time of infection. As with fungal attacks, behaviors such as summit diseases occur in insects that are attacked by NPV. Insects that are about to die will climb onto the plants where they died and Millions of polyhedra contained in the body fluids of dead and broken insects will fall to the bottom in the feeding zone (leaves, leaf debris) which may be eaten by other healthy caterpillars.

LITERATURE REVIEW

Compared with synthetic insecticides, NPV has many advantages. Insect viruses are specific to target insects, so they are safe for non-target insects and are not pathogenic for non-insect species (Laoh, et al, 2003; Setiawan et al, 2004; Bedjo, 2005). Several studies on *Helicoverva amigera* Nuclear polyhedrosis HaNPV virus that have been conducted at ITB have shown that on a laboratory scale it is effective in controlling this virus, *Helicoverpa armigera* and other insects of the order *Lepidoptera*. Several studie on ITB is Utilization of green mustard as a medium for integrated pest control agents *Spodoptera exigua* (Hariani, 2001), *Helicoverpa ramigera* exposed to HaNPV repeatedly at a dose of 800 pib was able to develop tolerance, based on food consumption and larval growth (Sanjaya, 2004), HaNPV resulted in 100% death of 3rd instar larva of *Spodoptera litura* at a dose of 8x10³ (Wahyuni, 2007),. Miranti, (2002) suggested that at a dose of 6x105 PIB/larvae, HaNPV resulted in 100% mortality in the third instar, 70% in the fourth instar, and 70% in the fifth instar. Next Br. Simanjuntak (2007) found that HaNPV was also pathogenic to *Spodoptera litura* and as observed in *Helicoverpa armigera*, mortality of *Spodoptera litura* due to HaNPV infection increased with increasing age of young larvae. Subsequent studies have shown that HaNPV is effective in controlling insects of the order *Lepidoptera* such as *Spodoptera litura, Spodoptera*

exigua, Helicoverpa, sea, and *Crocidolomia binotalis* (Miranti, 2002; Indrayani 2006, Simanjuntak, 2007; Wahyuni, 2007; Wulandary, 2008;). In addition, unlike synthetic insecticides which can cause insect pest resistance, the use of HaNPV does not cause insects to develop tolerance to this viral infection (Sanjaya, 2004). However, the effectiveness of controlling HaNPV insects from populations of the order *Lepidoptera* has not been tested in the field.

Real conditions in the field, there are several factors that can reduce or eliminate the effectiveness of HaNPV as a biological insecticide, such as UV rays in the sun can inactivate the virus or leaching due to rain. Based on this, we want to know how the application of NPV spraying frequency affects the protection of tomato plants against insects of the order *Lepidoptera*.

METHODS

Research material

Spodoptera litura

This insect is used to reproduce HaNPV because *Spodoptera litura* is sensitive to HaNPV and large in size, making it ideal for use as a medium for producing HaNPV in vivo (Miranti. 2008; Indriany. 1994; Miranti et al., 2006;). The *Spodoptera litura* used were collected directly from the tomato plantation area in Cibogo Village, Lembang, Bandung Regency, and in dayah Muara Garot, Indrajaya, Pidie Sigli, Aceh.

Insect virus (NPV)

The insect virus used in this study was *Helicoverpa armigera* Nuclear Polyhedrosis Virus (HaNPV). This virus is a natural pathogen of H. armigera, the collection of the Research Center for Bioscience and Biotechnology, ITB.

Experimental design

The experiment was carried out with one factor, namely the frequency of application of HaNPV with 5 experimental levels, namely,

P1 = treatment was not given synthetic insecticides and HaNPV (negative control),

- P2 = Treatment with synthetic insecticides (positive control),
- P3 = Treatment with HaNPV twice a week,
- P4 = treatment with HaNPV once a week,
- P5 = one month of treatment with HaNPV.

Virus applications was carried out on the underside of the leaves with the assumption that the HaNPV virus remained active in the field. With the given HaNPV concentration of 4x103 pib/ml, the volume of 50ml/tree is equivalent to 2x105 pib/tree.

The experiment was carried out by applying a Completely Randomized Design (CRD) and each treatment was repeated 50 times.

Parameter

1. Insect Infestation Pests of the order Lepidoptera

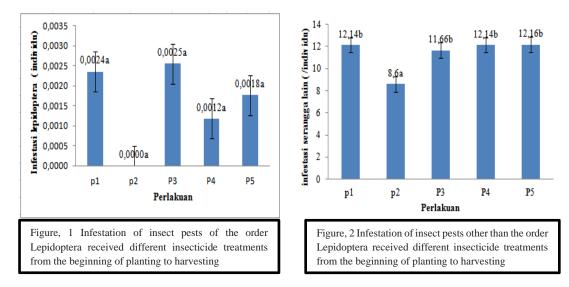
The collection of infected insects of the order *Lepidoptera* was carried out 2 times a week on all treatment plots, observations of insect pests of the order *Lepidoptera* were carried out until harvest time.

2. Insect Infestation Pests other than Order Lepidoptera

Infected insect pests were taken 2 times a week on all treatment plots, and each plant was observed for insect pests that came. Furthermore, insect pests that have been observed are grouped into insect pests of the order *Diptera, Coleoptera, Hymenoptera* and *Hemiptera*. Statistic analysis

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Data analysis with analysis of variance (ANOVA) and DUNCAN test if the significance value (p) < 0.05



RESULTS AND DISCUSSION

1. Insect Infestation Pests of the order Lepidoptera

The results of this study indicate that the use of HaNPV is effective in controlling the infestation of insect pests of the order *Lepidoptera* on tomato plants. Tomato plants treated with synthetic insecticides showed an average insect infestation of the order *Lepidoptera* with an individual level of 0.0000. Pest infestations were not significantly different from pest infestations on tomato plants treated with HaNPV twice a week, once a week, and once a month, the pest attack values for each individual were 0.0025, 0.0012 and 0.0018 individuals. It is proven that the application of HaNPV is as effective as synthetic insecticides, which is capable of controlling insect pests of the order *Lepidoptera*.

Insect pest infestation on tomato plants without synthetic insecticides or bioinsecticides (P1) was not significantly different from tomato plants that received synthetic insecticides (P2). The treatments gave the same results because (1) insect viruses are very easy to spread in the population and can be persistent in the long term (Sutarya, 1995; Indrayani and Winarno 1997; Sanjaya, 2004). (2) the plant plot is close between the treatment plot and the control plot p1. (3) The virus spreads through infected insects, the process of virus infection in the infected insect's body, this is supported by Miranti (2008) research which states that insect death due to NPV takes place slow when compared to deaths due to the use of synthetic insecticides.

This is because HaNPV takes time to replicate in the insect's body through a long infection process before causing death in the larvae. (4) Insects die due to viruses in control plants p1 The transfer of insect pests from p3, p4, p5 to plants p1 will result in p1 plants being protected from Lepidoptera. This is evidenced by the presence of dead Lepidoptera found in p1 plants. It is possible that the larvae that died were due to exposure to viruses in treatment plants as it was known that the application of the virus to insects could result in the death of the larvae within 4 to 3 weeks in the field (Bedjo, 1995; Miranti, 2002). the dead part of the body is torn apart, it is gray in color and emits a characteristic odor and liquid. Based on this, the P1 control plant could not be used as a negative control.

The application of HaNPV to tomato plants P3, P4 and P5 also did not show a significant difference, this proves that the frequency of application of HaNPV once a month (p5) is still effective in protecting tomato

plants from insect pests of the order *Lepidoptera* because HaNPV remains active. Application on the underside of the leaves was able to protect HaNPV to remain active for 1 month because it was protected from ultraviolet light which could inactivate HaNPV (Maraniorosch and Sherman, 1985). So it can be stated that the application of spraying under the leaves can protect the inactivation of HaNPV by ultraviolet light.

2. Insect Infestation Pests other than Order Lepidoptera

The Research also shows that the use of HaNPV is not effective in controlling insect pests except the ordo *Lepidoptera* (figure 2). Insect investations other than the order *Lepidoptera* on tomatos plants (Fig. 2) with the level of insect investation in the group p2 = 8.6/individual while the plants that were applied to HaNPV on plants p3=11.66/individual P4= 12.14/individual and P5= 12,16/ individual, these results indicate that tomato plants with the application of synthetic insecticides are more effective in controlling insect investations than *Lepidoptera*. Synthetic insecticides are able to control various kinds of pests including the orders *Diptera*, *Coleoptera*, *Hymenoptera*, and *Hemiptera* with the number of each insect pest being 150, 414, 53, and 1,679 pests.

Tomato plants with the application of HaNPV did not give effective results because the HaNPV used was specific to the order *Lepidoptera* but not specific to orders other than *Lepidoptera*. This statement is supported by research (Indriany, 1994; Miranti et al, 2008).

Specific NPV because when the NPV attacks the target insect, insect species that are sensitive to HaNPV infection are thought to have the same or similar receptors on *H. armigera* cells, the natural host of HaNPV, allowing the HaNPV virion to interact with the receptors of the insect's body cells, thus initiating infection at the stage of infection. mobile. (Miranti, 2008).

the results of insect pest infestations of *Lepidoptera*, it shows that the frequency of application of HaNPV gives effective results like synthetic insecticides, while for insect infestations other than the order *Lepidoptera*, the frequency of application of HaNPV does not give effective results like synthetic insecticides and it can be stated that HaNPV cannot be used as the only insecticide to control insect pests because HaNPV is pathogen-specific for *Lepidoptera*.

CONCLUSION

NPV has as weakness, which is easily degraded by sunlight, especially ultraviolet light. Exposure to ultraviolet light causes NPV to not survive for a long time in the field, only a few hours after being sprayed on plants because NPV is very sensitive to high temperature because it can reduce effectiveness. So to keep HaNPV in the field, the frequency of application is very important. For the development of further research, it is very important to educate farmers on the use of HaNPV, especially those in aceh, so that they can use HaNPV as a bioinsecticide that can make themselves and can use for a long time.

The application of HaNPV against infestations of the order *Lepidoptera* on tomato plants showed effective results such as synthetic insecticides, and the application of HaNPV was not effective in controlling pests other than the order *Lepidoptera* because HaNPV was specific to the order *Lepidoptera*. HaNPV is ineffective or unable to cause the death of non-Lepidoptera insects due to mismatches between ligands or receptors such as receptors on *Helicoverpa armigera* cells so that each HaNPV person cannot interact with the cell receptors of the non-*Lepidoptera* insect body and cannot infect the cellular stage.

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REFERENCES

- 1. Beckage, N. E., S. N. Thompson dan B. A. Federici. (1993): *Parasites and Pathogens of Insect*. Volume 2 : Pathogens. California : Academic Press, inc
- 2. Bedjo. (2005): Potensi, peluang, dan tantangan pemanfaatan *spodoptera litura* Nuclear polyhedrosis virus (*Sl*NPV) untuk pengendalian *Spodoptera litura* Fabricus pada tanaman kedelai
- 3. Choden, K. (2012): Evaluation and production of improved formulation of nucleopolyhedrosis virus of *Spodoptera litura*
- Christian, P. (1994): Recombinant Baculovirus Insecticides Catalysts for a Change of Heart. <u>Proceedings of the 1st Brisbane Symposium. Biopesticides : Opportunities for Australian Industry.</u> CJ. Monsour, S. Reid, and R.E. Teakle (eds.). June, 9-10 1994. Brisbane 40-50..
- 5. Indriany, L. (2006): Kajian tentang produksi *H. armigera* nuclear polyhedrosis virus (*HaNPV*) pada *Spodoptera litura* fabricius: pengaruh umur larva terhadap tingkat produksi *HaNPV*
- 6. Iqbal, M. (2013): Peningkatan patogenitas virus laten hasil isolasi dari kultur sel *midgut* larva *Spodoptera litura* terhadap larva *Spodoptera litura* melalui penginfeksian berulang
- 7. Miranti, M. (2000): *H. armigera* Nuclear Polyhedrosis virus Field Application : The influence of Concentration of Time of Application on Survival of *H. armigera* in Tomato plants in Green House. Jurusan Biologi, FMIPA, Universitas Padjadjaran
- 8. Miranti, M., dan W. Niloperbowo. Pengaruh dosis *H. armigera* Nuclear polyhedrosis Virus (*HaNPV*) terhadap konsumsi makan, berat badan dan berat pupa larva *H. armigera* (hubner)
- 9. Miranti, M., dan W. Niloperbowo. In vivo of *H. armigera* Nuclear Polyhedrosis Virus (*HaNPV*) in *Spodoptera litura* : The effect of viral serial passage in *S.litura* on production and Pathogenicity of the virus to *H.armigera*.
- 10. Miranti, M. (2008): Produksi *H. armigera* nuclear polyhedrosis virus (*HaNPV*) secara *in vivo* pada inang pengganti
- 11. Sartika. A.R. (2013): Virulensi nuclear polyhedrosis virus (NPV) terhadap ulat grayak (*Spodoptera litura* F.) (Lepidoptera : noctuidae) pada tanaman tembakau deli di rumah kaca
- 12. Sanjaya. Y, W. Niloperbowo., dan T. Anggraeni. (2004): konsumsi makan dan pertumbuhan larva *H. armigera* toleran terhadap pemaparan *H. armigera* Nuclear polyhedrosis virus (HaNPV)
- 13. Sastrosiswojo, S. (1993): Biological Control of The Diamondback Moth Under Indonesia's National IPM Program.In : <u>Proceedings International Symposium on the "Use of Biological control</u> <u>Agents Under integrated Pest Management"</u>. Fukuoka Japan. Oktober 4-10, 1993. 302-312
- 14. Simanjuntak, E. C. Br. (2007): Kepekaam larva *Spodoptera litura* fabricius terhadap infeksi *H. armigera* nuclear polyhedrosis virus (*HaNPV*).
- 15. Sutarya, R. (1995): Pengaruh konsentrasi Nuclear polyhedrosis Virus terhadap kematian ulat buah tomat (Helicoverpa armigera hubn).
- 16. Vehuliza. A. (2013): The biologicaltest of formulation of subculture *H. armigera* nuclear polyhedrosis virus (*HaNPV*) against *Crocidolomia pavonana* Fab. Larvae populationthat exposed to cabbage (*Brassica oleracea* var. capitata L.)
- 17. Wulandari. A. (2015): Pengaruh beberapa konsentrasi *Spodoptera litura Nuclear Polyhedrosis Virus (SI*NPV) JTM97C terhadap mortalitas *Helicoverpa armigera* Hubner (Lepidoptera : Noctuidae) pada tanaman kedelai
- 18. Wiwin setawati. (2004): pemanfaatan musuh alami dalam pengendalian hayati hama pada tanaman sayuran Lembang Bandung 40391
- 19. Wahyuni, W. (2007): Pengaruh dosis *H. armigera* nuclear polyhedrosis virus (*HaNPV*) terhadap produksi *HaNPV* pada *spodoptera litura* fabricius

20. Wulandary, I. (2008): Patogenitas *H. armigera* Nuclear Polyhedrosis virus (*HaNPV*) hasil subkultur pada *Spodoptera litura* Fabricius terhadap larva *H. armigera* Hubner dan *Spodoptera litura* fabricius.