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Field efficacy of nucleopolyhedrovirus against armyworm, *Spodoptera litura* F. on tobacco

(Keberkesanan nukleopolihedrovirus mengawal serangan *Spodoptera litura* F. di kawasan tembakau)

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Key words: *Spodoptera litura*, nucleopolyhedrosis, virus, tobacco, polyhedral inclusion body, field experiment

Abstrak

Keberkesanan *Spodoptera litura* nukleopolihedrovirus jenis tempatan untuk mengawal serangan ulat seribu pada pokok tembakau di ladang telah dibandingkan dengan racun serangga yang disyorkan (α-Cyphermethrin). Virus ini tidak bertindak setanding dengan racun-serangga yang disyorkan dan tidak dapat mengurangkan bilangan ulat seribu dengan ketara. Walau bagaimanapun, virus ini dapat mengurangkan peratus kerosakan daun tembakau akibat serangan ulat seribu. Pada tahun 1994, petak yang disembur dengan virus, campuran virus dengan racun serangga yang disyorkan dan petak tanpa rawatan masing-masing mencatat kerosakan daun tembakau sebanyak 23.10, 28.50 dan 37.10%. Namun begitu, pada tahun 1995, petak yang disembur dengan virus tidak dapat mengurangkan peratus kerosakan secara ketara. Perbezaan keputusan antara tahun juga terdapat pada berat daun hijau yang dikutip. Pada tahun 1994, petak yang disembur dengan campuran virus (SpltNPV) dan racun serangga yang disyorkan memperoleh hasil rendah yang ketara jika dibandingkan dengan hasil daripada petak tanpa rawatan tetapi hasil tidak berbeza dengan perlakuan lain. Namun begitu, hasil yang diperoleh pada tahun 1995 tidak ketara berbeza. Perbezaan pada corak cuaca antara tahun 1994 dengan 1995 berkemungkinan menjadi faktor utama kepada perbezaan keputusan pada tahun-tahun tersebut.

Abstract

The efficacy of an indigenous *Spodoptera litura* nucleopolyhedrovirus strain for controlling field armyworm infestation was evaluated against a recommended insecticide (α-Cyphermethrin). The virus did not act as effectively as insecticide. It did not significantly reduce armyworm larval counts. However, it could reduce tobacco leaves damage due to armyworm infestation. In 1994, plots that were sprayed with virus, mixture of virus and insecticide, and untreated plots resulted in 23.10, 28.50 and 37.10% leaf damage due to armyworm infestation, respectively. In 1995 on the other hand, weekly spraying with virus did not reduce the percentage of damage significantly. The varying results between years were also observed in fresh weight of harvested tobacco leaves. In 1994 plots that were treated with a mixture of SpltNPV and insecticide had significantly

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lower yield than untreated plots but not significantly different to other treatments. However, no significant difference in the yield was recorded in 1995. The differences in the weather pattern between years might contribute to the differences in the outcome of the experiment between the years.

Introduction

The armyworm, *Spodoptera litura* F. is a serious pest of important crops in Malaysia and Southeast Asia (Md. Jusoh and Lim 1981; Laviña et al. 2001). Uncontrolled infestation can result in total yield loss due to larval defoliation. In Malaysia, the voracious *S. litura* larvae which undergo five instars (Md. Jusoh and Lim 1981) are being managed through the use of many kinds of chemical insecticide. However, environmental and health problems associated with chemical insecticides have stimulated efforts to search for alternative control measures which include the use of insect virus.

Viruses, particularly those belonging to the Baculoviridae family, are among the most promising biological insecticides (Thompson et al. 1981; McKinley et al. 1989). These viruses occur naturally in insect population and are well known to cause high mortality on their host insects, particularly lepidopteran pests (Bilimoria 1991). One of these viruses is the *Spodoptera litura* nucleopolyhedrovirus (SpltNPV). It is very pathogenic to *S. litura* larvae but is not detrimental to the generalist predator, *Sycanus leucomesus* (Sajap et al. 1999; Sajap et al. 2000). SpltNPV is characterized by a polyhedral inclusion body (PIB) containing double stranded viral DNA. The inclusion body enables the virus to survive outside its host for a long period (Thompson et al. 1981). The virus infects its host when susceptible larvae consume polyhedra on contaminated foliage. The polyhedra dissolve in alkaline midgut of the larva, thereby releasing infectious virions. The infected host larvae succumb within several days, thus releasing virus particles into the environment shortly after the death of the host.

The virus has been successfully used for controlling field populations of armyworm in Taiwan, China, India and Egypt (Jones et al. 1994; Kao 1996). However, in Malaysia, there is no record on its efficacy against armyworm. This paper reports the results of field experiments to evaluate the potential of using SpltNPV as biological insecticide to control armyworm.

Materials and methods

Virus culture

The SpltNPV used in this study was originally obtained from infected armyworm larvae collected in a tobacco field (Mohd. Norowi and Jamiah 1996). The virus was then cultured on laboratory-reared armyworm. The virus was propagated through the infection of third instar armyworm which were fed with SpltNPV-contaminated diet. Virus-infected larvae were kept in a freezer (–5 °C) until use. An aqueous suspension of SpltNPV at the dosage of 6.0×10^8 PIB per litre was used. This dosage was based on the previous bioassay work reported by Sajap et al. (2000).

Field experiment

Experiments were conducted at Semerak Pasir Puteh, Kelantan, in 1994 and 1995 tobacco seasons. Recommended agronomic practices for flue-cured tobacco production (Musa et al. 1989) were followed with no insecticide application, except when determined by the experimental treatment. In both years, an area of 16 m x 80 m was selected, and divided into 16 experimental plots. Each plot consisted of five rows of 32 tobacco plants. The three middle rows were considered treatment rows and these were guarded on each side by an untreated row. Tobacco seedlings were transplanted on

20 January 1994, topping (removal of terminal buds when tobacco plant is flowering) was done in the third week of March and the first harvesting was conducted on 23 March 1994. There were four harvesting dates in 1994. In 1995 season, tobacco seedlings were transplanted on 25 January 1995, topping was also done in the third week of March and the first harvesting was carried out on 18 March 1995. There were five harvesting dates in 1995.

The experiment was designed as a randomized complete block with four treatments as follows:

1. Weekly sprays with water (untreated),
2. Weekly sprays with α-Cyphermethrin at the rate of 0.0033 litre per litre of water,
3. Weekly sprays with SpltNPV at the rate of 6.0 X 10^8 PIB per litre of water, and
4. Mixed application of SpltNPV (at the rate of 6.0 X 10^8 PIB per litre of water) with a recommended insecticide (as treatment 2) on the 6th and 9th week after transplanting.

The 6th and 9th week after transplanting are the peak armyworm population on tobacco as shown by a simulation study conducted by Mohd. Norowi (1994).

In 1994, the experiment was carried out with four replicates in each treatment, but in 1995 only three replications were considered because some of the tobacco plots in one of the replications were flooded. For each treatment, the plants were sprayed until run-off using a knapsack sprayer with solid cone nozzle.

Data collection

Larval count The number of armyworm larvae was counted weekly commencing from the third week after transplanting, and one or two days before the application of the various treatments. Larval census was carried out in the morning on 10 fix-sampled plants in each treatment row. Each armyworm was classified into four classes based on the larval stages, viz. class one for the first and second instars, class two for the third instar, class three for the fourth instar and class four for the fifth instar. The size of the larvae was the main criterion used to differentiate each instar.

Tobacco yield and damage To estimate the damage caused by the armyworm, 5 and 10 tobacco leaves per plot for 1994 and 1995, respectively, were randomly selected from the treatment rows. Tobacco leaves were harvested when they began to ripen. Harvested leaves were immediately weighed to determine the yield. The harvested leaves were then individually assessed to estimate the percentage of defoliation due to the armyworm.

Data analysis Larval count in each class, percentage of leaf damage due to armyworm defoliation and fresh weight of leaves were analysed using ANOVA. For larval count *(C)*, the total number in each plot was first transformed to $\log_{10}(C+1)$. ANOVA was carried out on the means of several sampling dates using the transformed values. An ANOVA was also performed on the mean percentage of leaf damage per plant. In the case of total fresh weight, ANOVA was carried out on the mean total weight per plant. Data collected in 1994 and 1995 were analysed separately. A *t*-test was also carried out to compare the means of percentage of damages and yields of 1994 and 1995. In addition, a multiple regression analysis was also carried out to quantify the larval stage that contributed significantly to the damage of tobacco leaves.

Weather data

The average mean of maximum temperature and total rainfall in the months of the tobacco season were obtained from a weather station located at MARDI Research Station at Jeram Pasu, which is located about 15 km from the experimental area.

Results

Armyworm larval population

Figure 1 shows the fluctuation of early stages (class one) of armyworm population on tobacco for each treatment in 1994 and 1995. Although the time of recruitment of armyworm into tobacco field and its initial larval counts were almost similar in both years, its population development for the rest of the season was different. This suggests that the pattern of armyworm survivorship to adult in 1994 was different from that of 1995. There were three peaks of armyworm counts in 1994 that occurred at about 30, 50 and 78 days after transplanting. However, there were only two peaks of armyworm counts in 1995 that occurred at about 30 and 50 days after transplanting. According to Mohd. Norowi (1994), each peak of armyworm counts might indicate the generation of armyworm on tobacco.

Efficacy of virus to control armyworm infestation

Larval count The summary of ANOVA of the armyworm larval count is shown in *Table 1*. As expected, in both years plots that were treated weekly with a recommended insecticide had a significantly ($p \leq 0.05$) reduced armyworm larval counts when compared with other treatments. The larval counts in the plots that were treated with virus varied between years. In 1994, no significant difference ($p \leq 0.05$) in the armyworm larval counts was recorded between the virus treated plots and the untreated plots except for the fourth larval class. In this class, the larval counts were reduced to about 8% and 36%, respectively, for plots treated with a mixture of SpltNPV and insecticide and plots sprayed weekly with SpltNPV only.

In 1995, there was no significant difference in larval counts from the untreated and the virus-treated plots. In fact, the virus-treated plots consistently had higher larval counts. Selective application of a mixture of insecticide and SpltNPV was able to reduce armyworm population as effectively as the weekly SpltNPV spray but not as effective as the recommended insecticidal spray. In addition, results of multiple regression analysis indicated that only the fifth instar significantly contributed to the feeding damage on tobacco (for 1994, $p < 0.001$, $r^2 = 56.3$; for 1995, $p = 0.035$, $r^2 = 24.4$). The other classes of instars did not significantly affect the damage ratings as shown in the changes of r^2 values.

Percentage of tobacco leaf damage due to armyworm All treatments significantly influenced the average mean damage of individual harvested leaf *(Table 2)*. Again, plots treated with insecticide had significantly ($p \leq 0.05$) lowest percentage of armyworm damage. In 1994, the damage recorded in plots treated with SpltNPV was significantly lower than that of the untreated plots. The recorded damage ratings were 23.10, 28.50 and 37.10% in the weekly SpltNPV spraying, selective spraying of a mixture of SpltNPV and insecticide, and control plots, respectively.

On the other hand, in 1995, although the insecticide treated plot had significant lower damage percentage, but weekly SpltNPV spraying did not reduce the percentage of damage significantly. However, the selective of a mixture SpltNPV and insecticide spray resulted in significantly lower damage as compared to that of control plots. A different result between years was also observed for fresh weight of harvested tobacco leaves *(Table 2)*.

In 1994, plots treated with a mixture of SpltNPV and insecticide had 472 g per plant of harvested green leaves, significantly ($p \leq 0.05$) lower than the untreated plots but not significantly different to other treatments. In 1995, there was no significant difference in the tobacco yields among the treatments.

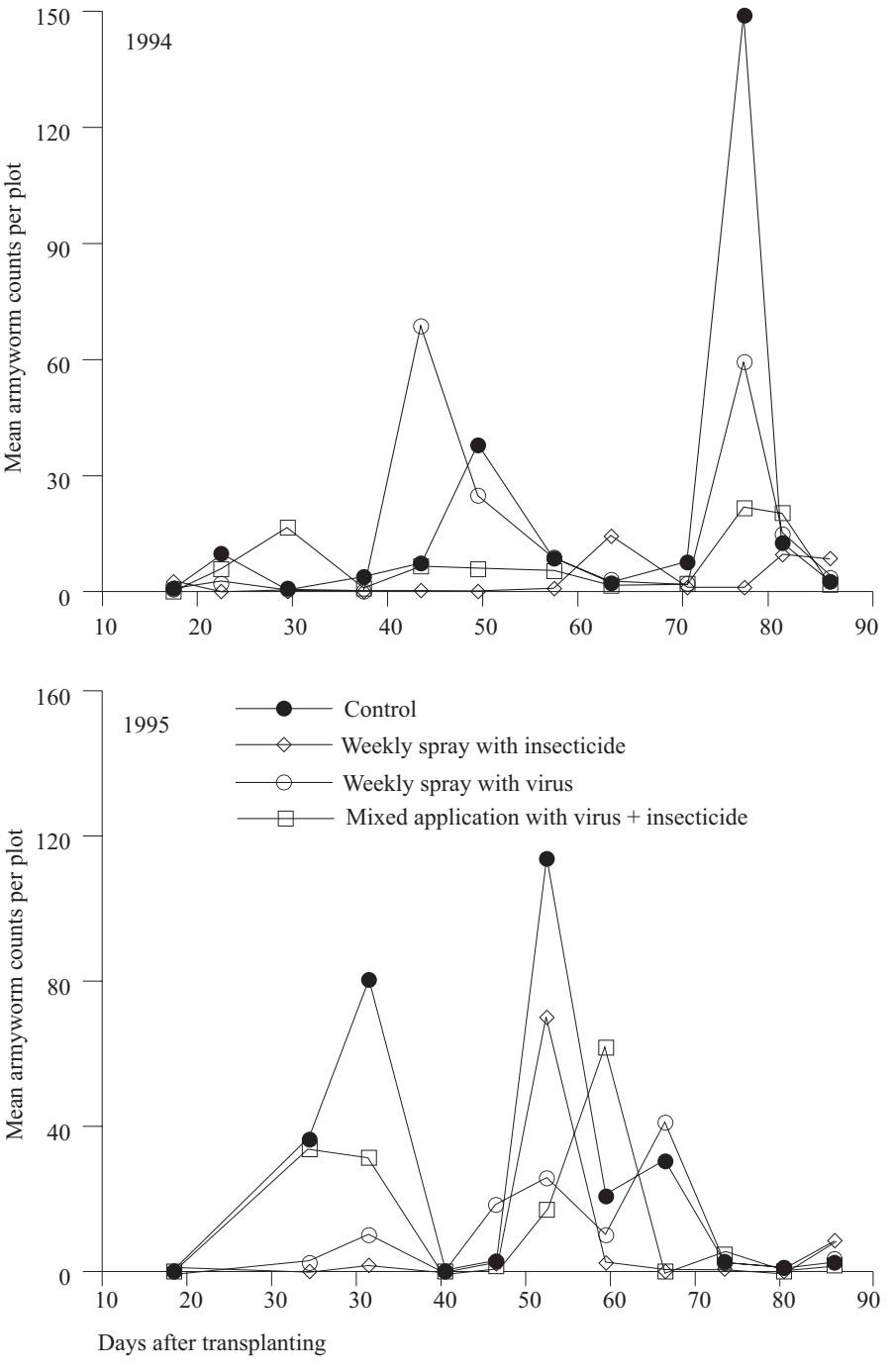

Figure 1. Seasonal abundance of early stages (class one) armyworm larvae in each treatment for 1994 and 1995

Table 1. Effects of treatment on armyworm larval counts on four classes of larval stages (1994–1995)

	1994				1995			
	1	2	3	4	1	2	3	4
Weekly spray with water	0.732a	0.359a	0.315a	0.390a	0.68a	0.141a	0.040b	0.020ab
Weekly spray with insecticide	0.285b	0.111b	0.138b	0.112c	0.275b	0.010b	0.010b	0.000b
Weekly spray with SpltNPV	0.556a	0.329a	0.265a	0.284b	0.588a	0.141a	0.132a	0.068a
Insecticide + SpltNPV applied on 6th and 9th week after transplanting	0.561a	0.263a	0.288a	0.369ab	0.502ab	0.105ab	0.059ab	0.030ab
Variance ratio	6.63	8.828	5.94	16.86	3.87	3.19	2.66	2.18
F-Probability	<0.001	<0.001	<0.001	<0.001	0.012	0.028	0.053	0.096
LSD	0.201	0.107	0.090	0.086	0.250	0.098	0.089	0.054

The values were logarithmically transformed to $\log_{10}(C+1)$, where C is total larval count
The means with different letters indicate significantly differences at $p < 0.05$
Armyworm larval classes: 1 = first and second instars, 2 = third instar, 3 = fourth instar, 4 = fifth instar

Effect of weather pattern on tobacco growth

In both years, the tobacco plants were transplanted from mid-January and harvested in mid-March through the end of April. The weather profiles for 1994 and 1995 tobacco seasons were slightly different *(Figure 2)*. In 1994, the weather was relatively hot and dry in January when tobacco seedlings were transplanted. The weather became wet towards the end of the season when tobacco leaves were harvested. In 1995 however, the temperature was slightly low and more rain was recorded in January when tobacco seedlings were transplanted but it was dry during harvesting.

The relatively hot and dry weather conditions occurred during transplanting in 1994 appeared to reduce tobacco growth rate at the early stage of growth. As a result, lower tobacco yield was obtained in 1994. The average total fresh weight of leaves per tobacco plant was 560.5 g in 1994, which is significantly lower than in 1995 (828.6 g) ($t = 5.98$, $p < 0.001$).

Discussion

Although the virus did not significantly reduce armyworm larval counts but it could reduce tobacco leaves damage due to armyworm infestation. Among others, weather influences the efficacy of the treatments and tobacco production system. As indicated by Mohd. Norowi (1994), weather is one of the most important components for tobacco production system. Evidently, weather, especially the amount of rainfall had a crucial impact on the efficacy of each treatment. The amount of rain also has a direct effect on the rate of tobacco growth. According to Wong et al. (1987), monthly rainfall distribution could determine the yield and quality of tobacco. They indicated that for an optimum tobacco production, a slightly wet condition during the early stage of the development is required to stimulate tobacco growth. When tobacco plants reach maturity and their

Table 2. Effect of treatments on leaf damage and fresh weight of tobacco leaves (1994–1995)

	Damage of harvested leaves per plant (%)		Mean yield per plant (g)	
	1994	1995	1994	1995
Weekly spray with water	37.10a	14.91a	622a	788a
Weekly spray with insecticide	8.70c	2.54b	573ab	841a
Weekly spray with SpltNPV	23.10b	13.04a	576ab	835a
Insecticide + SpltNPV applied on 6th and 9th week after transplanting	28.50b	4.71b	472b	851a
Variance ratio	16.11	18.77	3.02	0.28
F-Probability	<0.001	<0.001	0.87	0.84
LSD	8.49	4.01	116.20	185.10

The means with different letters indicate significant differences at $p < 0.05$

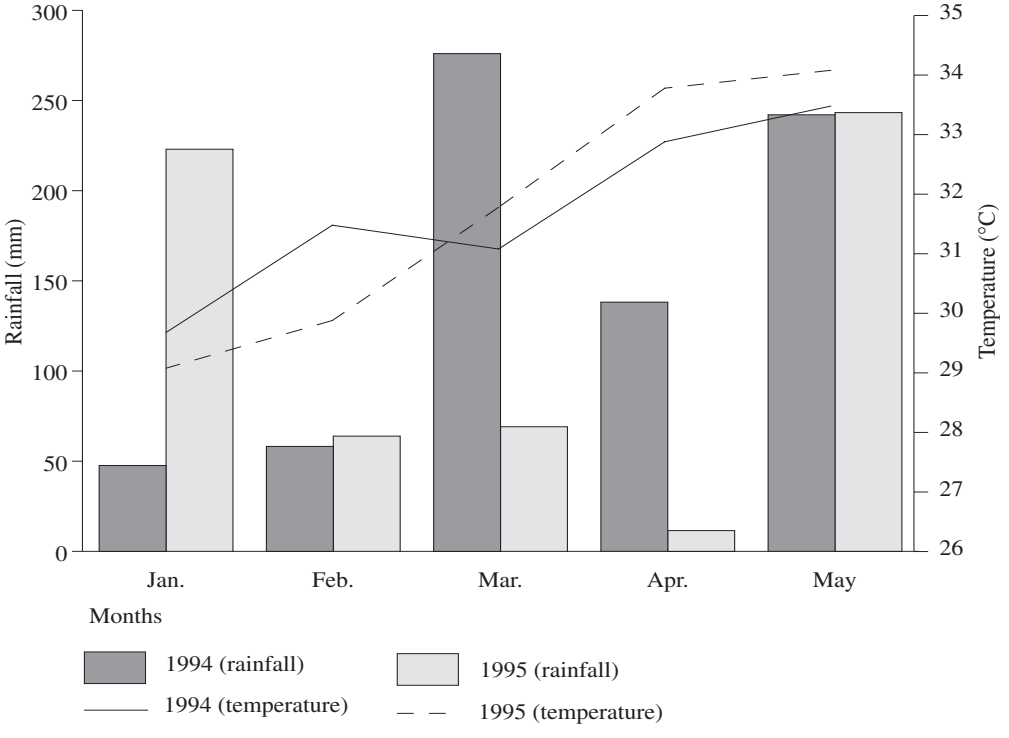

Figure 2. Monthly total rainfall and average daily maximum (°C) temperature during tobacco growth period of 1994 and 1995 recorded at the Jeram Pasu MARDI Research Station (about 15 km from the experimental sites)

leaves are ready to be harvested, a dry period is essential in order to avoid tobacco from absorbing more nutrients, which might affect leaf quality after curing.

The differences in weather pattern for 1994 and 1995 supported the above conclusion *(Figure 2)*. The rainfall distribution in 1994 was not only less suitable for tobacco production but also seemed to favour armyworm population development. Less rainfall in January resulted in dry weather conditions in the early stage of tobacco growth. As a result, tobacco growth was slow during this period that subsequently produced smaller tobacco plants. A slightly higher rainfall in March and April stimulated the plants to grow again. However, by this time tobacco had already been topped and more suckers (lateral buds) were produced. This phenological development might provide a favourable condition for the armyworm females to oviposit their eggs and thus produced its third population peak.

In contrast, in 1995 the weather pattern favoured tobacco growth but suppressed the armyworm population development. A relatively wet condition in the early stages of tobacco growth period resulted in higher rates of tobacco growth. As a result, tobacco plants were bigger. The dry condition in March and April prevented any significant growth of tobacco, especially the lateral buds. This phenological development did not favour armyworm oviposition. Consequently, only two peaks of armyworm counts were detected in 1995.

The difference in the pattern of tobacco growth between 1994 and 1995 seasons had a profound effect on the outcome of the trials. Firstly, it affected the tobacco yield. Smaller tobacco plants produced smaller harvestable leaves that resulted in lower yield. In contrast, bigger plants in 1995 season produced bigger harvestable leaves that result in higher yield. Secondly, it affected the level of armyworm damage. As the tobacco leaves were slightly smaller in 1994 compared with leaves obtained in 1995, the removal of an equal amount of leaf area by the same number of armyworm could result in higher rate of damage in 1994. In addition, the higher growth rate of tobacco in 1995 might increase the ability of tobacco to compensate some of the armyworm damage. Mohd. Norowi et al. (1990) indicated that tobacco could increase the growth of their remaining leaves when some of their leaves were removed. All these together resulted in very high armyworm damage in 1994 compared to 1995 season.

Tobacco growth rate also has a crucial effect on the performance of the treatments, especially the viral treatments. The explosion of armyworm population towards the end of the season in 1994 might neutralize the effect of viral treatment. As armyworm population was higher, more individual armyworm dispersed away from its original plots and subsequently reduced their intra-specific competition (Hassell and May 1990). Consequently, the effect of viral treatment was less obvious, as it requires a long time for the immigrating armyworm to be infected by the virus. In contrast, less armyworm population in 1995 might cause them to remain in their original plots and renders the effect of SpltNPV to be more pronounced in the 1995 trial compared to 1994. The higher means of larval count of the later instars in SpltNPV treated plots was possibly due to the less active infected larvae. Thus, they could be easily observed during sampling time.

The outcome of the experiment could be different if several environmental factors were quantified. For example, temperature and UV light can significantly affect the performance of the virus (McKinley et al. 1989; Lau et al. 2002; Sajap et al. 2002). Likewise the selection of proper nozzle size and shape also affect the performance of nucleopolyhedrosis (Ketunut et al. 2002). It is recommended that these factors shall be considered to ensure an effective use of the virus for controlling the pest in the future. The application of NPV in the late afternoon

might improve the performance of the virus because the temperature is cool and less chance of exposure to UV light.

Conclusion

Spodoptera litura nucleopolyhedrovirus (SpltNPV) could be a potential biological control agent of armyworm. Application of SpltNPV into the field was able to reduce armyworm population and thus reduce tobacco leaves damage. Tobacco plants may compensate the damage causes by armyworm infestation. Consequently, although infected armyworm larvae might cause slight damage to tobacco leaves, they did not significantly reduce the tobacco yield. The virus can be applied with insecticide and applied on selective dates to manage the armyworm infestation on tobacco.

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