BIODIVERSITAS
Volume 18, Number 2, April 2017
Pages: 582-588

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d180219

The potential of various isolates of *Spodoptera litura* Nuclear Polyhedrosis Viruses from East Java (Indonesia) to control *Spodoptera litura* on soybean

BEDJO
Indonesian Legumes and Tuber Crops Research Institute. Jl. Raya Kendalpayak Km 8, PO Box 66, Malang 65101, East Java, Indonesia.
Tel.: +62-41-801468, Fax.: +62-341-801496, ♥email: bj_yulismen@yahoo.co.id

Manuscript received: 24 October 2016. Revision accepted: 9 March 2017.

Abstract. *Bedjo. 2017. The potential of various isolates of* Spodoptera litura *Nuclear Polyhedrosis Viruses from East Java (Indonesia) to control* Spodoptera litura *on soybean. Biodiversitas 18: 582-588.* The study on potential of six isolates of *Sl*NPV which are originally from East Java to control *S. litura* on soybean was conducted at the Entomology Laboratory, Plant Protection Department of Indonesian Legumes and Tuber Crops Research Institute during May to August 2015. The experiments were conducted to evaluate the potential of six isolates of *Sl*NPV, namely: *Sl*NPV JTM 02-1, *Sl*NPV JTM 02-2, *Sl*NPV JTM 02-3, *Sl*NPV JTM 02-4. *Sl*NPV JTM 02-5 and *Sl*NPV JTM 97c (as control, a virulent strain uses five virus concentrations (1x10°, $1x10^6$, $1x10^8$, $1x10^{10}$, and $1x10^{12}$ PIBs/mL) to control the existence of *S. litura* instar-3. The data of the time for larvae to stop eating and their mortality were analyzed using MSTATC computer software and Hsinchi Probit program was used to analyze the 50% lethal concentration (LC50) and 50% lethal time (LT50). The study showed that the larvae's will to stopeating, their mortality, LC50, and LT50 were significantly influenced by the interaction between SINPV isolate and the inoculated virus concentration. These potential parameters were also different among the larvae instar of tested *S. litura*. The potential of each isolates was higher at instar-3 than the other. Besides that, *Sl*NPV JTM 97c, *Sl*NPV JTM 02-5 showed the highest potential to control *S. litura*. It can be seen from instar-3, LC50 that is effective to kill 50% of the larvae population of *Sl*NPV JTM 02-5 was between $1x10^{10}$ - $1x10^{12}$ PIBs/mL with LT50 between 170-207 Hours After Inoculation (HAI). It is revealed that *Sl*NPV JTM 02-5 has more potential as biopesticide to control *S. litura* than the other tested isolates.

Keywords: Mortality, Nuclear Polyhedrosis Virus, *Spodoptera litura*

INTRODUCTION

Armyworm of *Spodoptera litura* Fabricius is a terrifying pest of soybean in Indonesia. The pest attack can result in 40% of yield losses. The severe crop failure may occur when pest attacks at early stages of flowering and pod formation (Ditlintan 2004). The attempt to control this pest using insecticide also proven the increase of armyworm resistance against most of the conventional insecticides (Karuppaiah et al. 2013; Karuppaiah et al. 2016a). Indiscriminate applications of synthetic insecticide could result in economic and ecology loss (Tengkano and Suharsono 2005; Karuppaiah et al. 2016b). In order to overcome these problems, it is needed to identify alternative control methods which are suitable to overcome resistance and to keep ecological safety.

The use of armyworm nuclear polyhedrosis virus (NPV) is a viable alternative which could be employed in large scale to manage the armyworm menace in soybean. NPV is a pathogen in a variety of insects, especially the family of Noctuidae. This pathogen has a unique characteristic, namely, the existence of inclusion bodies like faceted crystals called polyhedra inside the nucleus of cells in body fat, hypodermic, tracheal matrix, and red blood (Sanjaya et al. 2010). Meanwhile, another type of NPV is a *Spodoptera litura* NPV (*Sl*NPV) that is scientifically called as *Borrelinavirus litura* (Virales: Borrelinaceae). Nowadays, it has been understood that the isolate of *Sl*NPV JTM 97c has effectiveness as equal as the lambda cyhalothrin insecticide (Bedjo et al. 2008). Based on the results of these studies, *Sl*NPV is obviously potential as biopesticide against armyworm. One of the bioinsecticides is *Sl*NPV JTM 97c which is originally from East Java, and it can be found on the dead armyworm infected by *Sl*NPV. *Sl*NPV is able to control the main pests such as soybean armyworm (*Spodoptera litura*) (Bedjo et al. 2008). Further research involving *Sl*NPV JTM 97c showed that it is responsible for the dead of armyworm and green leaf looper (*Crysodeixis chalcites*), leaf roller (*Lamprosema indicata*), and pod borer (*Etiella zinkcenella*) (Bedjo 2011). It shows that the isolate of *Sl*NPV JTM 97c is deadly for insect pests from the ordo of Lepidoptera, and it is different from other *Sl*NPV isolates of specific host.

The difference of isolates is suspected as the factor of differences on the level of the pathogenicity or the virulence of *Sl*NPV against armyworm. The difference of isolates could become one of the influential factors determining the level of virulence of NPV against insect host (Erayya et al. 2013; Hoffman and Frodsham 1993). The use of *Sl*NPV concentration at 15 x 1011 PIBs/mL in the laboratory could be 90% deadly to armyworm of *S. litura*, but, it only reached 25% in the field. The decrease in its effectiveness was caused by *Sl*NPV, which was non-resistant against ultraviolet radiation from the sun and it

also influenced the effectiveness of the virion (Morris 1971; Gupta et al. 2010). Moreover, the mortality level of larvae caused by NPV is also influenced by a number of polyhedra consumed by larvae (Ravinshankar and Venkatesha 2010; Bedjo 2011).

In addition to the difference of isolate, the concentrations of virus also influential on the virulence of *Sl*NPV against armyworm (Arifin 2012). Exploration research to obtain strains or *Sl*NPV isolate which is more virulence is necessary to be done, in order to accelerate the mortality rate of armyworm. Therefore, it is necessary to examine the potential of different isolate of *Sl*NPV originated from East Java to acquire virulent isolate on the concentration of virus which is more economic.

This study aimed to determine the type of isolate from East Java, Indonesia, namely *Sl*NPV which is the most virulent to control armyworms, as well as, to examine the virus concentration of *Sl*NPV as the most effective way to control armyworm larvae.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang, East Java, Indonesia from May to August, 2015.

The pathogenicity test of SlNPV JTM isolates in the laboratory used a completely randomized design (CRD) and was arranged with a treatment based on some factors. The first factor was the type of isolates (I), the isolates were from East Java and consisted of 6 kinds of isolates, namely: *Sl*NPV JTM 02-1 (Ia), *Sl*NPV JTM 02-2 (Ib), *Sl*NPV JTM 02-3 (Ic), *Sl*NPV JTM 02-4 (Id), *Sl*NPV JTM 02-5 (Ie) and *Sl*NPV JTM 97c (If) (as a virulent comparison). The second factor is the concentration of *Sl*NPV (K), consisting of five levels: 0 (No *Sl*NPV as a control) (K1), $1x10^6$ (K2), $1x10^8$ (K3), $1x10^{10}$ (K4), $1x10^{12}$ (K5) PIBs/mL. The combination of treatment consisted of 30 treatments. Each treatment was repeated three times.

The implementation research used armyworm instar-3 of 30 larvae per replicate for each treatment. The larvae were put into a vial, and each vial contains an armyworm. Furthermore, each vial is supplied with the leaves of soybeans that have been inoculated with the dipping method at various concentrations.

The variables measured were (i) time for the larvae to stop eating which was indicated in percent and observed at 1, 2, 4, 6, 8, 10, 12, and 24 hours after inoculation (HAI), (ii) the mortality of armyworm which was indicated in percent and observed at 0, 24, 48, 72, 96, 120, 144, and 168 HAI, (iii) lethal concentration (LC50) or the concentration of virus to be lethal at up to 50% of the population of larvae (N = 30) using Probit analysis (Chi 1997), and (iv) lethal time (LT50) or the time required to kill the larvae at up to 50% of the population (N = 30) using Probit analysis (Chi 1997).

RESULTS AND DISCUSSION

The results of research showed that in the virulent isolates of *Sl*NPV JTM 97c, the number of larvae to stop eating at 4 HAI at each concentration of the virus is, respectively, as much as $1x10^8$ (2.2%), $1x10^{10}$ (3.33%), and 1 x 10^{12} PIBs/mL (4.45%). With the same concentration of virus, the number of larvae that stop eating on *Sl*NPV JTM 02-4 was 0.00%, 0.00%, and 2.22% and at *Sl*NPV JTM 02-5 was 1.11%, 1.11%, and 3.33%. While in isolates of *Sl*NPV JTM 02-2 and *Sl*NPV JTM 02-3, it resulted in an earlier time to stop eating, i.e. at 6 HAI by 1.11% with a virus concentration of $1x10^{12}$ PIBs/mL.

The numbers of larvae that stop eating on five isolates were tested with increasing time of observation. At 24 HAI, the range of the number of larvae stop eating for each virus isolates in the five concentrations are from 00.00-14.44% (*Sl*NPV JTM 02-1), 0.00-17.78% (*Sl*NPV JTM 02-2), 0.00-21.11% (*Sl*NPV JTM 02-3), 0.00-34.45% (SlNPV JTM 02-4), 0.00-69.22% (*Sl*NPV JTM 02-5), and 0.00-80.00% (*Sl*NPV JTM 97c) (Table 1).

Based on the research result, the time of stop eating of larvae in isolates of *Sl*NPV JTM 02-5 and *Sl*NPV JTM 97c mostly occurred at 24 HAI at the virus concentrations of $1x10^8$, $1x10^{10}$ and $1x10^{12}$ PIBs/mL. The time of armyworm to stop eating occurred after 24 HAI for another third isolates. Therefore, based on the time of armyworm to stop eating, apart from *Sl*NPV JTM 97c which is known for its potential and effectivity, isolate of *Sl*NPV JTM 02-5 has a high potential as a biopesticide to control armyworm. In Figure 1, the result is demonstrated at 24 HAI, the number of larvae to stop eating by isolate of *Sl*NPV JTM 02-5 was always over other four isolates, except *Sl*NPV JTM 97c isolate.

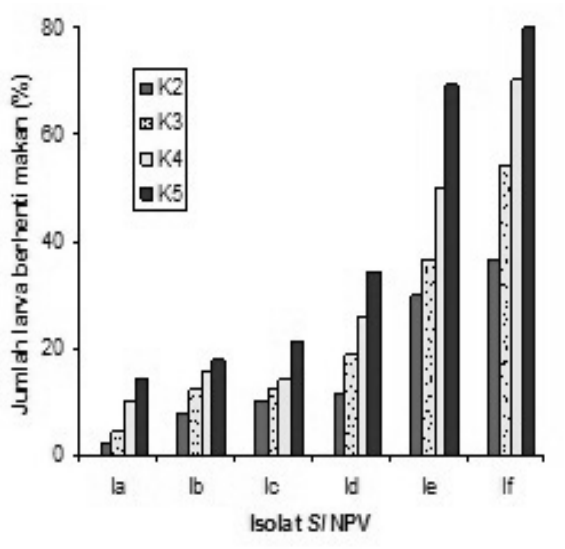

Figure 1. Amount of larvae of instar-3 *S. litura* which stop feeding at 24 HAI (N=30 larvae), after inoculated with six isolates of *Sl*NPV (Ia-If) on five-level concentrations of virus (K2-K5)

The results of these experiments indicate that the time to stop eating is influenced by the concentration of *Sl*NPV. The feeding duration was shortened if the concentration of inoculated *Sl*NPV increased. The larvae of *S. litura* stop eating because inoculated *Sl*NPV allegedly begin to infect the digestive system of insects. According to O'Neill (1995), in a little while after eating the virus, the armyworm will stop eating. The symptoms of *Sl*NPV infection on armyworms will be seen on one till three days. Bedjo et al. (2008) mentioned that the abdomen of larvae would be brownish white; while its back will be blackish brown. *S. litura* armyworm which was infected by *Sl*NPV was marked by its ability to stop eating. Armyworms infected by *Sl*NPV were generally characterized by the decrease ability to eat, slower movement, swollen body, due to viral replication of virus inside the body of an armyworm. Time of stop eating depended on the species of *Spodoptera*. Nurfadila (2004) stated that the result of *Sl*NPV infection on *S. exigua* is the damage of the intestinal epithelial nucleus cell. The damage on the nucleus cell is an indicator of viral replication in the nucleus cell (Rimadhani et al. 2013). The longer the incubation time affected the greater number of the newly formed viruses in the nucleus cell. The process of the final stages of infection was if the viral replication became faster and led to the death of *S. litura* larvae (Young 2000).

Table 2 showed the mortality of *S. litura* larvae which can be found since the observation of 24 HAI. It was equal to 1.1% to 3.33% in isolates of *Sl*NPV JTM 02-4, 3.33% to 4.23% at JTM *Sl*NPV 02-5, and 5.56 to 7.78% in *Sl*NPV JTM 97c. Afterward, on observations of 48 HAI, the mortality of *S. litura* larvae have been found in all isolates tested by *Sl*NPV. The mortality of larvae was getting faster, achieving ≥ 50%, by increasing the faster

Table 1. Percentage of larvae Instar-3 of *S.litura* that stop feeding at various time observations

Treatment		Percentage of Larvae Instar-3 of *S. litura* that Stop feeding				
***Sl*NPV Isolate**	**Concentration (PIBs/mL)**	**Observation (HAI)**				
		4	**6**	**8**	**12**	**24**
*Sl*NPV-JTM 02-1	$1x10^0$	0.00 d	0.00 d	0.00 h	0.00 j	0.00 o
	$1x10^6$	0.00 d	0.00 d	0.00 h	0.00 j	2.22 n
	$1x10^8$	0.00 d	0.00 d	0.00 h	1.11 j	4.44 m
	$1x10^{10}$	0.00 d	0.00 d	1.11 gh	4.45 hi	10.00 kl
	$1x10^{12}$	0.00 d	0.00 d	2.22 fg	6.67 ghi	14.44 ij
*Sl*NPV-JTM 02-2	$1x10^0$	0.00 d	0.00 d	0.00 h	0.00 j	0.00 o
	$1x10^6$	0.00 d	0.00 d	0.00 h	3.33 i	7.78 l
	$1x10^8$	0.00 d	0.00 d	0.00 h	5.56hi	12.12 jk
	$1x10^{10}$	0.00 d	0.00 d	2.22 fg	6.67 ghi	15.56 ij
	$1x10^{12}$	0.00 d	1.11 d	3.33 ef	7.78 fgh	17.78 hi
*Sl*NPV-JTM 02-3	$1x10^0$	0.00 d	0.00 d	0.00 h	0.00 j	0.00 o
	$1x10^6$	0.00 d	0.00 d	0.00 h	4.45 hi	10.00 kl
	$1x10^8$	0.00 d	0.00 d	0.00 h	5.56 hi	12.12 jk
	$1x10^{10}$	0.00 d	0.00 d	4.45 e	10.00 efg	14.44 fgh
	$1x10^{12}$	0.00 d	1.11 d	5.56 de	12.22 cde	21.11 efg
*Sl*NPV-JTM 02-4	$1x10^0$	0.00 d	0.00 d	0.00 h	0.00 j	0.00 o
	$1x10^6$	0.00 d	0.00 d	0.00 h	4.45 hi	11.11 jkl
	$1x10^8$	0.00 d	0.00 d	2.22 fg	7.78 fgh	18.89 ghi
	$1x10^{10}$	0.00 d	0.00 d	4.45 e	10.00 efg	25.56 ef
	$1x10^{12}$	2.22 bc	5.56 bc	8.89 bc	15.56 bcd	34.45 d
*Sl*NPV-JTM 02-5	$1x10^0$	0.00 d	0.00 d	0.00 h	0.00 j	0.00 o
	$1x10^6$	0.00 d	0.00 d	4.45 e	11.11def	30.00 de
	$1x10^8$	1.11 cd	4.44 c	7.78 cd	16.67 bc	36.67 d
	$1x10^{10}$	1.11 cd	4.44 c	8.89 b	21.00 b	50.00 c
	$1x10^{12}$	3.33 ab	7.78 b	12.22 ab	36.67 a	69.22 b
*Sl*NPV-JTM 97c	$1x10^0$	0.00 d	0.00 d	0.00 h	0.00 j	0.00 o
	$1x10^6$	0.00 d	1.11 d	4.45 e	14.14 cde	36.67 d
	$1x10^8$	2.22 bc	5.56 bc	10.00 bc	21.11 b	54.45 c
	$1x10^{10}$	3.33 ab	7.78 b	12.22 ab	32.22 a	70.33 ab
	$1x10^{12}$	4.45 a	11.11 a	15.56 a	38.89 a	80.00 a

Note: HAI: Hours After Inoculation. Numbers followed by the same letter are not significantly different at the Duncan test alpha level of 5%. Data in the transformation by the formula $\sqrt{X + 0.5}$ prior to analysis with MSTATC Program

concentration of inoculated virus. In isolates *Sl*NPV JTM 97c, the mortality ≥ 50% was achieved on the observation of 96 HAI with virus concentration of $1x10^{12}$ PIBs/mL, whereas the level of concentration of $1x10^{10}$ and $1x10^{8}$ PIBs/mL was reached on observations at 120 HAI, and at concentration of $1x10^{6}$ PIBs/mL the mortality ≥ 50% could be achieved on observations at 168 HAI. The isolates of *Sl*NPV JTM 02-5 were based on the time to stop eating and it is expected to have a high potential to control *S. litura*. Meanwhile, the mortality ≥ 50% was achieved on the observation at 120 HAI on a concentration virus of $1x10^{10}$ and $1x10^{12}$ PIBs/mL. On isolates of *Sl*NPV JTM 02-2, *Sl*NPV JTM 02-3, and *Sl*NPV JTM 02-4, the mortality of larvae of *S. litura* which was up to 168 HAI observations did not reach 50%.

The Probit analysis results using Chi (1997) showed LC50 values at the instar-3 *S. litura* for each isolate was different, moreover, LC50 values of each isolate were also different at each observation time (Table 3).

In Table 3 and Figure 2, it is indicated from six tested SlNPV isolates, the lowest concentration of the deadly virus up to 50% from the population of armyworm *Sl*NPV isolates are respectively demonstrated by *Sl*NPV JTM 97c, followed by *Sl*NPV JTM 02-5, *Sl*NPV JTM 02-4, *Sl*NPV JTM 02-3, *Sl*NPV JTM 02-1, and *Sl*NPV JTM 02-2. For example on experiment at 168 HAI, to kill 50% of the armyworm population (N = 30), isolates *Sl*NPV JTM 97c, *Sl*NPV JTM 02-5, *Sl*NPV JTM 02-4, *Sl*NPV JTM 02-3, *Sl*NPV JTM 02-1, and *Sl*NPV JTM 02-2, LC50 need the virus concentration of: $1x10^{6}$ (Y=2.3 + 3.2x), $1x10^{8}$ (Y=2.4 + 2.8x), $1x10^{15}$ (Y= 2.3 + 2.3x), $1x10^{18}$ (Y=2.3 + 2.2x), $1x10^{19}$ (Y=1.9 + 1.8x), and $1x10^{27}$ PIBs/mL (Y=2.5 + 1.7x).

Table 2. The mortality of instar-3 larvae of *S. litura* at various time of observation

Treatment		Mortality of instar-3 Larvae of *S. litura* (%)						
***Sl*NPV**	**Cons**	**Observation (HAI)**						
Isolate	**(PIBs/mL)**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
*Sl*NPV-JTM 02-1	$1x10^{0}$	0.00 e	0.00 i	0.00 o	0.00 o	0.00 j	0.00 n	0.00 n
	$1x10^{6}$	0.00 e	0.00 i	7.78 mn	10.00 n	12.22 i	13.33 m	13.33 m
	$1x10^{8}$	0.00 e	4.44 gh	10.00 klmn	13.33 lmn	14.44 i	14.44 lm	17.78 lm
	$1x10^{10}$	0.00 e	6.67 efg	11.11 jklm	14.45 klmn	16.67 ghi	18.89 jkl	24.44 jkl
	$1x10^{12}$	0.00 e	12.12 cde	14.45 ghijk	20.00 hij	22.22 f	23.33 hij	30.00 hij
*Sl*NPV-JTM 02-2	$1x10^{0}$	0.00 e	0.00 i	0.00 o	0.00 o	0.00 j	0.00 n	0.00 n
	$1x10^{6}$	0.00 e	3.33 h	6.67 n	10.00 n	12.22 i	13.33 m	13.33 m
	$1x10^{8}$	0.00 e	5.56 fgh	10.00 klmn	12.22 mn	13.33 i	15.56 klm	18.89 klm
	$1x10^{10}$	0.00 e	10.00 bcde	11.11 jklm	14.44 lmn	16.67 ghi	17.78 klm	20.00 klm
	$1x10^{12}$	0.00 e	11.11 bc	13.33 hijkl	17.78 hijkl	21.11 fg	23.33 hij	28.89 hij
*Sl*NPV-JTM 02-3	$1x10^{0}$	0.00 e	0.00 i	0.00 o	0.00 o	0.00 j	0.00 n	0.00 n
	$1x10^{6}$	0.00 e	4.44 gh	12.12 lmn	13.33 lmn	14.44 i	16.67 klm	17.78klm
	$1x10^{8}$	0.00 e	6.67 efg	13.33 hijkl	14.44 jklmn	15.56 hi	17.78 klm	22.22 klm
	$1x10^{10}$	0.00 e	10.00 bcd	16.67 fghi	20.00 hij	21.11 fg	23.33 hij	27.78 hij
	$1x10^{12}$	0.00 e	11.11 bc	20.00 def	22.22 ghi	24.44 ef	27.78 gh	34.44 gh
*Sl*NPV-JTM 02-4	$1x10^{0}$	0.00 e	0.00 i	0.00 o	0.00 o	0.00 j	0.00 n	0.00 n
	$1x10^{6}$	0.00 e	4.44 gh	10.00 klmn	16.67 ijklm	16.67 ghi	17.78 klm	18.89 klm
	$1x10^{8}$	0.00 e	7.78 cdef	15.56 fghij	20.00 hij	20.00 fgh	20.00 ijk	24.44 ijk
	$1x10^{10}$	1.11 d	10.00 bcde	18.89 efg	23.33 gh	23.33 ef	25.56 hi	32.22 hi
	$1x10^{12}$	3.33 c	11.11 bc	21.11cdef	26.67 fg	28.89 de	33.33 fg	42.22 fg
*Sl*NPV-JTM 02-5	$1x10^{0}$	0.00 e	0.00 i	0.00 o	0.00 o	0.00 j	0.00 n	0.00 n
	$1x10^{6}$	0.00 e	5.56 fgh	12.22 ijkl	21.11 hijk	32.22 d	34.44 f	33.33 f
	$1x10^{8}$	3.33 c	8.89 cdef	17.78 efgh	27.78 efg	44.45 c	46.67 de	52.22 de
	$1x10^{10}$	3.33 c	11.11 bc	23.33 cde	34.44 cde	49.99 bc	54.45 bcd	60.33 bcd
	$1x10^{12}$	4.23 b	11.11 bc	25.56 bcd	41.11 bc	53.34 b	61.11 b	67.00 b
*Sl*NPV-JTM 97c	$1x10^{0}$	0.00 e	0.00 i	0.00 o	0.00 o	0.00 j	0.00 n	0.00 n
	$1x10^{6}$	0.00 e	6.67 defg	17.78 efgh	32.22 def	44.44 c	45.56 e	51.11 e
	$1x10^{8}$	5.56 b	11.11 bc	26.67 bc	35.56 cd	50.00 bc	52.22 cde	61.11 cde
	$1x10^{10}$	6.67 ab	13.33 ab	31.11 ab	45.56 ab	54.45 b	60.33 bc	68.11 bc
	$1x10^{12}$	7.78 a	17.78 a	34.44 a	52.22 a	65.11 a	72.22 a	77.78 a

Note: HAI: Hours After Inoculation. Numbers followed by the same letter are not significantly different at the Duncan test alpha level of 5%. Data in the transformation by the formula $\sqrt{X + 0.5}$ prior to analysis with MSTATC Program

Table 3. The concentration of the deadly virus (LC50) of the isolates *Sl*NPV against instar-3 larvae of *S. litura* at 24-168 hours after inoculation

Isolate of *Sl*NPV	The concentration of the deadly virus (LC50) of the isolates *Sl*NPV against instar-3 larvae of *S. litura* at 24-168 hours after inoculation						
	24	**48**	**72**	**96**	**120**	**144**	**168**
Ia	-	1 x 10 1^{08} (Y=1.4 + 2.1x)	1 x 10^{53} (Y=2.7 + 1.1x)	1 x 10^{51} (Y=2.6 + 1.4x)	1 x 10^{51} (Y=2.8 + 1.3x)	1 x 10^{44} (Y=2.8 + 1.3x)	1 x 10 1^{9} (Y=1.9 + 1.8x)
Ib	-	1 x 10^{97} (Y=1.8 + 1.8x)	1 x 10^{68} (Y=2.6 + 1.2x)	1 x 10^{61} (Y=2.7 + 1.2x)	1 x 10^{61} (Y=2.9 + 1.2x)	1 x 10^{51} (Y=2.9 + 1.2x)	1 x 10^{27} (Y=2.5 + 1.7x)
Ic	-	1 x 10^{62} (Y=1.9 + 1.7x)	1 x 10^{48} (Y=2.4 + 1.6x)	1 x 10^{42} (Y=2.9 + 1.3x)	1 x 10^{38} (Y=2.9 + 1.3x)	1 x 10^{35} (Y=3.0 + 1.3x)	1 x 10 1^{8} (Y=2.3 + 2.2x)
Id	-	1 x 10^{67} (Y=2.1 +1.6x)	1 x 10^{42} (Y=2.6 + 1.5x)	1 x 10^{40} (Y=3.1 + 1.1x)	1 x 10^{32} (Y=3.0 + 1.3x)	1 x 10^{23} (Y=2.7 + 1.7x)	1 x 10 1^{5} (Y= 2.3 + 2.3x)
Ie	1 x 10 1^{55} (Y= 1.9 + 1.4x)	1 x 10^{63} (Y=2.3 + 1.5x)	1 x 10^{27} (Y=2.5 + 1.7x)	1 x 10 1^{6} (Y=2.7 + 1.9x)	1 x 10 1^{0} (Y=3.2 + 1.8x)	1 x 10^{8} (Y=2.9 + 2.3x)	1 x 10^{8} (Y=2.4 + 2.8x)
If	1 x 10^{497} (Y=2.6 + 0.9x)	1 x 10^{38} (Y=2.1 + 1.8x)	1 x 10 1^{9} (Y=2.8 + 1.7x)	1 x 10 1^{1} (Y=3.1 + 1.8x)	1 x 10^{7} (Y=3.6 + 1.6x)	1 x 10^{7} (Y=3.1 + 2.2x)	1 x 10^{6} (y= 2.3 + 3.2x)

Note: Data were analyzed by Probit analysis program Chi (1977). Similarities in parentheses (Y = a + bx) is a regression equation of each value LT50

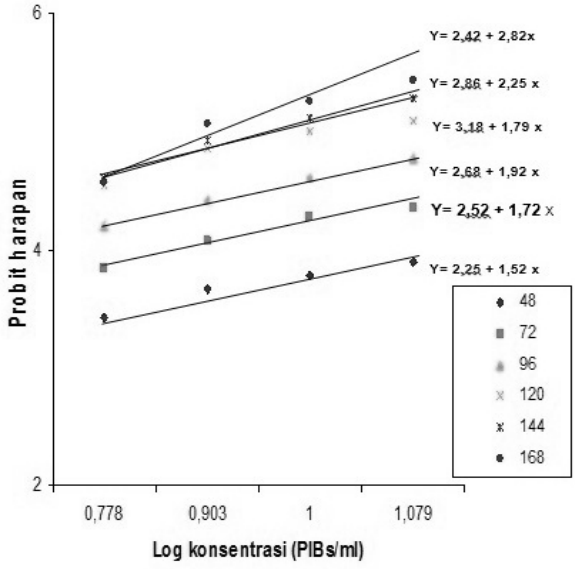

Figure 2. LC50 SlNPV JTM 02-5 on instar-3 *S. litura* at 48, 72, 96, 120, 144, and 168 HAI

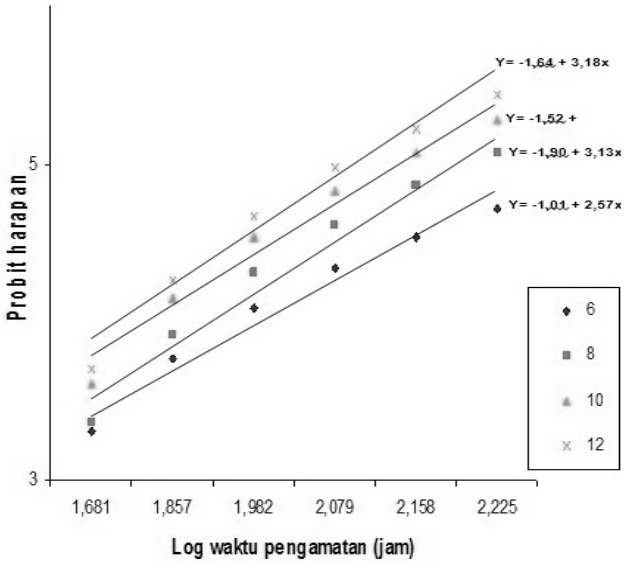

Figure 3. LT50 *Sl*NPV JTM 02-5 at instar-3 *S. litura* at virus concentration of 1 x 10^{6}, 1 x 10^{8}, 1 x 10^{10}, and 1 x 10^{12} PIBs/mL

From six isolates of *Sl*NPV that were tested, in addition to *Sl*NPV JTM 97c, only *Sl*NPV JTM 02-5 that has a high potential as biopesticide against *S. litura* instar-3. The potential is estimated based on the reliability of the LC50 values shown at 96-168 HAI observation time, for example, the value range of LC50 amounted 1x10^{8} - 1x10^{16} PIBs/mL. At the same time of observation, the isolate of *Sl*NPV JTM 97c has an LC50 value range of 1x10^{6} - 1x10^{11} PIBs/mL. This susceptibility of *Spodoptera litura* to *Sl*NPV may also vary depending on the type of host plant offered as a diet. Karuppaiah et al. (2016a) reported that host plant had effect on susceptibility of *S.litura* to different insecticides.

The Probit analysis results using Chi (1997) indicated that the value of LT50 on instar-3 *S. litura* for each isolate was different and LT50 values of each isolate were also different in every observation time.

Table 4. Time of death (LT50) of six *Sl*NPV isolates at five different doses on the instar-3 larvae of *Spodoptera litura*

Isolate of SlNPV	LT50 at various doses on the instar-3 larvae of *S. litura* (hour)				
	1 x 106	1 x 108	1 x 1010	1 x 1012	Et all dose
*Sl*NPV JTM 02-1	1844 (Y=1.6 + 1.0x)	985 (Y=1.3 + 1.2x)	654 (Y=1.2 + 1.3x)	372 (Y=0.9 + 1.6x)	597 (Y=0.8 + 1.5x)
*Sl*NPV JTM 02-2	1158 (Y=1.1 + 1.3x)	941 (Y=1.2 + 1.3x)	869 (Y=1.5 + 120x)	567 (Y=1.6 + 1.2x)	987 (Y=1.6 + 1.1x)
*Sl*NPV JTM 02-3	626 (Y=0.6 + 1.6x)	597 (Y=0.8 + 1.5x)	475 (Y=1.4 + 1.3x)	387 (Y=1.5 + 1.3x)	653 (Y=1.5 + 1.2x)
*Sl*NPV JTM 02-4	722 (Y=1.2 + 1.3x)	600 (Y=1.5 + 1.2x)	474 (Y=1.7 + 1.2x)	252 (Y=0.5 + 1.9x)	430 (Y=1.1 + 1.5x)
*Sl*NPV JTM 02-5	217 (Y=1.0 + 2.6x)	159 (Y=1.9 + 3.1x)	136 (Y=1.5 + 3.1x)	122 (Y=1.6 + 3.2x)	153 (Y=1.1 + 2.8x)
*Sl*NPV JTM 97c	157 (Y=1.0 + 2.7x)	138 (Y=1.1 + 2.9x)	123 (Y=1.5 + 3.1x)	104 (Y=2.0 + 3.5x)	127 (Y=1.4 + 3.1x)

Note: Data were analyzed by Probit analysis program Chi (1977). Similarities in parentheses (Y = a + bx) is a regression equation of each value LT50

In Table 4, it is indicated that out of six isolates that were tested with *Sl*NPV, the shortest time required to kill 50% of the population of *S. litura* isolates is demonstrated by *Sl*NPV JTM 97c, followed by *Sl*NPV JTM 02-5, *Sl*NPV JTM 02-4, *Sl*NPV JTM 02-3, *Sl*NPV JTM 02-1, and *Sl*NPV JTM 02-2.

For example, the virus concentration of 1 x 10^{12} PIBs/mL is needed to kill 50% of the population of armyworm (N = 30), thus, isolates of *Sl*NPV JTM 97c, *Sl*NPV JTM 02-5, *Sl*NPV JTM 02-4, *Sl*NPV JTM 02-1, *Sl*NPV JTM 02-3, and *Sl*NPV JTM 02-2, LC50 takes, respectively, 104 hours (Y = 2.0 + 3.5x), 122 hours (Y = 1.6 + 3.2x), 252 hours (Y = 0.5 + 1.9X), 372 hours (Y = 0.9 + 1.6x), 387 hours (Y = 1.5 + 1.3x), and 567 hours (Y = 1.6 + 1.2x) (Figure 3).

Table 4 also indicated that the time to kill 50% of the population of larvae rise as the increase of the lower concentrations of virus inoculated. The LT50 value range of each isolate *Sl*NPV JTM 97c, *Sl*NPV JTM 02-5, *Sl*NPV JTM 02-4, *Sl*NPV JTM 02-1, *Sl*NPV JTM 02-3, and *Sl*NPV JTM 02-2, is, respectively, 104-157 HAI, 122-217 HAI, 252-722 HAI, 372-1844 HAI, 387-6726 HAI, and 567-1158 HAI.

From the six isolates of *Sl*NPV which were tested, in addition to *Sl*NPV JTM 97c, it was only *Sl*NPV JTM 02-5 that has a high potential as biopesticide against instar-3 *S. litura*. The potential is estimated based on the reliability of LT50 values shown in virus concentration of 1x10^{12} PIBs/mL, and on the time required to kill 50% of the larvae population (N = 30) which was 122 HAI. At the level of concentration, the virus isolates *Sl*NPV JTM 02-4, *Sl*NPV JTM 02-1, *Sl*NPV JTM 02-3, and *Sl*NPV JTM 02-2, each takes 252, 372, 387, and 567 HAI.

REFERENCES

Arifin M. 2012. *Sl*NPV biopesticide to control armyworm for supporting self-sufficiency on soybean. Jurnal Pengembangan Inovasi Pertanian 5 (1): 22. [Indonesian]

Bedjo. 2008. *Sl*NPV isolate JTM 97c obtained from east Java to control armyworm on soybean. [Thesis]. Universitas Brawijaya, Malang. [Indonesian]

Bedjo. 2011. The effectiveness of *Sl*NPV to control leafhopper and pod borer on soybean. Prosiding Seminar Hasil Penelitian Tanaman Aneka Kacang dan Umbi. Malang. [Indonesian]

Chi H. 1997. Probit Analysis. Computer Software Programme. National Chung Hsing University, Taichung, Taiwan.

Ditlintan. 2004. Evaluation of soybean crop damage caused by pest attaches at 2003, 2002 and an average of 5 year period (1997-2001). Dir. Perlindungan Tanaman, Jakarta.

Erayya, Jagdis J, Sajeesh PK, Upadhyay V. 2013. Nuclear Polyhedrosis Virus (NPV), a potential biopesticide: A review. Res J Agric For Sci 1 (8): 30-33.

Gupta RK, Raina JC, Arora RK, Bal K. 2010. Selection and field effectiveness of Nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Hubner). Intl J Virol 6 (3): 164-178.

Hoffmann MP, Frodsham AC. 1993. Natural Enemies of Vegetable Insect Pest. Cooperative Extension. Cornel University, Ithaca, New York.

Karuppaiah V, Srivastava C, Subramanian S, Padaria JC, Ahuja DB. 2016a. Nucleotide polymorphism and modulation in carboxyl esterase activity in *Spodoptera litura* (F.) (Noctuidae: Lepidoptera). Indian J Entomol 78 (3): 252-256.

Karuppaiah V, Srivastava C, Subramanian S. 2016b. Effect of host plants on insecticide susceptibility and detoxification enzyme activity in *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera). Proc Natl Acad Sci India, Section B: Biol Sci 86 (3): 715-721.

Karuppaiah, V. and Srivastava, C. 2013. Relative toxicity of newer group insecticides to *Spodoptera litura*. Ann Plant Protect Sci 21 (2): 305-309.

Morris ON. 1971. The effect of sunlight, ultraviolet and gamma radiations and temperature on the infectivity of nuclear polyhedrosis virus. J Invert Pathol 18: 292-294.

Nurfadila. 2004. The effectiveness of inoculum of *Spodoptera exigua* Nuclear Polyhedrosis Virus (*Se*NPV) and their effect on the gut

epithelium of *S. exigua*. [Thesis]. Program Pascasarjana, Universitas Brawijaya, Malang. [Indonesian]

O'Neill G. 1995. Turbo charged viruses speed insect kill. J Ecosyst 82 Summer 1994/1995. CSIRO, Canberra, Australia.

Ravinshankar BS, Venkatesha MG. 2010. Effectiveness of *Sl*NPV of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) on different host plants. J Biopest 3 (1): 168-171.

Rimadhani AS, Darma B, Maryani CT. 2013. Virulence of Nuclear polyhedrosis virus (NPV) at armyworm (*Spodoptera litura* F.) (Lepidoptera: Noctuidae) in Deli tobacco plants in the greenhouse. J Agro Ekologi 1 (3): 678-679. [Indonesian]

Sanjaya Y, Machmudin D, Kurniawati ND. 2010. Histological study of *Sl*NPV infection on body weight and peritrophic membrane damage of *Spodoptera litura* larvae. Bioteknologi 8 (2): 78-85.

Tengkano W, Suharsono. 2005. *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on soybean and its control. Bul Palawija 10: 43-52.

Young SY. 2000. Persistence of viruses in the environment. Environ Entomol 3: 1035-1036.