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Virulence of Spodoptera Litura Nuclear Polyhedrosis Virus (SLNPV) with kaolin as carrier material on spodoptera litura and tetragonula laeviceps on soybean

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Abstract. The objective of research is determined the virulence of different concentrations of *Sl*NPV- JTM 97C with kaolin as carrier material on *S. litura and T. laeviceps* mortality *on soybean.* The Research was conducted in Plant Protection Laboratory of State polytechnic of Jember, from June to September 2020. The lethal concentration (LC_{50} and LC_{95}) *Sl*NPVusing Polo Plus 1.0, with the IRAC version 3, method number 008. The *S. litura* mortality test used Completely Randomind Design with 5 treatments and 3 replications. The treatments were contamination of larvae diet by *Sl*NPV with control (the third instar larvae without contamination *Sl*NPV), 0,1%, 0,2%, 0,3% and 0,5% . These concentrations are equivalent to the concentrations of 0.1.2x 10^8 PIBs/g, 2.4x 10^8 PIBs/g, 3.6 x 10^8 PIBs/g and 6.0 x 10^8 PIBs/g, respectively. Each test was repeated three times. Mortality percentage of larvae were observed as experiment data. Those data were analyzed by Analysis of Variant and Least Significant Difference (LSD) 5%. *Sl*NPV virulence test against *T. laeviceps* using a concentration that can cause *S. litura* mortality ≥ 70%. The results of the research indicated that LC_{50} of *Sl*NPV with kaolin as carrier against *S. litura* 3rd instar was 0.2%, LC_{95} was 0.5%, effective concentration of *Sl*NPV to control *S. litura* 3rd instar was 0.3% to 0.5%, *Sl*NPV concentration at 0.3% to 0.5%, non-toxic to *T. laeviceps.*

1. Introduction

Edamame soybean is one of the important food crop commodities in Indonesia. Armyworm attack is one of the obstacles in the cultivation of Edamame soybeans. [1] These pests can cause crop losses of up to 85%, and can even cause crop failure. Until now, armyworm control still relies on synthetic insecticides which are applied on a scheduled basis. The frequency of insecticide application needs to be taken into account so that ecologically and economically control measures are not detrimental because the continuous use of scheduled and excessive synthetic insecticides can kill natural enemy populations such as parasitoids and predators, problems of resistance and resurgence, and pollute the environment [2] .

Nucleo Polyhedroses Virus (NPV) has the potential to be developed into an effective, efficient, and environmentally friendly bioinsecticide. Nucleo Polyhedroses can survive in nature because the virus particles are encased in polyhedra and can spread naturally through the process of transmission. Larvae that died infected with NPV are often found hanging with the two artificial limbs attached to the leaves or twigs of plants. Based on several advantages of NPV compared to synthetic insecticides, the development of NPV biological agents for plant pest control in Indonesia has very good prospects.

One of the problems to increase the production of Edamame soybeans is the attack of armyworms (S. litura) because this pest is resistant to several synthetic insecticides are specific, selective, effective for pests that are resistant to synthetic insecticides and are safe for the environment [3].

At this time, there has been a phenomenon of pollinator decline called colony collapse disorder, which is the mass death of individual honey bees in one colony which generally attacks bee colonies in subtropical areas. The cause of this phenomenon is thought to be a combination of several factors, including pesticides [4].

To overcome the shortage of these pollinating insects, one of the efforts made is the use of pesticides that are safe against pollinating insects and effective against pests[4]. *SlNPVJTM 97C* is effective in controlling *S. litura*, easy to formulate, can be produced in vivo,. One way to increase the virulence of *Sl*NPV needs to be investigated regarding the virulence of *Sl*NPV concentrations with kaolin as a carrier against *S.litura* which is safe against pollinating insects such as *T. laeviceps*.

2. Material and Methods

The research was conducted in Plant Protection laboratory of State polytechnic of Jember, from June to August 2020.

2.1 NPV Propagation and Standardization

NPV propagation is based on the method, while NPV standardization is based on the [18]. Artificial feed slices (± 10 g) were put into a plastic vial and then dripped with a polyhedra suspension with a concentration of approximately 107 PiBs / ml. Then the 3rd instar caterpillars were put into the vial individually. Dead or dying caterpillars were collected and then extracted using a 100 mesh filter. The crude polyhedra suspension was purified using a centrifuge with a speed of 3500 revolutions/minute for 15 minutes.

The precipitate produced from several times of purification was made into a polyhedra stock suspension and stored in a refrigerator at 0 °C. The standard polyhedra suspension was diluted 10 times and then formulated in the form of flour with kaolin as a carrier. The trick is to drop a 25 ml suspension with 0.1% Triton x-100 agent to maintain the stability and persistence of Sl-NPV. then mixed with 100 g of kaolin gradually, stirring until blended. In this way, a polyhedra preparation with flour formula was obtained with a concentration of 1.2 X 108 PIBs/g [16].

2.2 LC_{50} and LC_{95} Test

The treatments were contamination of larvae diet by *Sl*NPV JTM 97C with control (the third instar larvae without contamination *Sl*NPV), 0,1%, 0,2%, 0,3% , 0,5% . These concentrations are equivalent to the concentrations of 0.1.2x 10^8 PIBs / g, 2.4x 10^8 PIBs/g, 3.6x 10^8 PIBs/g and 6.0x 10^8 PIBs/g, respectively. The number of tested insects, 10 per treatment. Each test was repeated three times. Percentage mortality of larvae was observed as experiment data. The Lethal Concentration (LC_{50} and LC_{95}) of *Sl*NPV JTM 97C using Polo Plus 1.0 , with the IRAC version 3, method number 008.

2.3 Mortality Test

The effect of *Sl*NPV JTM 97C concentration on *S. litura* used Completely Randomized Design with 5 treatments and 3 replications. The treatments were contamination of larvae diet by *Sl*NPV with control (the third instar larvae without contamination *Sl*NPV), 0,1%, 0,2%, 0,3% and 0,5% . These concentrations are equivalent to the concentrations of 0.1.2x 10^8 PIBs / g, 2.4x 10^8 PIBs/g, 3.6x 10^8 PIBs/g and 6.0x 10^8 PIBs/g, respectively. Each test was repeated three times. Percentage mortality of larvae was observed as experiment data. Those data were analyzed by Analysis of Variant and Least Significant Difference (LSD) 5%.

2.4 Insecticide Efficacy Test

If the first observation of the target pest population is not significantly different, then the efficacy of the tested insecticide is calculated using the Abbott formula [19].

$$EI = \frac{Ca - Ta}{Ca} \times 100\%$$

Note: EI = efficacy of the tested insecticide (%),, Ca = population of pests in control plot after insecticide application, Ta = population of pests in treatment plot after insecticide application.

If in the first observation the target pest population is significantly different between treatments, the efficacy of the insecticide tested is calculated by using the Henderson and Tilton formula.

$$EI = (1 - \frac{Ta}{Ca} \times \frac{Cb}{Tb}) \times 100\%$$

Note: EI = efficacy of the tested insecticide (%), Tb = population of pests in the insecticide treatment before application, Ta = population of pests in insecticide treatment after application, Cb = population of pests in controls before application, Ca = population of pests in controls after application. Insecticide efficacy criteria (EI): Insecticide treatment is said to be effective, if at least (½ n + 1) observations (n = total number of observations), the insecticide efficacy level is an EI value of ≥ 70%. Data were analyzed using SPSS 23.0 software

Test the effect of the effective concentration of *Sl*-NPV on *T. laeviceps* This test uses the Abbott formula as follows:

$$Mt\ (\%) = \frac{Mp - Mk}{100 - Mk} \times 100\ \%$$

Note: Mt = mortality corrected, Mp = mortality on treatment, Mk = mortality in controls. If Mt <30%: non-toxic to slightly toxic, Mt 30% to <80%: slightly toxic, 80% -99% Mt: toxic, Mt> 99%: very toxic.

3. Resuls and dicussion

The result and discussion in this study are presented in the following tables and figures.

Table 1. Toxicity of *Sl*-NPV to third instar *S. litura* larvae.

A ± GB	b ± GB	LC_{50} (SK 95%) (%)	LC_{95} (SK 95%) (%)
0,637±0,846	0,846±1,235	0,188 (0,131-0,250)	0,476 (0,331±1.340)

Note: a = intercept, b = slope of probit regression, GB = Standard Error, SK = Confidence Interval

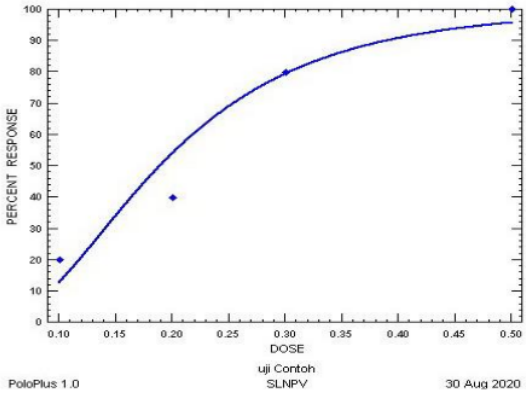

Figure 1. Graph of the Relationship between *Sl*-NPV Dosage and the percentage of mortality of *S. litura*

The results of the probit analysis show that the LC_{50} Sl-NPV (Lethal concentration of 50% of the population) for 3rd instar *S. litura* is 0.2% equivalent to a dose of 1.2 X 10^8 PIBs / g PIBs / ml, and LC_{95} is 0.5%% equivalent to a dose of 6.0 X 10^8 PIBs / g. This is because to obtain higher mortality requires a higher *Sl*NPV concentration (more and more Polyhedral Inclusion Body (PIB). According to the results of the study of *Sl*NPV-JTM 97C on *Croccidolomia binotalis* test insects, the concentration of *Sl*NPV-JTM 97C had a very significant effect on mortality and time to stop eating larvae. The higher the concentration of *Sl*NPV-JTM 97C isolate inoculated, the higher the percentage of mortality of *C. binotalis* larvae, and the shorter the time required for control. The correlation between concentration and mortality of *C. binotalis* was very strong. Likewise, the correlation between the time span of observation and mortality of *C. binotalis* was very strong [14].

3.1 Insecticide Mortality and Efficacy of Insecticide

Effect of *Sl*NPV concentration on mortality and insecticide efficacy (EI) of third instarn*S. litura* larvae.

Table 2. Mortality and Efficacy

*Sl*NPV Concentration	Mortality (%) 5th DAA	EI (%) 5th DAA
Aquadest (Control)	7 a	0
0,1%	20 b	0
0,2%	27 c	60
0,3%	40 d	80
0,5%	60 e	100

Note: numbers followed by different letters in the same column show significant differences according to the 5% LSD test. DAA is Day After Application, EI is Insecticide Efficacy.

Based on the 5% LSD test, it shows that *Sl*NPV treatment at a concentration of 0.1% to 0.5%, shows a significant difference. 80% insecticide efficacy indicated by a concentration of 0.3% to 0.5%, this indicates that the effective concentration for controlling *S. litura* is 0.3% to 0.5%.

Table 3. Effect of *Sl*NPV Efficacy on *T. laeviceps*

*Sl*NVP Concentration	Mortality corrected 5th DAA (%)
Aquadest (Control)	0
0,3%	20
0,5%	20

Note: if Mt <30%: non-toxic to slightly toxic, Mt 30% - <80%: slightly toxic, Mt 80 -99%: toxic, Mt> 99%: very toxic.

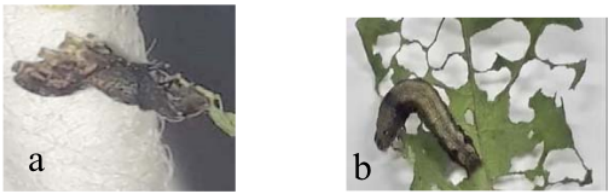

Figure 2. *S. litura* on control 5th DAA (a), *S. litura* die exposed to *Sl*NPV (b)

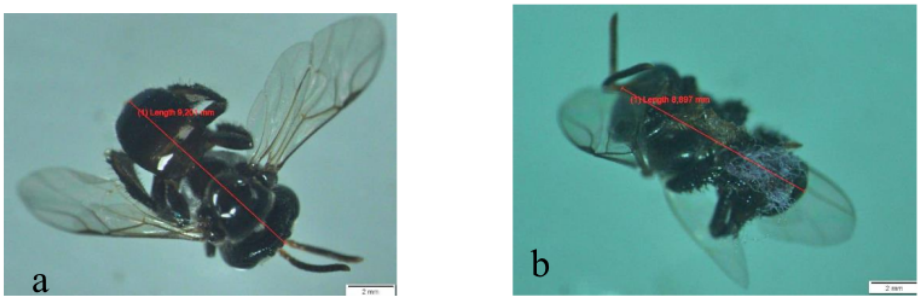

Figure 3. *T. laeviceps* on control (a). *T. laeviceps* exposed to *Sl*NPV (b)

4. Conclusion

The conclusions of this study are as follows**:**

1. LC_{50} of *Sl*NPV with kaolin as carrier against *S. litura* 3rd instar was 0.2%, LC_{95} was 0.5%
2. Effective concentration of *Sl*NPV to control *S. litura* 3rd instar was 0.3% to 0.5%.
3. *Sl*NPV concentration at 0.3% to 0.5%, non-toxic to *T. laeviceps*

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