Compatibility of Lemongrass Extract with *Spodoptera litura* Nuclear Polyhedrosis Virus Against *Spodoptera litura* on Soybean Plants

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ABSTRACT

Armyworm (*Spodoptera litura*) is an important pest that damages the leaves of soybean. *S/NPV* is one type of pathogen that has potential as a biocontrol agent to control armyworms because it is specific, selective, and effective. This study aimed to investigate the compatibility between the lemongrass and *S/NPV* in controlling armyworm. The Research was conducted at the Laboratory of Indonesian Legumes and Tuber Crops Research Institute (ILETRI) from February 2016 until April 2016. The study using completely randomized design with 7 treatments and 3 replication with a treatment that consists of control, *S/NPV*-JTM 97C 1.5×10^6 PIB/ml, 10% of lemongrass extract, a combination *S/NPV*-JTM 97C fixed concentrate of 1.5×10^6 PIB/ml with 2.5%, 5%, 7.5%, and 10% of lemongrass extract. The results showed that the combination of *S/NPV*-JTM 97C 1.5×10^6 PIB/ml with 10% lemongrass had the highest level of virulence, with 96% of mortality on larva 100% on the pupa stage, so that the combination of *S/NPV* and lemongrass extracts was compatible in controlling *S. litura*.

Keywords: Soybean; *S. litura*; *S/NPV*; lemongrass oil; compatibility

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INTRODUCTION

Spodoptera litura Fabricius (Lepidoptera: Noctuidae) is one of the important pests that damage soybean (Punithavalli et al., 2014). Yield loss due to *S. litura* pests attack can reach up to 80% (Marwoto and Suharsono, 2008). Based on these problems, an effective and efficient, and environmentally friendly control effort is needed, such as *Spodoptera litura* Nuclear Polyhedrosis Virus (*S/NPV*). *S/NPV* is a type of pathogenic virus that can potentially be a biological agent in controlling armyworm because it is specific, selective, effective for pests that have been resistant to insecticides, and is environmentally safe (Ayyub et al., 2019). However, one of the disadvantages is that

S/NPV, which is not formulated with other ingredients, is less effective when compared to that which is formulated; this is caused by reduced viability, stability, and pathogenicity of *S/NPV* (Trisnarningsih and Kartohardjono, 2016). The effectiveness of the *S/NPV* application can increase with formulation techniques by adding chemical compounds such as protective materials against solar radiation, grading, adhesives, antifeedant (stop appetite), and other compatible entomopathogens (Trisnarningsih and Kartohardjono, 2016).

Botanical pesticides are easily made with their environmentally friendly and biodegradable properties to be safe for humans and animals and reduce

environmental pollution (El-Wakeil, 2013). A combination of vegetable pesticides and S/NPV was carried out in a previous study, while the ingredients used as vegetable pesticides were soursop seed extract (Bedjo, 2015). This biopesticide has been known to have several benefits such as Lemongrass Extract (EDS) singly or in combination with plant extracts with insecticidal properties against the pest of *Callosobruchus maculatus* (Ojebode et al., 2016). This study aimed to investigate the compatibility and the effectiveness of the lemongrass leaf extract combined with S/NPV against *S. litura* in soybean plants.

MATERIALS DAN METODS

The study was conducted at the Laboratory of Indonesian Legumes and Tuber Crops Research Institute (ILETRI) from February 2016 until April 2016. This study used a Completely Randomized Design with seven treatments and was repeated three times (Table 1). Each experimental unit consisted of 10 larvae (3rd instar of *S. litura* larvae)

Rearing of *S. litura*

Propagation of test insects was carried out by collected *S. litura* eggs obtained from the field.

Maintenance was carried out in plastic jar containers whose walls were lined with soybean leaves for larvae to hatch. The larvae were kept until they become the third instar. The larvae were moved into a plastic jar (d= 5 cm and h= 5 cm). Each jar contained one *S. litura* larvae. Some larvae are stored and used as a propagation of NPV isolates.

Propagation of S/NPV-JTM 97C isolates

Propagation of S/NPV-JTM 97C isolates was done by pounding larvae that had been infected with S/NPV-JTM 97C by using mortar and pestil. The larvae were pounded until their bodies were destroyed, and then 1 ml of distilled water was added. The rough suspension was centrifuged at 3000 rpm for 2 minutes using a centrifuge. Then the supernatant was collected and separated by sediment. The supernatant was separated and then centrifuged at 6000 rpm for 20 minutes. The supernatant was again centrifuged in the same steps as before until a clean sludge was obtained. The final precipitate obtained was resuspended with sufficient distilled water. The suspension was diluted to facilitate the calculation of the concentration of Polyhedra Inclusion Bodies (PIBs).

Table 1. Treatment in this study

Code	Treatment
P0	Control
P1	10% lemon grass extract
P2	S/NPV 1,5x10 ⁶ PIB/ml
P3	S/NPV 1,5x10 ⁶ PIB/ml + 2.5% lemongrass extract
P4	S/NPV 1,5x10 ⁶ PIB/ml + 5% lemongrass extract
P5	S/NPV 1,5x10 ⁶ PIB/ml + 7.5% lemongrass extract
P6	S/NPV 1,5x10 ⁶ PIB/ml + 10% lemongrass extract

Lemongrass Extract

Lemongrass was separated from the branches and other impurities and then washed thoroughly. Dried lemongrass was blended to become a powder. The lemongrass powder was dissolved with methanol until completely submerged in a ratio of 1: 4 (w / v) while stirring with a glass stirring rod. Then shake for 1 x 24 hours. The extraction solution was filtered with filter paper. The lemongrass extract was obtained and then concentrated using a rotary vacuum evaporator at 65 ° C repeatedly. The product of the extraction was put into a sealed plastic bottle and stored.

Inoculation of Insects test

Spodoptera litura larvae were inoculated by feeding soybean leaves, which had been treated with the dipping method. The leaves used as feed are taken from soybean plants 35 days after planting. The leaf died was dipped in suspension at a concentration of 1.5×10^6 PIBs/ml. Then the leaves were dried and used as larvae died. Each test larvae was incubated in a rectangular palette (5 cm diameter).

Percentage of larvae stop eating was observed at 4, 8, 12, 18, 22 hours after application. When

observation stopped eating in the larvae that have been treated, the characteristics of the body surface color become dark. The eating activity was reduced and slow movement. Percentage mortality of *S. litura* larvae was observed every day after the application until the 7th day. The characteristics of larvae that die due to NPV are that the body becomes very soft and easily destroyed. The percentage of *S. litura* larvae that become pupae and adults was observed by counting the number of pupa and adults formed after being given treatment.

Data analysis

The data were analyzed with the F test at the 5% level and the further tests with the Least Significant Difference (LSD) at the 5% level by using SPSS software.

RESULTS AND DISCUSSIONS

Percentage of *S. litura* larvae that stop eating

The results showed that the *S. litura* larvae stopped eating at 4 hours after inoculation. In the treatment of 10% lemongrass extract (P2), the percentage of larvae that stopped eating was 13% and more significant than P3 up to P6 treatment. While

Tabel 2. Percentage of *S. litura* larvae that stop eating

Treatment	Number of <i>S. litura</i> (%)				
	4 HAI	8 HAI	12 HAI	18 HAI	22 HAI
P0	0 a	0 a	0 a	0 a	0 a
P1	13 a	17 a	17 a	43.3 b	100 c
P2	3 a	3 a	7 a	63.3 cd	90 b
P3	3 a	10 a	23 a	63.3 cd	100 c
P4	0 a	3 a	7 a	56.6 c	93.3 bc
P5	10 a	10 a	13 a	60 c	100 c
P6	17 a	20 a	20 a	73.3 d	100 c

Note: P0: Control, P1: 10 % lemongrass extract, P2: S/NPV-JTM 97C 1.5×10^6 PIB/ml, P3: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 2.5 % lemongrass extract, P4: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 5 % lemongrass extract, P5: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 7.5 % lemongrass extract, P6: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 10 % lemongrass extract and HAI (Hour After Inoculation). The data is transformed by the arcsin transformation formula $\sqrt{x + 0.5}$ and the numbers followed by the same letters in the same column show no significant difference at the 5% level of LSD test

Figure 1. Differences in mortality symptoms of *S. litura* larvae, (a) SINPV-JTM 97C, the larvae hang in an inverted "v" shape and secrete NPV, (b) a combination of SINPV-JTM 97C with lemongrass extract, reddish black larvae, and (c) lemongrass extract, shriveled and thin larvae.

the combination of SINPV-JTM 97C concentration 1.5×10^6 PIB/ml with 10% lemongrass extract (P7) was 17% and showed the highest value in all treatments (Table 1). These results were changed at 18 hours after inoculation, with the highest percentage in the combination of SINPV-JTM 97C concentration 1.5×10^6 PIB/ml with 10% lemongrass extract (P7). Each treatment showed a significant increase in the number of larvae to stop eating. After 22 hours after inoculation in P1 up to P6 treatment showed no significant difference (Table 2). The percentage value of larvae stopped eating was more significant than a single SINPV treatment. It showed the existence of synergism between SINPV and lemon extract. Compatibility occurred when SINPV and Lemongrass extract was combined. A factor

causing incompatibility between the two solutions was that Lemongrass extract has repellent properties (repellent). According to Ganjewala and Luthra (2009), Cymbopogon essential oils and constituents containing citral compounds (a mixture of geranial and neral) have been known to act as insecticides and insect repellents for a relatively long period. As a result of nature (repellent) possessed by fragrant lemongrass, the larvae had little or no eating the leaves given a combination treatment with SINPV so that the amount of polyhedral ingested and entering the body of the larvae were small.

Mortality of *S.litura* larva

The results showed that *Spodoptera litura* larvae mortality demonstrated a significant effect compared to control, where each treatment

Table 3. Percentage mortality of *S. litura* larvae

Treatment	Larvae mortality (%)						
	24 HAI	48 HAI	72 HAI	96 HAI	120 HAI	124 HAI	168 HAI
P0	0 a	0 a	0 a	0 a	0 a	0 a	0 a
P1	10 bc	20 c	27 d	40 c	63.3 b	73 b	73 ab
P2	7 ab	7 ab	10 bc	20 ab	56.6 b	86 b	86 b
P3	0 a	3 ab	10 bc	20 ab	63.3 b	76.6 b	86 b
P4	0 a	3 ab	7 ab	30 bc	73.3 b	80 b	90 c
P5	0 a	17 bc	23 cd	40 c	66.6 b	83.3 b	90 c
P6	17 c	30 c	33 d	46.6 d	73.3 b	86.6 b	96 c

Note: P0: Control, P1: 10 % lemongrass extract, P2: SINPV-JTM 97C 1.5×10^6 PIB/ml, P3: SINPV-JTM 97C 1.5×10^6 PIB/ml and 2.5 % lemongrass extract, P4: SINPV-JTM 97C 1.5×10^6 PIB/ml and 5 % lemongrass extract, P5: SINPV-JTM 97C 1.5×10^6 PIB/ml and 7.5 % lemongrass extract, P6: SINPV-JTM 97C 1.5×10^6 PIB/ml and 10 % lemongrass extract and HAI (Hour After Inoculation). The data is transformed by the arcsin transformation formula $\sqrt{x + 0.5}$ and the numbers followed by the same letters in the same column show no significant difference at the 5% level of LSD test

showed a percentage value above 85% at 124 and 168 HAI (Table 3). Combination treatment with 10% lemongrass extract showed the highest mortality rate of *S. litura* larvae. These results prove that the addition of lemongrass extract affects the NPV as the dose is added. On the 6th day after inoculation, the larvae were died with thick liquid coming out from its body and hanging to form an inverted "V." According to Bedjo (2017), the body of dead larvae will rupture and secrete a thick milk-brown liquid, which is NPV fluid and smells very unpleasant (Figure 1a). In a combination of NPV and Lemongrass extract, larval death symptoms still show characteristics of being infected with the NPV virus, i.e., the larval body looks reddish (Figure 1b). While the larvae that die due to single body lemongrass treatment, the body looks shiny, shrunken, and thin.

Percentage of Pupa Formed

The results showed that the pupae formed was slow in all treatment compared to the control (Table 4), which means that NPV and Lemongrass extract's larvae cause their life cycle to be longer. According to Hikail, Baeshen, & Said-Al Ahl (2017), the inhibition of toxic compounds found in botanical pesticides can disrupt the work system

that regulates insects' development and metamorphosis. The pupa formed is an abnormal pupa with black, puckered, and some rotten pupa colors. That is due to the death of the pupa before becoming an imago. It proves that NPV that is ingested and enters the larva's body can continue until the pupal stage but causes abnormalities. As a result, the tissue is damaged, which causes changes in pupa shape. Furthermore, according to Bedjo (2017a), pupae infected with SINPV at first the abdomen changes color to grayish-white, then the abdominal skin will become soft and cracked, and turbid fluid containing polyhedral comes out.

Percentage of Adults Formed

The results showed that *S. litura* adults showed an imago formed in a single 10% lemongrass treatment, a combination treatment of S/NPV-JTM 97C concentration of 1.5×10^6 PIB/ml with lemongrass (2.5% and 5%). The observations showed that larvae infected with S/NPV-JTM 97C could still live up to the imago stage even though their growth was longer than that of the control. Adults that appear in the treatment have a normal form. It might be caused by the S/NPV-JTM 97C dose applied is the middle dose, i.e., at a concentration of 1.5×10^6 PIB/ml so that the amount of

Table 4. Percentage of pupae formed in *S. litura* larvae

Treatment	Formed pupae (%)			
	9 DAI	10 DAI	11 DAI	12 DAI
P0	20 b	40 b	67 b	90 b
P1	0 a	0 a	7 a	17 a
P2	0 a	0 a	7 ab	3 a
P3	0 a	0 a	0 a	7 a
P4	0 a	0 a	3 a	3 a
P5	0 a	0 a	0 a	3 a
P6	0 a	0 a	3 a	3 a

Note: P0: Control, P1: 10 % lemongrass extract, P2: S/NPV-JTM 97C 1.5×10^6 PIB/ml, P3: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 2.5 % lemongrass extract, P4: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 5 % lemongrass extract, P5: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 7.5 % lemongrass extract, P6: S/NPV-JTM 97C 1.5×10^6 PIB/ml and 10 % lemongrass extract and HAI (Hour After Inoculation). The data is transformed by the arcsin transformation formula $\sqrt{x + 0.5}$ and the numbers followed by the same letters in the same column show no significant difference at the 5% level of LSD test

According to Asri, Rasminah, Rahardjo, & Sumitro (2013), in their development, the host individual can escape NPV infection's influence with the mechanism of "maturation resistance," in line with age will increase resistance or decrease sensitivity to NPV. The peritrophic membrane as a defense mechanism is still functioning optimally against insect pathogens that enter the body (Sanjaya et al., 2011).

CONCLUSIONS

The combination of S/NPV-JTM 97C with lemongrass extract is compatible on stop eating, mortality, pupa, and adult formation. The combination treatment of S/NPV-JTM 97C concentration (1.5×10^6 PIB/ml with 10% extract) showed the highest percentage value at stopping eating until the adults compared to the application of S/NPV-JTM 97C and single lemongrass. The treatment is compatible and synergistic, so that it is effective in controlling *S. litura*.

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