https://doi.org/10.48047/AFJBS.5.4.2023.392-400

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Efficacy and inclusion body productivity ability of Spodoptera litura Nucleopolyhedrovirus (SpltNPV) on armyworm Spodoptera litura Fabricius (Lepidoptera: Noctuidae) in the Mekong Delta of Vietnam

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Volume 5, Issue 4. Jun 2023

Received: 10 May 2023

Accepted: 25 May 2023

Published: 20 Jun 2023
doi:10.48047/AFJBS.5.4.2023.392-400

Abstract

To identify the infectivity of four Spodoptera litura nucleopolyhedrovirus (SpltNPV) isolates from diseased larvae of the armyworm: Spodoptera litura (SpltNPV-CT, SpltNPV-AG, SpltNPV-DT, and SpltNPV-KG), collected from the vegetable fields in Can Tho city, An Giang, Dong Thap, and Kien Giang provinces of Viet Nam against S. litura in laboratory conditions. Then, the best SpltNPV isolates will be screened for studies on the development stage of S. litura. The results showed that three isolates of SpltNPV-CT, SpltNPV-AG, and SpltNPV-KG were highly effective on armyworms, over 94% of larvae killed at 9 days after inoculation (DAI) using the droplet feeding method on the 2nd instar larvae. The isolate of SpltNPV-KG had a percent mortality on the 3rd instar of 100% at 7 DAI. Two isolates of SpltNPV-CT and SpltNPV-KG controlled the 4^{th} instar larva of S. litura from 82.1 to 100% at 10 DAI. The maximum yield of occlusion bodies of the fourth Nucleopolyhedrovirus was isolated from 2.87 x 10^9 to 6.09 x 10^9 OBs/larva (4^{th} instar larvae). The LD_{50} value of SpltNPV-KG isolate for the 4^{th} instar larva of S. litura was determined as 1.06 x 10^8 occlusion bodies/ml.

Introduction

The armyworm, Spodoptera litura (Fabricius), is a common pest among lepidopterans and belongs to the family Noctuidae [1]. The S. litura larvae become serious pests by feeding on many economically valuable crops such as soybean, cabbage, mung bean, groundnut, and corn. Feeding by insects causes damage to the leaves, stems, and reproductive parts of plants, which results in farmers losing plenty of their produce and income [2]. The S. litura seriously limits the yields in Korea and the Mekong Delta region of Vietnam.

The increasing number of S. litura infestations in the Mekong Delta is due to suitable weather and a rise in intensive farming. Usually, farmers count on using chemicals to deal with these infestations, but applying synthetic insecticides in large and random quantities has had unfavorable results [3].

Whereas, using synthetic insecticides in large quantities and random quantities showed unfavorable outcomes [4]. Therefore, S. litura now often resists various categories of insecticides, making it difficult to use. As a result of overusing pesticides, polluted the environment, affecting other living creatures, and hazardous chemical leftovers are causing adverse effects on food crops and human health, ultimately disturbing the ecosystem. Considering this disturbance, it is important to look for sustainable and eco-friendly ways to handle pests . Using natural treatments to control the prevalence of associated diseases is required and beneficial. The use of entomopathogenic viruses from the Baculoviridae family has increased due to their specific targeted function and effectiveness on insects from the Lepidoptera group [5]. Arthropod infections, particularly Lepidoptera insects, are caused by double-stranded DNA viruses called baculoviruses. In this group, members of the genus Nucleopolyhedrovirus (NPV) produce occlusion bodies (OBs) to surround the virions and carry from one host to another [6]. The S. litura nucleopolyhedrovirus (SpltNPV) can infect only S. litura larvae and has displayed a high level of virulence [7]. If weakened larvae take in OBs, the virus spreads in their bodies, causing disease, tissue death, and mortality, which keeps pest numbers under control [8].

The SpltNPV being used as a biopesticide offers several advantages [8]. Its specificity means diseases reach pests and not beneficial insects or pollinators. Besides, SpltNPV is easy to degrade and does not leave residues, which supports the IPM and sustainable agriculture concepts. Several trials conducted in different regions have demonstrated that SpltNPV decreases the population of S. litura, making it seem like a reliable biocontrol agent [7, 9].

In Vietnam, examining SpltNPV types in the environment helps create biopesticides suited to the local environment [10]. Identifying and characterizing viruses found locally can increase the success of biocontrol by matching pests and local conditions. The IBs in various SpltNPV strains is necessary for choosing the best production methods and obtaining plentiful, high-quality NPV formulations. The SpltNPV has some promising qualities, though many other things can affect its operation in the field. Viruses can become unstable and less infectious when the environment contains different temperatures, high humidity, or UV radiation. To illustrate, findings show that direct sunlight can inactivate SpltNPV, so it is important to add protection to the SpltNPV to keep it from degrading in UV light [11]. At the same time, a larva's developmental stage impacts the virus, since young ones are more likely to get infected.

Several elements, such as the larval stage hosts are in, the amount of virus in the insects, and the temperature during infection, can all affect the ability of IBs to help spread SpltNPV [12]. It has been shown that "occy strains" can produce better and larger insect-bait at 25°C when larvae are infected during their early final larval growth phase. Therefore, it produces SpltNPV for biopesticides on a large scale in an eco-friendly manner. Besides, genetic variation can change the behavior of SpltNPV as well as its functions [7]. Examining animal isolates from different regions has shown that the viral pathogenicity, how fast the disease kills the animal, and the amount of toxin secreted are all different. Different plant types and pests must be considered because the best strains vary greatly from region to region. Solitary lacewing virus biopesticides could make a big dent in using chemical insecticides in vegetable farming in the Mekong Delta. Adding SpltNPV to IPM systems can help reduce pests, safeguard the environment and keep food safe.

This study aims to assess how effective and how much silk it can produce four local strains of SpltNPV taken from infected S. litura larvae raised in the Mekong Delta of Vietnam. To find the best choices for biopesticide production, this study investiages the virulence, IB yield and stability in the environment of each strain. The findings will help develop effective biological control methods to control S. litura, maintaining sustainable agriculture in the area.

MATERIALS AND METHODS

Insect rearing

S. litura larvae were collected from fields in Can Tho city and propagated in the laboratory at the Department of Plant Protection, College of Agriculture & Applied Biology, Can Tho University. The larvae were fed on an artificial diet and reared in a plastic box (30 x 20 x 8 cm) under room conditions with a light regime (Light for 16hrs: Dark for 8hrs), until pupation. An Individual adult was transferred into paper bags (20 x 30 cm) with a 10% sugar solution for food. Newly laid eggs were sterilized with 3% formaldehyde for 10 min and washed with sterile distilled water. Hatched larvae were fed an artificial diet and used for experiments.

Nucleopolyhedrovirus

SpltNPV was isolated from a diseased larva collected from a vegetable field in An Giang, Dong Thap, Kien Giang provinces, and Can Tho city. The OBs were produced in the fourth instars of S. litura on artificial diet. The virus-killed larvae were triturated on 0.1% (wt/v) sodium dodecyl sulfate (SDS) and centrifuged at 500 rpm for 5 min, and the supernatant was centrifuged at 3000 rpm for 10 min. The resulting pellet comprising viral OBs was resuspended in sterile distilled water, counted using a Thoma hemocytometer under a phase-contrast microscope [13], and stored at 4^0C before use.

* Efficacy of four strains of SpltNPV on the Spodoptera litura

RCD conducted experiments using viruses collected in four provinces of the Mekong Delta, including Can Tho city, Kien Giang, Dong Thap, and An Giang provinces. Each experiment with 4 replications, each repetition containing 24 larvae of S. litura. The concentrations in each bioassay were 5 x 10^6 OBs/ml and control (sterile distilled water). The droplet feeding method fed the larvae with an OBs suspension [14, 15]. The second, third, and fourth instars, beginning to molt, as determined by head capsule, were transferred to plastic containers without food. After 12 h, the newly molting larvae were collected. Each larva was infected by feeding 2 µl (second instar), 2.5 µl (third instar), 3.0 µl (fourth instar) of an OBs suspension containing 10% sugar water + 1% red coloring (Kyoritu Food Co. Ltd., Tokyo). Larvae that completely ingested the droplet of suspension were individually transferred to 30 ml cups containing fresh artificial diet. Larvae were reared and observed daily for mortality and calculated using by formulas of Abbott et al., [16]. The cadavers were collected, transferred to sterile vials, and sample were frozen immediately. The cadavers were homogenized individually and transferred to 50 ml centrifuge tubes, and then the volume was made up to 25 ml with distilled water. After that, the enumeration of polyhedra of the following parameters were calculated:

$$\text{Yield/larva (OB)} = \frac{\text{OB/ml x suspension volume (ml)}}{\text{Total number of cadavers}}$$

Yield per 100 inoculated larvae (OB) = yield/larva x corrected larval mortality (%)

$$\text{Productivity ratio} = \frac{\text{Yield/larva (OB)}}{\text{Ob inoculated/larva}}$$

The effect of the SpltNPV on the stage of Spodoptera litura

The experiment used virus isolates, which were best chosen in experiment one. Second, third and fourth instars larvae were individually placed on a fresh artificial diet, after 24 h, each larvae was infected by feeding 2 µl (second instars), 2.5 µl (third instars), 3.0 µl (fourth instars) of OBs suspension containing 10% sugar water + 1% red coloring (Kyoritu food Co. Ltd., Tokyo). The concentrations used in the experiments were 10^4, 10^5, 10^6, 10^7 and 10^8 OBs/ml. Mortality was recorded daily or until moth emergence.

Statistical analysis

Data in percentages were transformed to arcsine $\sqrt{\text{percent}}$ and the data from OB yield were subjected to log - transformed and then analyzed. The larval counts were also transformed to $\sqrt{x + 0.5}$ values. Analysis of variance (ANOVA) and all mean comparisons (by DMRT) were done using MSTAC software. Mortality data were analyzed by probit analysis using the microcomputer program POLO-PC [17].

Results and Discussion

Percent mortality of Nucleopolyhedrovirus against S. litura

Table 1 shows that all SpltNPV isolates were efficacious to second instar larvae in laboratory conditions.

At the time of three days after treatment, the use of droplet feeding method with the concentration of 5 x 10^6 OBs/ml, virus strains collected in Kien Giang showed high efficiency (43.1%) but not significantly different at 1% with isolates collected in An Giang (36.1%) and Can Tho (20.8%) and completely different compared with collected at Dong Thap (5.6%).

Table 1. Efficacy of SpltNPV against S. litura second instar larva

Treatments	Corrected mortality (%) at days after inoculation					
	3	4	5	6	8	9
A	36.1[a]	45.8[b]	62.1[b]	81.5[b]	94.3[b]	94.3[a]
B	20.8[a]	37.5[b]	47.8[b]	66.2[b]	89.9[b]	97.0[a]
C	5.60[b]	12.5[c]	12.6[c]	14.3[c]	28.7[c]	31.7[b]
D	43.1[a]	73.6[a]	95.8[a]	97.1[a]	100[a]	100 [a]
CV (%)	2.4	3.4	2.9	4.1	2.0	2.1
F	**	**	**	**	**	**

Means followed by a common letter in the same column are not significantly different at 1% (**) level by DMRT. CV: Coefficient of variation, A: virus strain collected in An Giang province (SpltNPV-

AG), B: virus strain collected in Can Tho city (SpltNPV-CT), C: virus strain collected in Dong Thap province (SpltNPV-DT), D: virus strain collected Kien Giang province (SpltNPV-KG)

At the time of 4, 5 and 6 days after treatment, the effectiveness of SpltNPV isolates increased; the results showed that virus strains collected in Kien Giang increased the most from 43.1% (3 DAI) to 97.1% (6 DAI). After 8 days result showed that the strain collected of Kien Giang increased 100% optimal efficiency compared with the remaining strains from An Giang, Can Tho, Dong Thap that achieved 94.3%, 89.9% and 28.7% respectively; in the three strains, the strain collected in Dong Thap had the lowest effectiveness after 9 days of treatment achieved 31.7%.

Table 2. Efficacy of SpltNPV against S. litura third instar larva

Treatment (Obs/mL)	Corrected mortality (%) at days after inoculation		
	3	5	7
A	1.40^{b}	11.1^{c}	47.1^{c}
B	5.60^{bc}	33.8^{b}	78.3^{b}
C	12.5^{ab}	35.4^{b}	41.2^{a}
D	19.5^{a}	74.7^{a}	100^{a}
CV	53.9	22.0	13.0
F	**	**	**

Means followed by a common letter in the same column are not significantly different at 1% (**) level by DMRT, CV: Coefficient of variation, A: virus strain collected in An Giang province (SpltNPV-AG), B: virus strain collected in Can Tho city (SpltNPV-CT), C: virus strain collected in Dong Thap province (SpltNPV-DT), D: virus strain collected Kien Giang province (SpltNPV-KG)

The tested third and fourth larvae of S. litura fed on sugar droplets treated with a suspension containing 5 x 10^6 of OBs/mL. Table 2, Table 3 showed that the corrected mortality of larvae increased with time after inoculation. Most of the larvae were killed up to 100% (SpltNPV-KG isolate) at 7 days (third instar) and 10 days (fourth instar).

Table 3. Efficacy of SpltNPV against S. litura fourth instars larvae

Treatment (Obs/mL)	Corrected mortality (%) at days after inoculation			
	3	5	7	10
A	0.0^{b}	0.0^{d}	13.9^{c}	36.9^{c}
B	1.4^{b}	14.4^{c}	49.3^{b}	82.1^{b}
C	5.6^{a}	23.1^{b}	34.0^{a}	42.7^{c}
D	5.6^{a}	30.4^{a}	84.7^{a}	100^{a}
CV (%)	56.9	14.6	20.9	6.9
F	**	**	**	**

Means followed by a common letter in the same column are not significantly different at 1% (**) level by DMRT, CV: Coefficient of variation, A: virus strain collected in An Giang province (SpltNPV-AG), B: virus strain collected in Can Tho city (SpltNPV-CT), C: virus strain collected in Dong Thap province (SpltNPV-DT), D: virus strain collected Kien Giang province (SpltNPV-KG)

The result of the above experiment makes it possible to select NPV strain collected in Kien Giang province that has high effectiveness in controlling S. litura as the biological control agent.

*** Occlusion bodies yield of Nucleopolyhedrovirus**

Table 4. Effect of larval stage of S. litura on the yield of SpltNPV isolates at an inoculation dose of 5 x 10^6 OBs/larva in laboratory condition

Isolates	Larval instars	Yield/larva (x 10^9OB)	Yield per 100 inoculated larva (x 10^{11}OB)	Productivity ratio (x 10^3)
SpltNPV-AG	Second	1.72^b	1.63^c	0.34^b
	Third	2.81^b	1.04^c	0.56^b
	Fourth	4.24^{ab}	1.97^c	0.85^b
SpltNPV-CT	Second	2.12^{bc}	2.05^{bc}	0.42^b
	Third	2.43^b	1.99^{bc}	0.49^b
	Fourth	3.94^{ab}	3.24^{ab}	0.79^b
SpltNPV-DT	Second	2.51^b	0.79^c	0.50^b
	Third	2.66^b	2.45^b	0.53^b
	Fourth	2.87^b	1.22^b	0.57^b
SpltNPV-KG	Second	2.50^b	2.50^b	0.50^b
	Third	1.62^c	0.68^c	0.32^b
	Fourth	6.09^a	6.10^a	1.22^a

In a column, for each isolate means followed by different letters are significantly different ($p< 0.05$) by DMRT

Results showed a significant influence of larval age on the percentage of larval mortality, yield/larvae, yield per 100 inoculated larvae, and productivity ratio of isolates (Table 4). Among the larval ages evaluated, the fourth instars of isolates SpltNPV-AG, SpltNPV-CT, and SpltNPV-KG showed the highest yield/larva, which were 4.24 x 10^9, 3.94 x $10^{9,}$ and 6.09 x 10^9 OB/ larva (respectively), followed by SpltNPV-DT isolate by an amount of 2.87 x 10^9 OB/ larva. The initial larval age was critical for obtaining higher OB yield. Also, among the isolates tested in this study, the SpltNPV-KG isolate achieved the maximum productivity ratio 1.22 x $10^{3,}$ which was 1.44 and 1.54 times more than SpltNPV-AG and SpltNPV-CT isolates. However, the maximum OBs yield was collected from the fourth larva for all the isolates tested in this study, from 2.87 x 10^9 to 6.09 x 10^9OBs/larva, the yield per 100 larva inoculated larvae were1.97 to 6.10 x 10^{11} OBs.

Considerable studies had previously stated the same trends on different insects [18-20]. Most of them attained high productivity ratio of virus yield as larval age increased. The time required for multiplication of the virus in the host's body is most related to the age of larvae inoculated. Monobrullah and Nagata et al., reported that 9 days old S. litura larvae at the fourth instar treated with 4.8 x 10^6 OB/larva through diet resulted in the maximum productivity of the NPV [21].

Parameters of dose - mortality analysis of S. litura larvae inoculated with SpltNPV-KG

Table 5. Log-dose-probit parameters for SpltNPV-KG against S. litura larvae instar using droplet feeding

Stage at inoculation	Total no. of larvae used	DAI[a]	Slope	LD_{50} (OBs/mL	Limit 95% (OBs/mL)		χ^2	df
					Upper	Lower		
Second instar	360	5	0.28	2.4×10^5	4.05×10^7	2.15×10^5	4.71	2
Third instar	360	5	0.30	2.5×10^7	6.7×10^8	1.5×10^6	4.51	2
Fourth instar	360	5	0.30	1.06×10^8	3.1×10^8	3.4×10^6	4.9	2

DAI: Days after inoculation

The percentage of mortality of S. litura larvae increases increasingly associated with doses of SpltNPV, parameters of the log do probit mortality larvae in the Table. 5 the slopes of the regression lines varied from 0.28 to 0.3 among treatments, but their no significant differences among the instars ($p>0.05$). However, the median lethal dose (LD_{50}) increased with increasing larval instar at inoculation. The LD_{50} values of the second, third, and fourth larvae are 2.4×10^5, 2.5×10^7 and 1.06×10^8 OBs/larva (respectively). SpltNPV has dual pathology depending on the inoculation dose. Inoculation of a S. litura larvae with a relatively low dose (e.g., approx. 10^2 OBs/larvae) of SpltNPV caused chronic disease. However, inoculation with a high dose (e.g., approx. 10^6) of SpltNPV resulted in acute larval death.

Table 6. The effect of SpltNPV-KG on the stage of S. litura in the laboratory condition

Monitoring targets	NPV	Control	Difference
The rate of incomplete pupae (%)	22.7	4.2	18.5
The rate of dead pupae (%)	21.0	5.9	15.1
The rate of pupae transfer to the moth (%)	45.5	90.5	45.0
Spawning time of female moth (days)	4.5	6.0	1.5
The amount of laid eggs (eggs/female)	175.8	1250.8	1075.0

The SpltNPV virus affected the molting of S. litura in the pupal stage and the fecundity. The results of virus transmission on the S. litura shown in Table 6 show that:

When the S. litura is infected at the lower levels that are insufficient to cause death, the worms would be alive to the pupal stage, the rate of incomplete pupae accounted for 22.7% (control 4.2%).

Some of S. litura continued to die at the pupal stage, with this ratio of about 21% (control 5.9%). SpltNPV continuously to affected the moth, the ratio of the pupae transferred to moth completely (no defect) was only 45.5% while the control was 90.5%. Besides, SpltNPV also affected spawning time and fertility: 175.8 eggs / female and 1250.8 eggs / female adult (control)

Thus, it can be seen that the S. litura infected by the NPV virus often causes death, but the individuals that survive can have a change in developmental time and reduction in size, fecundity, vitality and egg viability [22-24].

In addition, another important factor is that SpltNPV causes chronic infection when the larvae are lightly infected, reduces the ability of pupation, molting of pupae, and can pass through the next generation. This creates the infection inherited from one generation to another, from individuals through other individuals in the digestive tract. In particular, it causes acute diseases when they infected amount of OBs if an infected small amount of OBs then the adults would reduce the fecundity. The next generation may also die (vertical infection). According to the studies, the Baculovirus group can also be transmitted vertically from the moth to the larvae. This transmission occurs because the eggs are virus-infected inside [25-27].

Conclusion

The SpltNPV as an established viral agent of pest control, SpltNPV has some beneficial characteristics as a viral agent to control S. litura larvae. The most important characteristic of SpltNPV is that it kills the host more quickly. Larvae died at 7 days after inoculation of second, third, and fourth instar with 5 x 10^6 OBs/ml, about 100% larvae died (SpltNPV-KG isolate). As crop damage depends largely on the speed of attack of armyworm, application of SpltNPV may contribute to further reduction of crop damage by reducing the survival time of infected S. litura larvae.

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