Journal of Biological Control, 26 (3): 234–239, 2012

Research Article

In vivo enhancement of nucleopolyhedrovirus infection in *Helicoverpa armigera* (Hübner) by the granulovirus of *Spodoptera litura* Fabricius

S. JEYARANI*, N. SATHIAH and P. KARUPPUCHAMY

Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

** Corresponding author: E-mail:jeyajawahar@sify.com*

ABSTRACT: Biopesticides based on baculoviruses offer great scope as promising viral insecticides against *Helicoverpa armigera* (Hübner). However, an important limitation in the use of baculoviruses is their reduced efficacy against grown up larvae. The infectivity of NPV against grown up larvae were reported to be enhanced by the presence of a viral enhancing factor (VEF) in granuloviruses (GV). Hence, we investigated the possible interaction of GVs of *Spodoptera litura* (SlGV), *Agrotis segetum* (AsGV), *Plutella xylostella* (PxGV), *Achaea janata* (AjGV) and *Chilo infuscatellus* (CiGV) separately with the NPV of *H. armigera* (HearNPV) against second, third, fourth and fifth instar *H. armigera.* Of the GVs tested with HearNPV, SlGV alone synergized HearNPV action with reduced LC_{50} values. The integration of SlGV with HearNPV resulted in 9.38, 10.93, 10.70 and 13.32 fold reduction in LC_{50} values against the second, third, fourth and fifth instars respectively. Tests conducted with heat inactivated (at 75, 80, 85 and 121°C for 10 min) and heat shielded SlGV could give similar effects with HearNPV indicating that the cause for the virulence enhancement rests with the capsular protein and not the virions of the SlGV, since the virions could be inactivated at temperatures more than 70°C. Integration of SlGV and HearNPV was found to shorten the LT_{50} values.

KEY WORDS: *Helicoverpa armigera,* Granulovirus, nucleopolyhedrovirus, enhancing factor.

(Article chronicle: Received: 3-127-3-2012-2012 Revised: 1-9-2012 Accepted: 14-9-2012)

INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is a major pest on a wide range of crops in Europe, Africa, Asia and Australia. Annual yield losses due to this pest have been estimated to be over USD2 billion worldwide (Sharma, 2005) and 300-400 million per annum in India alone, this is apart from the expenses spent on insecticides to control the insect pest (Shanower *et al*., 1999). Biopesticides based on baculoviruses offer great scope as promising viral insecticides against *H. armigera* due to their host specificity, lack of residue and their unlikely resistance development by the target insects, besides being safe to the environment. However, an important limitation in the use of baculoviruses as biological control agents is their slow speed of action, as for many baculoviruses it may take four to fourteen days to kill their host (Gröner, 1986). Granuloviruses (GV) are known to enhance the infectivity of NPVs and shorten the kill time due to the presence of a viral enhancing factor (VEF). Ping *et al.,* (1999) found that larval mortality of *Argyrogramma agnate* (Staudinger) was enhanced from 57.1 percent to 94.4 per cent and the LT_{50} was reduced from 8.6 days to 5.8 days when bioassayed with viral enhancing factor of TnGV along with AcMNPV. Similarly, Shapiro (2000) reported that the *Spodoptera frugiperda* GV (SfGV) reduced the LC_{50} of *Lymantria dispar* NPV (LdMNPV) by 13 fold. However, Hackett *et al.* (2000) reported that multiple viral infections may result in antagonistic, additive or synergistic effects. Hence, keeping these in view, investigations were carried out to determine the interaction of GVs of *Spodoptera litura* (SlGV), *Agrotis segetum* (AsGV), *Plutella xylostella* (PxGV), *Achaea janata* (AjGV) and *Chilo infuscatellus* (CiGV) separately with the NPV of *H. armigera* (HearNPV) against second, third, fourth and fifth instar *H. armigera.*

MATERIALS AND METHODS

Test insect cultures

The larvae used in the studies were obtained from the Biocontrol Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore and were maintained on chickpea based semi-synthetic diet (Shorey and Hale, 1965).

Multiplication and Standardization of virus inoculum

The HearNPV and GVs *viz*., SlGV, AsGV, PxGV, AjGV and CiGV maintained in the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore were used for this study. Since, the samples of different viruses were stored under refrigerated condition (4°C) for various periods, initial passage was made in early fifth instar. The virus was propagated *in vivo* by diet surface treatment method and the POB (polyhedral occlusion bodies) and OB (occlusion bodies) strength was assessed using a haemocytometer (Weber, England) of depth 1mm and 0.02 mm respectively for NPV and GV (Evans and Shapiro, 1997). The stock suspensions were stored at 4°C for further studies.

Interaction effect of HearNPV and granulovirus (GV)

Preliminary experiments were conducted to determine the interaction of GVs of *S. litura* (SlGV), *Agrotis segetum* (AsGV), *P. xylostella* (PxGV), *Achaea janata* (AjGV) and *C. infuscatellus* (CiGV) @ 786.35 OB/mm^2 (field dose) with serially diluted HearNPV doses of 19.659, 3.932, 0.786, 0.157, 0.031 and 0.006 POB/m^2 against second instar *H. armigera* larvae. The changes in LC_{50} were worked out. Similarly, the procedure was repeated for third, fourth and fifth instar larvae with ten fold increase in the HearNPV dosages for each instar. Each dose was tested with 30 larvae of uniform size. The potentiation effect (if any) of GV on HearNPV against each instar was worked out by the formula:

$$\text{Potentiation Index} = \frac{LC_{50} \text{ of HearNPV alone}}{LC_{50} \text{ of HearNPV+GV}}$$

Interaction effect of heat inactivated SlGV and heat shielded HearNPV against *H. armigera*

Tests were also conducted to determine the possible causes for the enhanced infectivity rests with the infective unit of the SlGV or with the capsular protein. Hence, bioassays were conducted with combination of SlGV (786.35 OB/mm^2) exposed to temperatures *viz*., 75, 80, 85 and 121°C for 10 min and heat shielded HearNPV @ LC_{50} doses. The experiment was conducted against second, third, fourth and fifth instar larvae of *H. armigera* and each treatment was replicated three times with thirty larvae each. Observations on mortality were recorded from 3 to 10 days after treatment. The doses studied against different instars are given below:

Instar	HearNPV (POB/mm^2)	SlGV (OB/mm^2)
Second	0.017	
Third	0.157	786.35
Fourth	1.241	
Fifth	16.055	

Interaction effect of lowest doses of SlGV with HearNPV against *H. armigera*

To determine the lowest doses of GV that could give maximum mortality with HearNPV against different instars of *H. armigera*, different doses of SlGV (1/10 and 1/100 fold less than the field dose) were combined simultaneously with LC_{50} dose of HearNPV. This was compared with the individual effects so as to study the relative speed of kill. Three replications with thirty insects were maintained for each treatment. Observations on the mortality were recorded at 12 h intervals from third to tenth day of treatment. A control was maintained separately.

Treatment	HearNPV (POB/mm^2)*	SlGV (OB/mm^2)
1.	LC_{50}	–
2.	–	7.86
3.	–	78.63
4.	LC_{50}	7.86
5.	LC_{50}	78.63

* HearNPV @ LC_{50} doses for respective instars studied

Statistical analysis

The concentration and time mortality responses of various experiments were subjected to probit analysis (Finney, 1962) using a statistical package for Social Sciences (SPSS), Ver. 10.00 SPSS Inc., USA. The analysis of variance in different experiments were carried out in IRRISTAT ver. 3.1., Biometric unit, IRRI, Philippines and the means were separated by Duncan's new Multiple Range Test (DMRT) (Duncan, 1966) available in the package.

RESULTS AND DISCUSSION

Interaction effect of HearNPV and granuloviruses (GV)

Of the GVs tested with HearNPV, SlGV alone synergized HearNPV action with reduction in LC_{50} values, while, the interaction was neutral with AsGV, PxGV, AjGV and CiGV against different instars tested (Table 1). Results of the bioassays revealed that the combination of SlGV with HearNPV potentiated the efficacy of HearNPV with a potentiation index of 9.38, 10.93, 10.70 and 13.32 fold against the second, third, fourth and fifth instars respectively than other GVs tested (Table 2). The HaGV was reported to enhance the NPV caused infections of the heterologous host *viz*., cabbage looper, *Trichoplusia ni* (Hübner) (Granados, 1990) and *Lymantria*

Table 1. Interaction effect of different GVs with HearNPV against second, third, fourth and fifth instar *Helicoverpa armigera* larvae

Treatments[$ab]	LC_{50} (POB/mm^2)			
	II Instar	III Instar	IV Instar	V Instar
HearNPV	0.015	0.153	1.241	16.055
HearNPV + PxGV	0.016	0.131	1.710	16.481
HearNPV + CiGV	0.017	0.129	1.430	15.649
HearNPV + SlGV	0.0016	0.014	0.116	1.205
HearNPV + AjGV	0.016	0.134	1.489	15.325
HearNPV + AsGV	0.016	0.134	1.951	17.815

[$] Number of insects used per treatment was 180
[a] LC_{50} doses of HearNPV for respective instars were used
[b] In all the GV treatments, the dose used was 786.35 OB/mm^2

Table 2. Potentiation effect of different GVs with HearNPV against second, third, fourth and fifth instar *Helicoverpa armigera* larvae

Treatments[$ab]	Potentiation Index			
	II Instar	III Instar	IV Instar	V Instar
HearNPV	1.00	1.00	1.00	1.00
HearNPV + PxGV	0.94	1.17	0.73	0.97
HearNPV + CiGV	0.88	1.19	0.87	1.03
HearNPV + SlGV	9.38	10.93	10.70	13.32
HearNPV + AjGV	0.94	1.14	0.83	1.05
HearNPV + AsGV	0.94	1.14	0.64	0.90

[$] Number of insects used per treatment was 180
[a] LC_{50} doses of HearNPV for respective instars were used
[b] In all the GV treatments, the dose used was 786.35 OB/mm^2

Table 3. Effect of heat inactivated and heat shielded SlGV with HearNPV against *Helicoverpa armigera*

Treatments	% Mortality[$]			
	II Instar	III Instar	IV Instar	V Instar
HearNPV* + SlGV heat inactivated (75°C)	86.67[a]	87.78[a]	78.89[ab]	76.67[a]
HearNPV* + SlGV heat inactivated (80°C)	85.56[a]	85.56[a]	82.22[a]	77.78[a]
HearNPV* + SlGV heat inactivated (85°C)	84.44[a]	82.22[a]	76.67[b]	74.44[a]
HearNPV* + SlGV heat inactivated (121°C)	56.67[b]	54.45[b]	51.11[c]	50.00[b]
HearNPV* + SlGV without inactivation	85.56[a]	86.67[a]	80.00[a]	76.67[a]
HearNPV* alone	57.78[b]	51.11[b]	45.56[d]	44.44[c]
SlGV** without inactivation	0.00[c]	0.00[c]	0.00[c]	0.00[c]

$ In a column, means followed by similar letters are not significantly different ($P = 0.05$) by DMRT.
* HearNPV @ LC50 dose for respective instars
** SlGV @ 786.35 OB/mm^2 for all the instars

dispar (Linnaeus, 1758) NPV (LdNPV) (Shapiro, 2000). This is in agreement with the present findings. It was already reported that the integration of HaGV with HearNPV could reduce the infectivity of HearNPV in the homologous host, *H. armigera* (Jeyarani, 2010) and hence in the present investigations the HaGV was not tested.

Interaction effect of heat inactivated and heat shielded SlGV with HearNPV against *H. armigera*

Tests were conducted to determine whether the possible causes for the enhanced infectivity rests with the infective unit of the SlGV or with the capsular protein. The bioassays with heat inactivated and heat shielded SlGV with HearNPV showed that both the SlGV could give similar effects. The results of the study indicated that even after heat inactivation at temperatures 75 or 80°C, SlGV effectively potentiated the action of HearNPV and the results were comparable to a combination of heat shielded SlGV. The interaction effect of HearNPV with heat inactivated SlGV at 121°C was statistically inferior to heat inactivation at lower temperature (Table 3). From this, it is inferred that the cause for the virulence enhancement rests with the capsular protein and not the virions which could be inactivated at temperatures more than 70°C. This is in confirmation with the findings of Tanada (1959) against *Pseudaletia unipuncta* (Haworth). They found that the heat inactivated PuGV was still capable of enhancing the virulence of unheated PuNPV and suggested that the GV retained its synergistic property within the inclusion body. However, SlGV heated at 121°C does not show any virulence enhancement which might be attributed to the denaturation of capsular protein at 121°C.

Interaction effect of lowest doses of SlGV with HearNPV against *H. armigera*

Investigations revealed that combination of lowest doses of SlGV with HearNPV also increased the mortality of different instars significantly than HearNPV alone. Among them, combination of LC_{50} dose of HearNPV with SlGV at 78.63 OB/mm^2 recorded significantly the highest mortality consistently against all the instars tested. It was followed by simultaneous treatment of LC_{50} dose of HearNPV with SlGV at 7.86 OB/mm^2. Considering the lethal time response, integration of SlGV was found to shorten the LT_{50} values for NPV (Tables 4). Tanada (1985) and Hukhara *et al*. (1987) demonstrated the synergistic effect of the Hawaiian strain of *P. unipuncta* GV on PuNPV. Lepore *et al*. (1996) subsequently isolated a protein factor (enhancing) from *T. ni* GV that enhanced NPV infection in *T.ni.* It was also reported that the *Spodoptera frugiperda* (J.E. Smith) GV (SfGV) reduced the LC_{50} of LdNPV by 13 fold but had no effect on LT_{50} (Shapiro, 2000). This was in support of the present findings where in the LC_{50} of HearNPV was reduced by 5.75 to 13.32 folds against different instars when combined with SlGV. Similarly, Chakraborty *et al*. (2003) showed that the virulence of *Mythimna separata* (Walker) NPV (MsNPV) could be enhanced by 3.33 (Log_{10}) index with a reduction of LC_{50}- by nearly 2000 times, when MsNPV was combined with a constant dose (0.01 ml) of viral enhancing protein fraction from SlGV. This was in agreement with the present findings.

The potentiation of HearNPV by the SlGV may be due to the ability of the enhancin of GV to mediate the attachment of virus particles to epithelial cells or they may act as proteolytic enzymes that could destroy the

Table 4. Interaction effect of HearNPV with different doses of SlGV against second, third, fourth and fifth instar larvae of *Helicoverpa armigera*

Treatments*		II Instar		III Instar I		V Instar		V Instar	
HearNPV (dose)	SlGV (OB/mm^2)	% Mortality	LT_{50} (h)	% Mortality	LT_{50} (h)	% Mortality	LT_{50} (h)	% Mortality	LT_{50} (h)
LC_{50}	7.86	72.22 ^{b}	92.83	64.44 ^{b}	94.04	63.33 ^{b}	95.91	58.89 ^{b}	97.82
LC_{50}	78.63	86.67 ^{a}	87.03	76.67 ^{a}	88.20	72.22 ^{a}	89.88	65.56 ^{a}	95.11
LC_{50}	–	58.89 ^{c}	99.52	53.33 ^{c}	100.31	50.00 ^{c}	102.92	47.78 ^{c}	104.46
–	7.86	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
–	78.63	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* In a column, means followed by similar letters are not significantly different ($P \leq 0.05$) by DMRT.

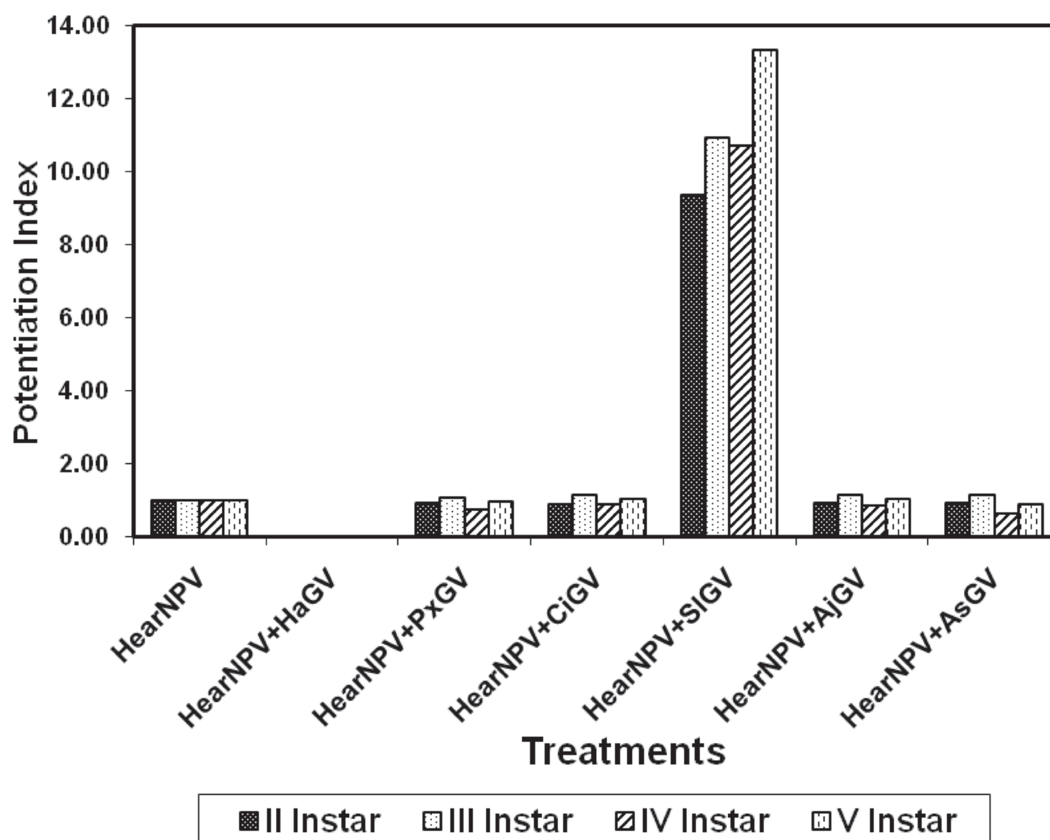

Fig. 1 – Potentiation of HearNPV by GVs

protective peritrophic membrane of the insect midgut or due to both activities. The potential utility of these novel proteins indicates that the enhancins may play an important role in the formulation of future biopesticides. Further, the fractionation of enhancing protein from SlGV and the gene responsible for the same has to be investigated.

REFERENCES

Chakraborty M, Narayanan K, Sivaprakash MK. 2003. *In vivo* enhancement of nucleopolyhedrovirus of oriental armyworm, *Mythimna separata* using viral enhancing factor of a granulosis virus of *Spodoptera litura*. In: *Proc Natl Symp Bio-Management Insect Pests*. 29–31 March, 2003, Annamalai University, Annamalainagar, India. pp. 43–44.

Duncan OD. 1966. Path analysis: Sociological examples. *American J Sociol.* **72**: 1–6.

Evans HF, Shapiro M. 1997. Viruses. pp 17-54. In: Lacey L. (Ed.). *Manual of Techniques in Insect Pathology*. Academic Press, London.

Finney DJ. 1962. Probit Analysis. Cambridge University Press, Cambridge and New York.

Granados RR. 1990. Baculovirus proteins and viral pesticides containing same. U.S. Patent No. **4:** 973, 667.

Gröner A. 1986. Specificity and safety of baculoviruses. pp. 177–202. In: Granados RR, Federici BA. (Eds.), *The biology of baculovirus, biological properties and molecular biology*. Boca Raton, FL: CRC Press.

Hackett KJ, Boore A, Deming C, Buckley E, Camp M, Shapiro M. 2000. *Helicoverpa armigera*. Granulovirus interference with progression of *H. zea* larvae. *J Invertebr Pathol.* **75**: 99–106.

Hukhara T, Tamura K, Zhu Y, Abe H, Tanada Y. 1987. Synergistic factor shows specificity in enhancing nuclear polyhedrosis virus infections. *Appl Entomol Zool.* **22**: 235–236.

Jeyarani S, Karuppuchamy P. 2010. Investigations on the enhancing efficacy of granulovirus on nucleopoly-hedrovirus of *Helicoverpa armigera* (Hübner). *J Biopest*. **3**(1): 172–176.

King ABS. 1994. *Heliothis/Helicoverpa* (Lepidoptera: Noctuidae). pp. 39–106. In: Matthews GA, Tunstall,

J. P. (Eds.), *Insect Pests of Cotton*. CAB International, UK.

Lepore LS, Roelvink PR, Granados RR. 1996. Enhancing the granulosis virus protein that facilitates nucleopolyhedrovirus (NPV) infections, is a metalloprotease. *J Invertebr Pathol.* **68**: 131–140.

Ping L, Lin MX, Ping XJ, Wen JX, Bai YL, Rong GJ. 1999. Cloning, expression and bioassay of the gene encoding viral enhancing factor of *Trichoplusia ni* granulosis virus. *Chinese J Biol Control*, **15**: 188–189.

Shanower TG, Romeis J, Minja EM. 1999. Insect pests of pigeonpea and their management. *Ann Rev Entomol*. **44**: 77–96.

Shapiro M. 2000. Effect of two granulosis viruses on the activity of the gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *J Econ Entomol.* **93**: 1633–1637.

Sharma HC. 2005. *Heliothis/Helicoverpa* Management: pp: 469. Emerging Trends and Strategies for Future Research. Oxford and IBH Publishers, New Delhi, India.

Shorey HH, Hale RL. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. *J Econ Entomol.* **58**: 522–524.

Tanada Y. 1959. Synergism between two viruses of the armyworm, *Pseudalatia unipuncta* (Haworth) (Lepidoptera : Noctuidae). *J Invertebr Pathol.* **1**: 215–231.

Tanada Y. 1985. A synopsis of studies on the synergistic property of an insect baculovirus. A tribute to Edward A. Steinhaus (Founders Lecture). *J Invertebr Pathol.* **45**: 125–138.