Investigations on the enhancing efficacy of granulovirus on nucleopolyhedrovirus of *Helicoverpa armigera* (Hübner)

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ABSTRACT

The implementation of microbial control of insect pests especially with nucleopolyhedroviruses (NPVs) is less popular among the farmers owing to its slow action and 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, investigations were carried out to find out the effect of *Helicoverpa armigera* Granulovirus (HaGV) on the nucleopolyhedrovirus of *H. armigera* (HaNPV) against second, third, fourth and fifth instar larvae. Results of the studies revealed that treatment of HaNPV, HaGV applied at LC_{50} doses alone or a combination of HaNPV (LC_{50}) + HaGV (LC_{25}) significantly caused higher mortalities due to polyhedrosis or polyhedrosis mixed with granulosis than other individual treatments tested at LC_{25}, LC_{50} or their combinations. Data on the LT_{50} values against different instars indicated that combination of HaNPV + HaGV and HaGV alone at different doses resulted in lowered mortality and extension in survivorship time. Only in HaNPV treated at LC_{50} doses, the LT_{50} was found to be significantly the shortest recording 99.52, 100.31, 102.92 and 104.46 h against second, third, fourth and fifth instar larvae respectively. It was found that the efficacy of HaNPV was not enhanced by the HaGV and the decrease in the infectivity of HaNPV by HaGV might be due to out competing nature of HaGV for tissue sites or it might possess a viral factor such as a protein or peptide, for inhibiting viral replication. The efficacy of HaNPV was also not enhanced by the heat inactivated HaGV.

Key words: *Helicoverpa armigera*, Granulosis virus, nucleopolyhedrovirus, enhancing factor

INTRODUCTION

The cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is a major pest on a wide range of crops with high mobility and fecundity (Rao *et al*., 2001). Insecticide treatments have been widely used and are indispensable for its control in almost all crops, which has resulted in insecticide resistance. Biopesticides based on baculovirus group especially the nucleopolyhedrovirus (NPV) offer great scope against *H. armigera*. However, the implementation of microbial control of insect pests especially with nucleopolyhedroviruses (NPVs) is less popular among the farmers owing to its slow action and reduced efficacy against grown up larvae. Granuloviruses are known to enhance the infectivity of NPVs due to the presence of a viral enhancing factor (VEF). Whitlock *et al*. (1996) demonstrated that an extract of HaGV capsular protein enhanced infection due to the NPV. Shapiro (2000) reported that the LC_{50} of *Lymantria dispar* L. MNPV (LdMNPV) was reduced by nearly 300 fold (HaGV at 10^{-2} dilution) and the LT_{50} was reduced by as much as 18 percent. Webb *et al*. (2001) and Xian Guo *et al*. (2001) found that the enhancin present in HaGV with LdMNPV enhanced the *L. dispar* larval mortality by 10 per cent. However, Hackett *et al*. (2000) reported that at a high dosage of HaGV with low dosage of *Heliothis zea* (Boddie) NPV (HzNPV), the HaGV interfere with the progression of HzNPV and the competition between HaGV and HzNPV are dependent on relative dosage of each virus. Hence, keeping these in view, investigations were carried out to find out whether *Helicoverpa armigera* Granulovirus (HaGV) is having any enhancing effect on the nucleopolyhedrovirus of *H. armigera* (HaNPV).

MATERIALS AND METHODS

Test insect cultures

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

HaNPV and HaGV Multiplication and Standardization

The HaNPV and HaGV 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 and OB strength was assessed using a haemocytometer of depth 1mm and 0.02 mm respectively for NPV and GV (Weber, England) (Evans and Shapiro, 1997). The stock suspensions were stored at 4°C for further studies.

Determination of LC_{25} and LC_{50} of HaNPV and HaGV against different instars of *H. armigera*

Preliminary bioassays were conducted to determine the LC_{25} and LC_{50} values of HaNPV and HaGV against different instars of *H. armigera* by diet surface treatment method with the following doses;

Instar	Dose range	
	HaNPV (POB/ml)	HaGV (OB/ml)
Second	$5x10^5 – 1.6x10^2$	$2x10^7 – 6.4x10^3$
Third	$5x10^6 – 1.6x10^3$	$2x10^8 – 6.4x10^4$
Fourth	$5x10^7 – 1.6x10^4$	$2x10^9 – 6.4x10^5$
Fifth	$5x10^8 – 1.6x10^5$	$2x10^{10} – 6.4x10^6$

Observation on the mortality was recorded at 24 h intervals from 3 to 10 days after treatment.

Effect of HaGV with HaNPV against *H. armigera*

To determine the lowest doses of GVs that could give maximum mortality with HaNPV against *H. armigera*, different doses of HaGV (LC_{25} and LC_{50}) were combined simultaneously with LC_{25} and LC_{50} doses of HaNPV. 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 3 to 10 days after treatment. A control was maintained separately. The experiment was conducted against second, third, fourth and fifth instars of *H. armigera*. HaNPV and HaGV @ LC_{25} and LC_{50} doses for respective instars were used for the study.

Effect of heat inactivated HaGV on HaNPV against *H. armigera*

To determine the interaction between the heat inactivated HaGV on HaNPV, tests were conducted with combination of HaGV @ field dose (786.35 OB/mm^2) exposed to temperatures *viz.*, 75, 80, 85 and 121°C for 10 min and HaNPV @ LC_{50} doses. Each treatment was replicated three times with thirty larvae. Observations on mortality were recorded from 3 to 10 days after treatment. The experiment was conducted against second, third, fourth and fifth instar larvae of *H. armigera* and the HaNPV doses used were 0.017, 0.157, 1.241 and 16.055 POB/mm^2 respectively.

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

Determination of LC_{25} and LC_{50} values

Results revealed that high degree of susceptibility of early instars than older instars to HaNPV and HaGV. Investigations on the dose-mortality response of second, third, fourth and fifth instar of *H. armigera* larvae to HaNPV showed that the LC_{25} values were 0.001, 0.008, 0.075 and 1.221 POB/mm^2, while the LC_{50} values were 0.017, 0.157, 1.241 and 16.055 POB/mm^2 respectively (Table 1). Corresponding to an increase in stage of larvae, there was marked increase in LC_{25} and LC_{50} value.

Laboratory bioassay on the susceptibility of *H. armigera* to HaGV revealed that early instars were more susceptible than older instars. Against second, third, fourth and fifth instar larvae, the LC_{25} values were 4.508, 26.865, 244.571 and 1458.019 OB/mm^2 and the LC_{50} values recorded were 74.183, 440.031, 3131.059 and 12102.271 OB/mm^2 respectively (Table 1). Whitlock (1978) reported that the lower dosage of HaNPV and HaGV took longer time to exert their full effect on the older stage than early stage larvae and dosage that gave LC_{25} and LC_{50} increased with increased age of the larvae. It was reported that *H. armigera* larvae was not as susceptible to its GV as HaNPV (Kuppusamy, 1994) which was in conformation with the present findings. Chen *et al*. (1992) reported that the

Table 1. LC_{25} and LC_{50} values for HaNPV and HaGV against different instars of *H. armigera*

Instar	Concentration			
	HaNPV (POB/mm^2)		HaGV (OB/mm^2)	
	LC_{25}	LC_{50}	LC_{25}	LC_{50}
Second	0.001	0.017	4.508	74.183
Third	0.008	0.157	26.865	440.031
Fourth	0.075	1.241	244.571	3131.059
Fifth	1.220	16.055	1458.019	12102.271

early instars of *Hyphantria cunea* were more susceptible to their GVs than older ones. Easwaramoorthy and Santhalakshmi (2000) found that susceptibility of *Chilo infuscatellus* to CiGV decreased with increase in age. The highest LC_{50} values for the HaGV in the present findings may be attributed to the high JH titre in the GV infected larvae.

Integrated effect of HaGV and HaNPV against *H. armigera*

Studies conducted to find out the influence of simultaneous treatment of HaGV and HaNPV at LC_{25} and LC_{50} doses alone and their combination on reciprocal basis against different instars of *H. armigera* showed that HaNPV, HaGV applied at LC_{50} doses alone or a combination of HaNPV (LC_{50}) + HaGV (LC_{25}) significantly caused higher mortalities than other individual treatments tested at LC_{25}, LC_{50} or their combinations. In the course of investigations, it was found that the proportion of larvae dying due to polyhedrosis or polyhedrosis mixed with granulosis was higher in combined treatment of HaNPV and HaGV at LC_{50} + LC_{25} and LC_{50} + LC_{50} doses. However, the larval mortality due to granulosis alone was higher in LC_{25} + LC_{50} doses tested. Data on the LT_{50} values against different instars indicated that combination of HaNPV + HaGV and HaGV alone at different doses resulted in extension in survivorship time and was detrimental. Only in HaNPV-LC_{50} treatment the LT_{50} was found to be significantly the shortest irrespective of the instars (Tables 2-5).

Tanada (1959) reported that the PuGV enhanced the infectivity of PuNPV against *Pseudolatia unipuncta* even in fourth and fifth instar larvae and Goto (1990) reported the same in *Xestis c-nigrum*. However, HaGV when combined with HaNPV decreased the mortality rates with increased LC_{50} values against all instars tried. Hackett *et al*. (2000) reported that simultaneous infections of HaNPV and HaGV were found to lower the mortality than each of the virus tested separately. In contrast, simultaneous infection of TnGV and TnNPV enhanced the NPV infection in *T. ni* larvae (Lepore *et al*., 1996).

In the present investigations, it was observed that as the GV concentration was lesser than or equal to NPV, the mortality due to polyhedrosis was more than granulosis. Hackett *et al*. (2000) reported that the overall mortality rate was lower in cases of HaGV with HaNPV and HzNPV than in cases where separate viruses were used. This could be attributed to an interfering factor present in one or both of the viruses. It was suggested that the faster acting NPV can prevent infection by GV. However, this is not in agreement with the present findings. The presence of HaGV in the mixed suspension had a suppressing effect on the HaNPV infection and the incubation period of NPV increased. This is in agreement with the findings of the Whitlock (1977). They also reported that the interfering factor does not exist in the capsules of the viruses, since the inactivated viruses did not have an effect on the other infective viruses.

Integrated effect of heat inactivated HaGV on HaNPV against *H. armigera*

Tests were conducted to determine whether the possible causes for the enhanced infectivity rests with the infective unit of the HaGV or with the capsular protein by using heat inactivated viruses. Since, virions could be inactivated at temperatures more than 70°C; the temperature was fixed as 75, 80, 85 and 121°C for inactivation of HaGV. The results of the study indicated that even after heat inactivation, HaGV had not enhanced the action of HaNPV and the results were similar to that of the individual effects (Table 6). However, Roelvinck *et al*. (1995) demonstrated the enhancing factor in HaGV. It was also reported that the LC_{50} of LdMNPV was reduced by as much as 300 fold and the LT_{50} was reduced by 18 per

Table 2. Combined effect of HaNPV and HaGV against second instar *H. armigera*

Treatments*		% Mortality of larvae infected with				LT_{50}
HaNPV	HaGV	Polyhedrosis	Mixed	Granulosis	Cumulative	(h)
LC_{25}	-	27.78 [b]	-	-	27.78 [c]	-
LC_{50}	-	56.67 [a]	-	-	56.67 [a]	99.52 [a]
-	LC_{25}	-	-	20.00 [c]	20.00 [c]	-
-	LC_{50}	-	-	44.44 [a]	44.44 [b]	373.04 [b]
LC_{25}	LC_{25}	7.78 [d]	7.78 [d]	4.44 [e]	20.00 [c]	469.37 [f]
LC_{50}	LC_{25}	27.78 [b]	23.33 [a]	5.56 [e]	56.67 [a]	422.01 [c]
LC_{25}	LC_{50}	5.56 [d]	11.11 [c]	26.67 [b]	43.34 [b]	430.68 [e]
LC_{50}	LC_{50}	17.78 [c]	17.78 [b]	11.11 [d]	46.67 [b]	427.68 [d]

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

Table 3. Combined effect of HaNPV and HaGV against third instar *H. armigera*

Treatments*		% Mortality of larvae infected with				LT_{50}
HaNPV	HaGV	Polyhedrosis	Mixed	Granulosis	Cumulative	(h)
LC_{25}	-	31.11 b	-	-	31.11 c	-
LC_{50}	-	58.89 a	-	-	58.89 a	100.31 a
-	LC_{25}	-	-	18.89 c	18.89 d	-
-	LC_{50}	-	-	42.22 a	42.22 b	390.75 b
LC_{25}	LC_{25}	6.67 e	6.67 b	3.33 e	16.67 d	474.25 f
LC_{50}	LC_{25}	25.55 c	21.11 a	3.33 e	49.99 b	424.84 c
LC_{25}	LC_{50}	3.33 f	8.89 c	25.56 b	37.78 c	433.64 e
LC_{50}	LC_{50}	13.33 d	15.56 b	11.11 d	40.00 b	430.68 d

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

Table 4. Combined effect of HaNPV and HaGV against fourth instar *H. armigera*

Treatments*		% Mortality of larvae infected with				LT_{50}
HaNPV	HaGV	Polyhedrosis	Mixed	Granulosis	Cumulative	(h)
LC_{25}	-	30.00 b	-	-	30.00 c	-
LC_{50}	-	53.33 a	-	-	53.33 a	102.92 a
-	LC_{25}	-	-	21.11 b	21.11 c	-
-	LC_{50}	-	-	43.33 a	43.33 b	427.99 b
LC_{25}	LC_{25}	8.89 e	5.56 c	5.56 d	20.00 c	484.41 f
LC_{50}	LC_{25}	24.44 c	22.22 a	6.67 d	53.34 a	433.23 c
LC_{25}	LC_{50}	4.44 f	7.78 c	26.67 b	38.89 b	449.18 e
LC_{50}	LC_{50}	16.67 d	14.44 b	12.22 c	43.33 b	442.59 d

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

Table 5. Combined effect of HaNPV and HaGV against fifth instar *H. armigera*

Treatments*		% Mortality of larvae infected with				LT_{50}
HaNPV	HaGV	Polyhedrosis	Mixed	Granulosis	Cumulative	(h)
LC_{25}	-	28.89 b	-	-	28.89 c	-
LC_{50}	-	56.67 a	-	-	56.67 a	104.46 a
-	LC_{25}	-	-	16.67 c	16.67 d	-
-	LC_{50}	-	-	40.00 a	40.00 b	447.21 b
LC_{25}	LC_{25}	10.00 d	8.89 b	4.44 e	23.33 c	490.33 c
LC_{50}	LC_{25}	27.78 b	20.00 a	7.78 d	55.56 a	449.13 b
LC_{25}	LC_{50}	6.67 e	5.56 c	30.00 b	42.23 b	456.04 b
LC_{50}	LC_{50}	17.78 c	17.78 a	10.00 d	45.56 b	452.57 b

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

Table 6. Effect of heat treatment of HaGV on the integration effect of HaGV with HaNPV against *H. armigera*

Treatments	% Mortality$			
	II Instar	III Instar	IV Instar	V Instar
HaNPV* + HaGV heat inactivated (75°C)	58.89 a	54.45 a	53.33 b	56.67 a
HaNPV* + HaGV heat inactivated (80°C)	57.78 b	56.67 a	57.78 a	54.45 a
HaNPV* + HaGV heat inactivated (85°C)	58.89 a	53.33 bc	54.45 b	54.45 a
HaNPV* + HaGV heat inactivated (121°C)	56.67 b	54.45 b	51.11 c	53.33 a
HaNPV* + HaGV without inactivation	56.67 b	58.89 a	56.67 a	53.33 a
HaNPV* without inactivation	57.78 b	51.11 c	54.45 b	55.56 a
HaGV** without inactivation	61.11 a	59.78 a	56.67 a	45.45 b

$ In a column, means followed by similar letters are not significantly different (P = 0.05) by DMRT. * HaNPV @ LC_{50} dose for respective instars, ** HaGV @ 786.35 OB/mm^2 for all the instars

cent, while SfGV reduced the LC_{50} of LdMNPV by 13 fold but had no effect on LT_{50} (Shapiro, 2000). The present findings indicate that the HaGV was not synergistic with the homologous host NPV (HaNPV). The decrease in the infectivity of HaNPV by HaGV may be due to out competing nature of HaGV for tissue sites or it may possess a viral factor such as a protein or peptide, for inhibiting viral replication.

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