J. Biol. Control, 19(2): 193-196, 2005

Research Note

Economics of *Hp*NPV production using field collected and laboratory reared *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae)

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ABSTRACT: **The economics of mass production of the nucleopolyhedrovirus of the teak defoliator, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae) (*Hp*NPV) using the laboratory-reared and field-collected (from teak plantations) host larvae was compared. The data were based on 7645 laboratory-reared (LR) larvae and 8925 field-collected (FC) larvae. The virus production was carried out using fifth instar larva inoculated with $1x10^6$ OBs (Viral Occlusion Bodies)/ larva. The virus yield/ larva in LR and FC larvae was $4.8x10^8$ and $3.6x10^8$ OBs, respectively. The virus productivity ratio in LR and FC larvae was 241 ± 55 and 178 ± 100.85 OBs, respectively. The cost of production of *Hp*NPV per larva was higher in the case of LR (Rs. 0.82/larva) in comparison with FC (Rs.0.72). However, the cost of *Hp*NPV required for spraying in one hectare at the rate of 1.63×10^{11} OBs worked out to be Rs. 279 and Rs. 317 in the case of LR and FC, respectively. A marginal difference in the virus yield was found affecting the cost of the virus produced.**

KEY WORDS: *Hyblaea puera*, *Hp*NPV, mass production, nucleopolyhedrovirus, teak defoliator

Nucleopolyhedroviruses (NPVs) have been recognized as an effective control measure against lepidopteran pests, which is environmentally safe. Because of their specificity to arthropod pests, NPVs are preferred to be the ideal microbial agents for development as biopesticides. One of the prime requisites to use a baculovirus for pest management is the availability of the virus inoculum in large quantities. In general, the common practice is to replicate the virus in the homologous host reared on artificial diet (Cherry *et al.*, 1997). An alternative is to make use of the field population of the host. In the case of teak defoliator, *Hyblaea puera* (Cramer) during the pre-outbreak period, healthy larvae are generally available in plenty, which are useful for virus multiplication.

The paper deals with nucleopolyhedrovirus of the teak defoliator, *H. puera*. This baculovirus known as *Hp*NPV is a potential biocontrol agent against the teak defoliator (Sudheendrakumar *et al.*, 1988; Nair *et al.*, 1998). An attempt was made to workout the economics of *Hp*NPV production using the host insects obtained from two sources, 1. laboratory culture and 2. teak defoliator infested teak plantation. The study was focused on the cost

of virus production and virus productivity with regard to the host larvae from two different sources.

Healthy fifth instar larvae of *H. puera* were used for *in vivo* mass production. The larvae were made available from the following two sources:

Host culture

The host larvae were collected from the teak plantations in Nilambur, Kerala during April-June 2001. During the occurrence of large-scale outbreak population, early fifth instar larvae were collected from the infested trees in plastic containers of 100-litre capacity and transported to the laboratory. The larvae were screened for over-size and parasitization (usually by *Palexorista solennis*) visually. The selected larvae were used for *Hp*NPV multiplication.

A continuous culture of *H. puera* was maintained on an artificial diet (Mathew *et al.*, 1990). The larvae were reared up to the third instar on teak leaf and then transferred individually to insect rearing tubes (8x 5cm) containing artificial diet (1.5 ml). The larvae intended for *Hp*NPV production, were reared up to the fifth instar stage on artificial diet and then transferred to the *Hp*NPV production laboratory.

Production of *Hp*NPV

The virus inoculum used in the study was obtained from a virus stock maintained in the Entomology Laboratory, Kerala Forest Research Institute sub-centre, Nilambur. Insect rearing tubes (8 x2.5cm) containing 3 ml of semi-synthetic diet were prepared and arranged in trays. The diet surface was sprayed with purified *Hp*NPV inoculum at the rate of $1x10^6$ OBs/ rearing tube using an atomizer. The fifth instar larvae were transferred individually into the inoculated diet tubes and kept for incubation at 26° ± 3°C. The larvae, which died after 84 h post inoculation were retrieved and stored at - 20° C until processed further. The frozen larvae were macerated in the homogenizer (OMNI 5000 International) with sterile 0.1 per cent sodium dodecyl sulphate (SDS) to prevent the clumping of the viral occlusion bodies (OBs). The homogenate was filtered using a muslin cloth to remove coarse insect debris. The filtrate was again filtered using muslin cloth in three layers. The virus suspension was centrifuged at 1000rpm for 10 minutes to remove the large contaminant particles and the supernatant was collected and again centrifuged at 7000rpm for 25min in a REMI R-24 centrifuge (with angle rotor) to produce a pellet, comprising mainly of the virus. For removing the SDS the pellet was resuspended in distilled water. On settlement of the occlusion bodies at the bottom, the supernatant was removed. This process was repeated thrice and the retrieved occlusion bodies were enumerated using a Neubauer haemocytometer and stored at 4°C.

The data on the economics of *Hp*NPV production generated are based on 7645 laboratory-reared larvae and 8925 field-collected larvae processed in different batches. The actual cost involved for diet chemicals, skilled labour and transport of larvae were recorded. The *Hp*NPV production parameters (larval retrieval and *Hp*NPV yield/larva, productivity ratio) for the laboratory-reared and field-collected larvae were compared statistically using one-way ANOVA (Least Significant Difference).

The *Hp*NPV production parameters using the laboratory-reared and field-collected larvae are given in Table 1. About 10 per cent of the laboratory-reared larvae and 18 per cent of the field-collected larvae were found unsuitable for virus production. In the case of the larvae used for inoculation, there was substantial difference in the number of larvae retrieved between the laboratory- reared (91%) and field-collected (84%). The rejected larvae included those, which did not complete feeding and those got bacterial infection.

The productivity ratio did not vary significantly between the laboratory-reared larvae and field-collected larvae (241±55 OBs and 178 ± 100 OBs, respectively) (Table 1). The productivity ratio obtained for *Hp*NPV is very low in comparison with many other NPVs like *Spodoptera exigua* NPV-$1.2x10^6$ OBs (Smits and Vlak, 1988), *Helicoverpa zea* NPV- $1x10^5$OBs (Sheigh, 1989), *Mamestra brassicae* NPV-$1.28x10^3$ (Evans *et al.*, 1981) and *Euproctis chrysorrhoea* NPV ($1x10^3$ OBs) (Kelly *et*

Table 1. *Hp*NPV mass production parameters of laboratory reared and field collected larvae

Sl. No.	*Hp*NPV mass production parameters	Source of larvae	
		Laboratory-reared	Field-collected
1.	Larvae inoculated	6950	7319
2.	Larvae retrieved (%)		84.2 ± 11.2 (86-96)
3.	Virus yield (OBs)/larva	$4.8x10^8 \pm 1X10^8$ ($3.8x10^8$-$6x10^8$)	$3.6x10^8 \pm 2x10^8$ ($1x10^8$-$5.8x10^8$)
4.	Productivity ratio (virus yield/ virus dosage)	241 ± 55(190-300)	178 ± 100.9 (50-250)
5.	Estimated virus production cost/larva (Rs.)	0.82	0.70
6.	Virus production (OBs) per Rupee	$5.85 x 10^8$	$5.14 x 10^8$
7.	Production cost of inoculum for spraying* 1 ha teak plantation (Rs.)	279	317

*When ultra low volume spraying done @ $1.63 x 10^{11}$ /ha
Dosage estimated based on LD_{50} value for 3rd instar larvae
Figure in parentheses shows the range values.

al., 1989). The productivity is generally linked to the host larval age and the dosage used. When undertaking this study, these aspects were not fully addressed. The fifth instar larvae were used in this study with the understanding that the virus yield is positively correlated with the larval weight (Smits and Vlak, 1988). The inoculum rate of $1x10^6$ used in this study could probably be on the higher side. It has been reported that a very high inoculum rate may affect the virus productivity as the larvae get killed before virus replication is complete (Shapiro, 1982). The harvesting time is also critical as a late harvest may affect the viral retrieval due to loss. Hence a better virus yield could be expected if the production is carried out with an optimized inoculum rate, larval age and harvesting time.

The split-up of the cost of production of virus/ larva is given in Table 2. The all-inclusive cost for virus production from one larva was Rs. 0.82 for laboratory-reared and Rs. 0.7 for field-collected larvae There was no significant difference in the virus yield/larva between the two methods though the virus yield under the laboratory-reared set showed a higher value. Based on the cost of production, the virus produced per one rupee in the case of laboratory reared and field collected sets is estimated to be $5.85 x 10^8$ and $5.14 x 10^8$ OBs, respectively. Accordingly the cost of virus inoculum required for spraying in one hectare plantation for targeting third instar larval population was estimated which in the case of laboratory-reared and field-collected larvae was Rs. 279 and 317, respectively. This difference in the cost can only be due to the difference in the viral yield/larva between the two methods even though it was marginal. This finding suggests that the cost of virus production is influenced by the viral yield/ larva. Another factor influencing the cost of production is the low percentage of larval retrieval caused by factors like bacterial infection. An improved rearing condition could probably enhance the larval retrieval and thereby reduce the cost of virus production.

The study showed that both the laboratory-reared and field-collected larvae are suitable for virus production. However, it appears that the laboratory-reared larvae would be the ideal source for virus production on a regular basis as the availability of

Table 2. Break-up of expenditures involved in mass-producing *Hp*NPV

Task/materials		Expenditure (Rs.)	
		Laboratory	Wild
Larval collection	Labour	0.00	0.10
	Transport	0.00	0.15
Diet	For rearing III rd instar up to V th instar and through post infection	0.26	0.26
	For rearing V th instar through post infection	0.10	0.00
Inoculation		0.09	0.00
Retrieval of infected larvae (labour cost)		0.09	0.09
Washing of rearing tubes (labour cost)		0.10	0.10
Larval transfer to the diet tubes	V th instar	0.09	0.00
	III rd instar	0.09	0.00
TOTAL		0.82	0.70

the larvae from teak plantations is only seasonal and its quality may be questionable. Hence, it appears that a better quality control could be ensured if the virus production is carried out using laboratory-reared larvae.

ACKNOWLEDGEMENTS

This study was carried out as part of a Research Project on *Hp*NPV funded by the Department of Biotechnology, Govt. of India. For facilities we thank Director, KFRI.

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