Standardization of Mass Production in Three Isolates of Nucleopolyhedrovirus of *Helicoverpa armigera* (Hübner)

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**Abstract:** The effect of four major parameters, viz., larval age and weight, inoculation dose, incubation temperature and time of harvesting the larvae, on the production of three isolates of nucleopolyhedrovirus of *Helicoverpa armigera* (HaNPV), viz., Ooty (OTY), Coimbatore (CMB) and Negamum (NGM) were evaluated. Early 5th instar larvae recorded the maximum yield of virus per larva when inoculated with a dose of \(5 \times 10^6\) POB larva\(^{-1}\) and incubated at a temperature of 25°C. Also, highest POB yield was recorded when virosed larvae were harvested as cadavers. However, among the isolates tested in this study, CMB isolate collected from Tamil Nadu, India, showed the highest yield per larva in all of the conditions.

**Key words:** Nucleopolyhedrovirus, geographical isolates, *Helicoverpa armigera*, mass production

**INTRODUCTION**

Since viral pathogens are obligate in nature, they must be necessarily multiplied on their natural live hosts from which they have been collected (Narayana, 2003). The selection of a virus for pest management depends not only on its bioefficacy but also on the ease of mass production (Sherman, 1985). Nucleopolyhedroviruses find extensive use in assisted control programmes for early management of pests (Carner and Yearian, 1989; Rabindra *et al.*, 2003; Dolinski and Lacey, 2007). While propagation of the virus in cell culture continues to receive increasing attention (Shuler *et al.*, 1995), *in vivo* production has been found to be the only feasible method for large scale propagation of virus (Hunter *et al.*, 1998). There has been continuous flow of information on the *in vivo* production of baculoviruses, notably the nuclear polyhedrosis viruses in many insects (Shapiro, 1986; Ebora *et al.*, 1990). However, to increase the yield of three HaNPV isolates collected from India and selection the highly promising viral isolate under *in vivo* conditions a series of laboratorial studies were conducted. The present investigations were therefore undertaken to evaluate the effect of stage of the host larvae, incubation temperature and dose of virus inoculum as well as the time of harvest on the *in vivo* yield of three HaNPV isolates.

**MATERIALS AND METHODS**

The *H. armigera* culture used in the study was maintained on a semi-synthetic diet based on Shoney and Hale (1965) in Bio-Control Research Laboratories (BCRL), Bangalore, India. Three Indian isolates of HaNPV collected from Coimbatore (CMB), Negamum (NGM) and Ooty (OTY) of Tamil Nadu state, India, were used in this study during the year 2005. These isolates were passaged through early fifth instar larvae of host insect at 25±1°C to get uniformity in their virulence since they had been stored in the refrigerator for prolonged period of time. All the experiments were performed in insect virology laboratory of Project Directorate of Biological Control (PDBC), Bangalore, India.

**Effect of larval age and weight on virus yield:** Mid 4th, late 4th, early 5th and mid 5th instar larvae of *H. armigera* were used to study the influence of insect age on virus yield. Semi-synthetic diet without formaldehyde was prepared and filled in 5 mL glass vials up to 1/3rd height of the vial. A quantity of 10 µL of virus suspension (\(5 \times 10^6\) POB larva\(^{-1}\)) was applied onto the diet surface using a micropipette providing a dose of 1965.78 POB mm\(^{-2}\). The suspension was spread uniformly

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over the diet surface with the polished blunt end of a
glass rod (6 mm). Larvae in the four age groups were
weighed individually in an electronic balance and
transferred to treated diet. The treatments were replicated
twice and included a control for each age group and
isolate. Each treatment had 40 insects. After inoculation,
the larvae were incubated at 25±1°C in an incubator. The
mortality in different treatments was recorded at 24 h
interval. Upon death, the cadavers were collected,
transferred to sterile vials and the number of harvestable
cadavers in each treatment was recorded. The samples
were frozen immediately.

The cadavers were homogenized individually using
a glass pestle and mortar with distilled water. The
homogenate was transferred to a measuring cylinder
and volume made up to 25 mL with distilled water.
Enumeration of polyhedra was performed in this stage
and the following parameters were calculated:

\[ \text{Yield larva}^{-1} (\text{POB}) = \frac{\text{POB mL}^{-1} \times \text{Suspension volume (mL)}}{\text{Total No. of cadavers}} \]

Yield per 100 inoculated larvae (POB) = Yield larva\(^{-1}\) ×
Corrected larval mortality (%)

Productivity Ratio (POB) = \frac{\text{Yield larva}^{-1}}{\text{POB inoculated larva}^{-1}}

**Effect of period of larval harvest on virus yield:** Early 5th
instar larvae of *H. armigera* were allowed to feed on diet
surface treated with viral dose of 1965.87 POB mm\(^{-2}\) and
incubated at 25±1°C for each NPV isolate. The infected
larvae were harvested five, six, seven and eight days after
inoculation. Also, a treatment of harvesting the cadavers
was included for comparison. An untreated control was
also maintained. Each treatment was replicated three times
with 40 larvae per replication.

The larval cadavers were transferred to sterile vials,
plugged with cotton and frozen immediately. The
cadavers were then processed and POBs were enumerated
as described earlier.

**Statistical analyses:** Analyses of variances (ANOVA)
were carried out using SAS software version 6.12 and
means were separated by Duncan’s Multiple Range Test
(DMRT). All data in percentage were transformed to
arcsin \(\sqrt{\%}\) and the data from POB yields were
subjected to log transformation and then analyzed. The
larval counts were also transformed to \(\sqrt{x+0.5}\) values. The
probit analyses in various experiments were carried out in
a Statistical Package for Social Sciences (SPSS), version
10.0 for windows.

**RESULTS**

**Virus yield in relation to larval age and weight:** A
significant influence of larval age and weight on the per-
cent larval mortality, yield larva\(^{-1}\), yield per 100 inoculated
larvae and productivity ratio of the isolates was observed
in the assay (Table 1). Of the different larval ages, early
5th instar recorded significantly the highest yield and
productivity with all the three isolates tested. Mid 4th, late
4th and mid 5th instars registered decreased production of
POB.

The initial larval weight was critical for obtaining
higher POB yield in all the isolates tested in this study.
The maximum POB yield was related to the early 5th
instar larvae with initial weight range of 65.46-68.13 mg
(Var. 1).

**Virus yield in relation to inoculation dose:** Optimization
studies on inoculation dose revealed that larval mortality
increased as the inoculum dose advanced. Highest
mortality of 83.77, 82.90 and 82.05% was recorded at
inoculum dose of 1965.87 POB mm\(^{-2}\) of NGM, OTY and
CMB isolates, respectively (Table 2). Similarly, the virus
yield per larva and for 100 inoculated larvae was
significantly higher at this dose of all the three isolates.
The productivity ratio of all the isolates decreased
progressively as the dose of inoculation advanced.
Table 1: Effect of larval stage on the yield of HaNPV isolates at an inoculation dose of $5 \times 10^6$ POB larva$^{-1}$ and an incubation temperature of 25±1°C

<table>
<thead>
<tr>
<th>HaNPV isolates</th>
<th>Larval stage</th>
<th>Mean harvestable cadavers (out of 40)</th>
<th>Yield larva$^{-1}$ ($\times 10^6$ POB)</th>
<th>Yield per 100 inoculated larvae ($\times 10^3$ POB)</th>
<th>Productivity ratio ($\times 10^3$ POB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMB</td>
<td>Mid 4th</td>
<td>39.67</td>
<td>2.69d</td>
<td>2.67c</td>
<td>0.54d</td>
</tr>
<tr>
<td></td>
<td>Late 4th</td>
<td>39.67</td>
<td>4.12c</td>
<td>4.09b</td>
<td>0.83c</td>
</tr>
<tr>
<td></td>
<td>Early 5th</td>
<td>34.33</td>
<td>6.73a</td>
<td>5.73a</td>
<td>1.25a</td>
</tr>
<tr>
<td></td>
<td>Mid 5th</td>
<td>29.33</td>
<td>5.81b</td>
<td>4.22b</td>
<td>1.16b</td>
</tr>
<tr>
<td>NGM</td>
<td>Mid 4th</td>
<td>40.00</td>
<td>1.79d</td>
<td>1.79c</td>
<td>0.36d</td>
</tr>
<tr>
<td></td>
<td>Late 4th</td>
<td>38.00</td>
<td>3.45c</td>
<td>3.27b</td>
<td>0.69c</td>
</tr>
<tr>
<td></td>
<td>Early 5th</td>
<td>33.67</td>
<td>6.45a</td>
<td>5.38a</td>
<td>1.29a</td>
</tr>
<tr>
<td></td>
<td>Mid 5th</td>
<td>30.33</td>
<td>4.55b</td>
<td>3.42b</td>
<td>0.91b</td>
</tr>
<tr>
<td>OTY</td>
<td>Mid 4th</td>
<td>40.00</td>
<td>1.95d</td>
<td>1.95c</td>
<td>0.39d</td>
</tr>
<tr>
<td></td>
<td>Late 4th</td>
<td>35.33</td>
<td>3.56c</td>
<td>3.12c</td>
<td>0.71c</td>
</tr>
<tr>
<td></td>
<td>Early 5th</td>
<td>33.33</td>
<td>5.83a</td>
<td>4.83a</td>
<td>1.17a</td>
</tr>
<tr>
<td></td>
<td>Mid 5th</td>
<td>29.33</td>
<td>4.63b</td>
<td>3.33b</td>
<td>0.92b</td>
</tr>
</tbody>
</table>

In a column, for each isolate means followed by the same letter (a-d) are not significantly different (p = 0.05) by Duncan’s Multiple Range Test (DMRT).

Table 2: Effect of dose of inoculum on the yield of HaNPV isolates achieved from early 5th instar larvae at an incubation temperature of 25±1°C

<table>
<thead>
<tr>
<th>HaNPV isolates</th>
<th>Dose (POB mm$^{-2}$)</th>
<th>Larval mortality (%)</th>
<th>Yield larva$^{-1}$ ($\times 10^6$ POB)</th>
<th>Yield per 100 inoculated larvae ($\times 10^3$ POB)</th>
<th>Productivity ratio ($\times 10^3$ POB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMB</td>
<td>1965.87</td>
<td>82.05a</td>
<td>6.87a</td>
<td>5.64a</td>
<td>1.37c</td>
</tr>
<tr>
<td></td>
<td>393.17</td>
<td>81.30a</td>
<td>6.13b</td>
<td>4.90b</td>
<td>6.13b</td>
</tr>
<tr>
<td></td>
<td>78.63</td>
<td>74.56b</td>
<td>2.51c</td>
<td>1.87c</td>
<td>12.57a</td>
</tr>
<tr>
<td>NGM</td>
<td>1965.87</td>
<td>83.77a</td>
<td>6.49a</td>
<td>5.44a</td>
<td>1.30c</td>
</tr>
<tr>
<td></td>
<td>393.17</td>
<td>80.39b</td>
<td>5.30b</td>
<td>4.26b</td>
<td>5.30b</td>
</tr>
<tr>
<td></td>
<td>78.63</td>
<td>74.56c</td>
<td>2.32c</td>
<td>1.75c</td>
<td>11.58a</td>
</tr>
<tr>
<td>OTY</td>
<td>1965.87</td>
<td>82.90a</td>
<td>5.87a</td>
<td>4.86a</td>
<td>1.17c</td>
</tr>
<tr>
<td></td>
<td>393.17</td>
<td>80.33b</td>
<td>4.95b</td>
<td>4.01b</td>
<td>4.95b</td>
</tr>
<tr>
<td></td>
<td>78.63</td>
<td>74.56c</td>
<td>2.10c</td>
<td>1.63c</td>
<td>10.95a</td>
</tr>
</tbody>
</table>

In a column, for each isolate means followed by the same letter (a-c) are not significantly different (p = 0.05) by DMRT.

Fig. 1: Effect of larval stage and weight on the yield of Ha NPV isolates at an inoculation dose of $5 \times 10^6$ POB larva$^{-1}$ and an incubation temperature of 25±1°C

Virus yield in relation to incubation temperature: The maximum yield of virus per larva and yield per 100 inoculated larvae were obtained for all the viral isolates when larvae were incubated at 25°C after virus inoculation as compared with that of room temperature and 30°C (Table 3). The productivity was
Table 3: Effect of incubation temperature on the yield of HaNPV isolates achieved from early 5th instar larvae inoculated with $5\times10^6$ POB larva$^{-1}$

<table>
<thead>
<tr>
<th>HaNPV isolates</th>
<th>Temperature (°C)</th>
<th>Mean harvestable cadavers (out of 40)</th>
<th>Yield larva$^{-1}$ ($10^5$ POB)</th>
<th>Yield per 100 inoculated larvae ($10^5$ POB)</th>
<th>Productivity ratio ($10^5$ POB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMB</td>
<td>Room$^*$</td>
<td>34.33</td>
<td>5.65b</td>
<td>4.80b</td>
<td>1.13b</td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>33.67</td>
<td>6.87a</td>
<td>5.66a</td>
<td>1.37a</td>
</tr>
<tr>
<td></td>
<td>30°C</td>
<td>34.00</td>
<td>5.10b</td>
<td>4.26b</td>
<td>0.92b</td>
</tr>
<tr>
<td>NGM</td>
<td>Room$^*$</td>
<td>35.33</td>
<td>5.38b</td>
<td>4.73b</td>
<td>1.06b</td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>35.67</td>
<td>6.37a</td>
<td>5.54a</td>
<td>1.27a</td>
</tr>
<tr>
<td></td>
<td>30°C</td>
<td>33.33</td>
<td>4.91b</td>
<td>4.29b</td>
<td>0.96b</td>
</tr>
<tr>
<td>OTY</td>
<td>Room$^*$</td>
<td>35.67</td>
<td>4.89b</td>
<td>4.34b</td>
<td>0.98b</td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>34.33</td>
<td>5.98a</td>
<td>5.04a</td>
<td>1.26a</td>
</tr>
<tr>
<td></td>
<td>30°C</td>
<td>33.00</td>
<td>4.55b</td>
<td>3.95c</td>
<td>0.91b</td>
</tr>
</tbody>
</table>

$^*$: The temperature ranged 28-31°C during the period of study. In a column, for each isolate means followed by the same letter (a-c) are not significantly different ($p = 0.05$) by DMRT.

Table 4: Effect of harvest period on the yield of HaNPV isolates achieved from early 5th instar larvae inoculated with $5\times10^6$ POB larva$^{-1}$ at an incubation temperature of 25±1°C

<table>
<thead>
<tr>
<th>HaNPV isolates</th>
<th>Harvest period (DAI) $^\dagger$</th>
<th>Mean harvestable cadavers (out of 40)</th>
<th>Yield larva$^{-1}$ ($10^5$ POB)</th>
<th>Yield per 100 inoculated larvae ($10^5$ POB)</th>
<th>Productivity ratio ($10^5$ POB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMB</td>
<td>Five</td>
<td>5.33</td>
<td>1.48d</td>
<td>0.17e</td>
<td>0.30d</td>
</tr>
<tr>
<td></td>
<td>Six</td>
<td>11.33</td>
<td>3.44c</td>
<td>0.91d</td>
<td>0.69c</td>
</tr>
<tr>
<td></td>
<td>Seven</td>
<td>20.00</td>
<td>4.30c</td>
<td>2.06c</td>
<td>0.87c</td>
</tr>
<tr>
<td></td>
<td>Eight</td>
<td>33.33</td>
<td>4.96b</td>
<td>4.09b</td>
<td>0.99b</td>
</tr>
<tr>
<td>NGM</td>
<td>Five</td>
<td>6.00</td>
<td>1.25d</td>
<td>0.16d</td>
<td>0.25d</td>
</tr>
<tr>
<td></td>
<td>Six</td>
<td>14.00</td>
<td>3.11c</td>
<td>0.98d</td>
<td>0.62c</td>
</tr>
<tr>
<td></td>
<td>Seven</td>
<td>23.00</td>
<td>3.87bc</td>
<td>2.18c</td>
<td>0.77c</td>
</tr>
<tr>
<td></td>
<td>Eight</td>
<td>34.33</td>
<td>4.39c</td>
<td>3.75b</td>
<td>0.86b</td>
</tr>
<tr>
<td>OTY</td>
<td>Five</td>
<td>5.90</td>
<td>1.02d</td>
<td>0.13e</td>
<td>0.20d</td>
</tr>
<tr>
<td></td>
<td>Six</td>
<td>12.33</td>
<td>2.82c</td>
<td>0.77d</td>
<td>0.56c</td>
</tr>
<tr>
<td></td>
<td>Seven</td>
<td>21.67</td>
<td>3.29bc</td>
<td>1.75c</td>
<td>0.66c</td>
</tr>
<tr>
<td></td>
<td>Eight</td>
<td>33.00</td>
<td>4.01b</td>
<td>3.27b</td>
<td>0.86b</td>
</tr>
</tbody>
</table>

$^\dagger$: Days after inoculation. In a column, for each isolate means followed by the same letter (a-c) are not significantly different ($p = 0.05$) by DMRT.

Fig. 2: Effect of incubation temperature on the yield of HaNPV isolates and larval mortality achieved from early 5th instar larvae inoculated with $5\times10^6$ POB larva$^{-1}$

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also the highest at 25°C. The yield larva\(^{-1}\), yield per 100 inoculated larvae and productivity ratio were minimum at 30°C.

Data on the larval mortality showed a rise in incubation temperature resulted in a significant increase in the per cent kill in all the isolates (Fig. 2).

**Effect of periods of larval harvest on virus yield:** There was a significant increase in larval mortality with increasing time of larval harvest in all the three viral isolates (Table 4). The yield larva\(^{-1}\), yield per 100 inoculated larvae and productivity ratio were the highest when virosed larvae were harvested as cadavers. As the time of harvest decreased, POB yield larva\(^{-1}\), yield per 100 inoculated larvae and productivity ratio were reduced (Fig. 3).

**DISCUSSION**

**Virus yield in relation to larval age and weight:** The yield and productivity parameters of the viral isolates were determined by larval age and weight at the time of inoculation. Of the different age groups studied, early 5th instar with a weight range of 65.46-68.13 mg recorded significantly the highest yield of 6.73×10\(^5\), 6.45×10\(^5\) and 5.83×10\(^5\) POB larva\(^{-1}\) in the case of CMB, NGM and OTY isolates, respectively. Mid 4th, late 4th and mid 5th instar larvae registered lower POB production (Table 1). Tenkle and Byrne (1989) found an exponential increase in the yield of the virus with age of the larvae of *H. armigera*. A 100-fold increase in the yield of the virus was noted in larvae of age 6 days compared to one day old larva. Ignoifo (1966) estimated that at least 6×10\(^5\) POB were produced per larva in later instars of *H. zea*. Dhandapani (1990) reported in a study on *H. armigera* that the yield of POB larva\(^{-1}\) inoculated at the 5th instar stage was 2.4×10\(^5\). Early 5th *S. litura* larvae was found to be an optimum stage for inoculation of virus to maximize the virus productivity (Tuan et al., 1998; Subramanian et al., 2001). Monoburullah and Nagata (2000) reported that 9-day-old *S. litura* larvae weighing 125-155 mg treated with 4.8×10\(^5\) POB larva\(^{-1}\) through diet resulted in maximum productivity of the NPV. Shieh (1989) reported that from *H. zea* larvae with the initial larval weight of 50-120 mg, the highest POB yields could be obtained. At this weight range, the larvae could continue their normal growth as
that of healthy insects until a day before death resulting in a higher amount of POB yields. These observations are in agreement with the present findings. When the larvae of *H. armigera* were allowed to feed on a constant dose, Whitlock (1977) found an inverse relationship between larval age at the time of inoculation and the mortality. In the present studies, mortality was significantly highest in the 4th and mid 4th instar larvae followed by early 5th and mid 5th instars. However, in all the *HaNPV* isolates tested, yield per 100 inoculated larvae was the highest in the early 5th instar (Table 1).

**Virus yield in relation to inoculation dose:** A significant influence of inoculation dose on the per cent larval mortality and yield larva⁻¹ was observed with all the isolates tested. The highest mortality occurred with the highest inoculation dose of 1965.87 POB mm⁻², recording 82.05, 83.77 and 82.90% for the isolates CMB, NGM, OTY, respectively. These results are in agreement with the findings of Narayanan and Jayaraj (2002) who observed a marked difference between doses as well as a significant interaction between dosages and larval instars. A mean number of 2.5×10⁹ POB was harvested from late 4th instars when inoculated at low dose of 1.1×10⁶ POB cup⁻¹ compared to 1.2×10¹⁰ POB when inoculated at a higher dose of 1.1×10⁶. A virus concentration of 3×10¹⁰ POB mL⁻¹ by diet incorporation technique (Tuan et al., 1998) and 1×10⁹ POB mL⁻¹ by diet surface contamination method (Subramaniam et al., 2001; Kumar and Rabindra, 2003) were found to be optimum for in vivo production of the virus. However, the productivity ratio progressively decreased as the dose of inoculation increased. This is in partial agreement with the findings by Bell (1991) who reported that a lower dose of virus can be used for achieving higher yield.

**Virus yield in relation to incubation temperature:** Data on evaluation of different incubation temperature on the yield of three *HaNPV* isolates indicated that, maximum yield larva⁻¹ was obtained at 25°C followed by room temperature and 30°C (Table 3). Similarly, the yield per 100 inoculated larvae and productivity was also maximum at 25°C. However, the maximum larval mortality occurred at room temperature followed by 30 and 25°C (Fig. 2).

Insects systems function optimally within a limited range of temperature (Chapman, 1998). Synchrony in larval development during virus multiplication is considered important, as the larvae are sensitive to temperature fluctuation. Any variation in optimal temperature will directly affect the larval growth as well as viral multiplication. So, handling this synchronization to get the maximum benefit in terms of yield and productivity of the virus was one of the major objectives in the present studies. Optimization of larval growth during the virus incubation will allow the virus to attain rate of its multiplication. Studies conducted by O’Reilly and Miller (1989) indicated that prolongation in larval growth, even beyond the period of a normal larval stage, would benefit the viral reproduction. Present findings are in agreement with that of previous studies.

Cherry et al. (1997) indicated that the productivity was maximum at an incubation temperature of 25°C for *S. itura* and *S. exempta*. Huang (1995) reported that a temperature range of 24–27°C was optimum for 3rd and 4th instar *S. itura* larvae inoculated with 3.85×10⁶ and 3.85×10⁷ POB mL⁻¹, respectively, to maximize the SNPV yield. Incubation of early 5th instar *S. itura* larvae dosed with 3932.4 POB mm⁻² at 25°C enhanced the NPV productivity to 6.62×10¹⁰ POB yield per 100 inoculated larvae, while it was only 1.779×10⁹ at 35°C (Subramaniam et al., 2006).

Many laboratory studies have demonstrated that nucleopolyhedrovirus is inactivated by exposure to high temperature. McLeod et al. (1977) stated that increase in temperature from 15 to 45°C increased the LD₅₀ values of *H. zea* NPV (29.8-349.2 POB mm⁻³ of diet surface). Stairs (1978) indicated that high temperatures caused direct inactivation of the virus and adversely affected the viral replication. Johnson et al. (1982) demonstrated the inhibition of virus activity against the velvet bean caterpillar, *Anticarsia gemmatalis*, at the extremes of temperature of 10 and 40°C. Kelly and Entwistle (1988) found an approximate linear relationship between the *Mamestra brassicae* NPV and the incubation temperature. Histopathological studies by Sathiah (2001) revealed that at 25°C, the growth of fat body in virus-inoculated larvae progressed normally during the early stages of infestation providing adequate substrate for the growth and multiplication of the virus. In *H. armigera* larvae, the virus multiplied at a slow pace at 25°C allowing the fat bodies to proliferate simultaneously. At higher temperatures the virus multiplied faster destroying the fat body before it could grow to provide greater substrate volume. Therefore, a good mass production facility should possess a temperature-controlled incubation chamber to provide a constant temperature of 25±1°C.

**Virus yield in relation to period of harvest:** Harvesting of viroosed larvae is usually considered as a laborious and time consuming job in all the virus production units. Therefore attempts were made to optimize the period of harvest as it has influence on biological activity of the virus and growth of secondary contaminants like bacteria. The POB yield was higher when viroosed larvae were
harvested as cadavers than when harvested at different days after inoculation (Table 4). The POB yield per 100 inoculated larvae and productivity ratio were also higher in the case of all isolates when harvested from cadavers. The finding of this study is in contrast with that of Smith and Vlak (1988) who reported that production of *S. exigua* NPV did not increase after seven days of inoculation. However, Satish (2001) and Narabendhi (2004) state that harvesting the viroseed larvae as cadavers will enhance the yield production of *HaNPV*.

Ignofo and Shapiro (1978) found that the activity of *H. zea* NPV processed from dead hosts was 7-9 times higher than those from live viroseed larvae. Similarly, Shapiro and Bell (1981) evaluated the yield and biological activity of *Lymantria dispar* NPV at different times after infection. They showed that POB harvested from dead larvae were up to 7 times more active than from living infected larvae. Also, the virus yield increased up to about 2×10^3 POB larva⁻¹ during the first 11 days and then remained constant.

CONCLUSIONS

Virus mass production *in vivo* in host larvae is the pragmatic method as of today. A variety of parameters have been addressed for increasing productivity of the *HaNPV*. Present study revealed that factors like larval age and weight, dose of the inoculum, the incubation temperature and the period of harvest could enhance the *in vivo* yield of the virus. Of the larval age evaluated early 5th instar larvae recorded the maximum yield. The inoculation dose of 165.87 POB mm⁻³ and the incubation temperature of 25°C registered the highest *in vivo* virus yield. Also, the POB yield was recorded maximum range when viroseed larvae were harvested as cadavers. However, among the isolates tested in this study, CMB isolate collected from Tamil Nadu, India, showed the highest yield in all of the conditions tested compared to the other *HaNPV* isolates.

REFERENCES


