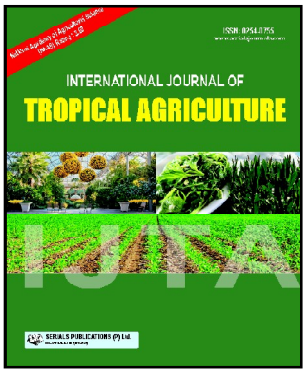

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Conservation of Baculovirus for the control of Bihar Hairy Caterpillar, *Spilarctia obliqua* (Walker) (Arctiidae: Lepidoptera)

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Abstract: The caterpillars of *Spilarctia obliqua* are susceptible to species specific virus, *Spilarctia obliqua* nuclear polyhedrosis virus (SoNPV). In this paper, methodology on mass production of SoNPV viral pesticide is discussed from the view point of field utility and conservation of the viral pathogen. This initiative is taken by virtue of eco-friendly approach of viral pesticide. The average yield of polyhedra of third & fourth instars is 3 X 10^8 POBs and 7 X 10^9 POBs per dead larva respectively. The viability of the polyhedra was 24 months under the indoor conditions of Imphal.

Key words: Bihar Hairy Caterpillar, biocontrol, *Spilarctia obliqua*, SoNPV, viral pesticide

INTRODUCTION

Spilarctia obliqua (Walker) (Arctiidae: Lepidoptera) is a polyphagous pest mainly feeding on economically important plants. The caterpillars of this pest feed voraciously on the foliage of the crops including that of oilseeds and pulses. In order to control this pest, the microbial pathogen has been found suitable (Battu *et al.,* 1991; Chaudhari, 1997; Singh and Varatharajan, 2001). SoNPV is a naturally occurring soil pathogen and becomes virulent when ingested by the caterpillar along with the leaf. Natural epizootic by SoNPV has been observed during drought period, but for the purpose of controlling the pest it is necessary to spray the virus on the crop. For the large scale application of viral pesticide, it is imperative to produce the concerned pathogen by "*in vivo*" process and store them under low temperature. Although *in vitro* method of producing viral pesticide is ideal, the production cost is more and hence the *in vivo* method is preferred for developing countries like India. In this paper,

methodology relating to mass production of SoNPV viral pesticide (Manipur isolate) and storage methods are discussed.

MATERIALS AND METHODS

S. obliqua larvae infest nearly 21 plant species under the climatic conditions of the valley region of Manipur (Rajen & Varatharajan, 2005). Its fecundity ranges from 600-1160 eggs per female. A colony consisting of about 480 larvae (IV instar) were mass reared on the leaves of *Phaseolus vulgaris* L., *Ricinus communis* L. , *Helianthus annuus* L. and *Ipomoea carnea* Jace @ 120 larvae per host, each with 3 replications @ 40 larvae per replication. To inoculate the larva with virus, the leaves were sprayed with pathogenic suspension of *So*NPV (Manipur Isolate) containing $5x10^5$ POBs/ml (Polyhedral Occlusion Bodies) and after air-drying; the base of the twig was inserted in to a small conical flask with water (Ingobi *et al.,* 2007). The insect culture was maintained in an insect cage (30 x 30 x 30 cm^3) at room temperature of 24±2°C with 75± 5% R.H and photoperiod of 13-14hrs day length (Rajen, 2002). The field collected caterpillars (IV instar), which were kept under starvation for 4hrs were released on the respective host @120 individuals per culture. For subsequent use, only fresh leaves of the respective plant host (virus untreated) were provided. Care was taken to remove excreta and dead larvae daily.

Mode of infection

- Virus enters the gut of the larva along with the virus contaminated foliage
- Virions enter into the nucleus of epithelial cells where they multiply
- Leading to spreading of viral infection within the body and eventually insect dies

Purification of occlusion bodies (POBs) (Sudhakar *et al.,* 1997)

1. Macerate the putrefied virus infected larvae.
2. Filter through muslin cloth.
3. Centrifuge at 500 rpm for 2 minutes.
4. Centrifuge the supernatant at 10,000 rpm for 25 minutes.
5. Wash the pellet with 0.1% SDS solution.
6. Centrifuge at 10,000 rpm for 25 min.
7. Wash the pellet with water.
8. Repeat the steps 6 & 7 for 3 times.
9. Finally the semi-pure pellets were collected & stored in amber colour bottle at 4° C.

Virus Production Method

1. Inoculate the larvae by leaf surface contamination method.
2. Allow the larvae to feed on leaf.
3. Collect the dead larva & store it in water; putrefy it for a week.
4. Grind the cadaver & filter it.
5. Remove debris by centrifuging.
6. Centrifuge the filtrate @ 10,000 rpm for 10 mintutes.
7. Wash the precipitate is washed again with water 2 or 3 times.
8. Stored at low temp.
9. Dilute the POBs & use it as per requirement for the field concerned.

RESULTS AND DISCUSSION

Lethal time (LT_{50})

Experiments on time factor versus mortality rate of *S. obliqua* larvae due to viral infection were carried out by Varatharajan and Ingobi (2002) and Ingobi *et al.*, (2007). They observed that the lethal time to kill 50% (LT_{50}) of their population ranged from 6.4 to 8.99 days for III - V instars indicating a gradual increase in tolerance of the larvae with their

advancing age/stage. For instance, LT_{50} of the virus inoculated IV instar of *S. obliqua* reared on *P. vulgaris* was 6.45 days, while that of *I. carnea* was 7.5 days. The LT_{50} data indicated that it is ideal to inoculate IV instar so that the yield of polyhedra production in the larva will be more during V instar stage. It is also shown that inoculating I to III and V instars for mass production of polyhedra will not be so advantageous because by the time they manifest symptoms of viral infection, V instar may pupate, while early stages of larvae invariably provide appreciably lesser yield than IV instar. For instance, the average yield of polyhedra of III & IV instars are 3 X 10^8 POBs and 7 X 10^9 POBs per larva respectively and therefore, IV instar is preferred for mass production.

Crowding effect

Based on the concept of crowding effect, an attempt has been made to find out how does it influence the infection rate. The crowding effect on viral infection indicated that the larval mortality due to viral infection was 18.4, 44.6 and 54.7% in the cultures with caterpillar density of 50, 100 and 150 larvae respectively (Table 1). This revealed that as the larval density increased in each culture, the viral infection also increased significantly. Crowding effects in general, bound to augment the infectivity because of the factors like limited space within the culture set up, restless movement of the larvae, oozing of haemolymph and behavior of cannibalism of the infected larvae etc. Even though, the viral infectivity increased in proportion with larval density, it is counterproductive when the larval density exceed beyond 150 per culture due to cannibalism; which appears to be unique feature among certain virus infected caterpillar species. In order to overcome this problem, the virus inoculated larvae are usually reared individually in a mini cup or ice tray as in the case of *Helicoverpa armigera* (Sathiah & Jayaraj, 1998) and *Agrotis segetum* (Vargas *et al.*, 1995). However, with respect to *S. obliqua*, the rate of cannibalism was appreciably low (4.8%) and therefore, the study inferred that approximately 150 larvae would be optimum for mass rearing.

Viability of SoNPV

Unlike chemical pesticide, the pathogenic pesticides can't be stored for long period in view of their dwindling viability. Therefore, pathogen's viability needs to be tested and that is of paramount importance in order to achieve good control over pest. Keeping this view in mind the storage effect of polyhedral bodies of SoNPV was studied at different periods of time and the efficacy was evaluated in term of LT_{50} against IV instar larvae. The LT_{50} value did not vary much till 24 months of storage but it was found to be 15 days for the polyhedra stored for 30 months. Amrapali and Sarwshri (2011) also reported that population reduction in *Helicoverpa armigera* was appreciably high when fresh HaNPV was sprayed in comparison to those of stored one. Parasnath and Chakravorthy (2004) also found the lowest average population of *H. armigera* when sprayed with the fresh NPV formulations. Studies relating to viability of viral pathogen in the present work revealed that SoNPV is viable for at least 24 months. However the pathogen can be augmented using the simple *in vivo* system and fresh stock can be used in combination with preserved one for field application. The above experiment on SoNPV has been taken by virtue of its eco-friendly approach, target specific action of

Table 1
Effect of larval density on percentage larval mortality

Sl no.	*No. of larvae per culture*	*Mean larval mortality due to viral infection* *
1.	50	18.4
2.	100	44.6
3.	150	54.7
	CD at 1% level	9.5 (ANOVA)

*Percentage larval mortality 7 days after post infection

SoNPV, besides having multifaceted advantages of compatibility with other pesticides.

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