## Bio control:: Mass production::Virus

### Production of the nuclear polyhedrosis virus of *Helicoverpa armigera*

**Introduction**

NPV infected larva

In India, *Helicoverpa armigera* is of major importance damaging a wide variety of food, fibre, oilseed, fodder and horticultural crops. The nuclear polyhedrosis virus of*H. armigera* (HaNPV) is currently used for the management of *H. armigera* on chickpea, cotton, pigeon pea, tomato and sunflower. Mass production of Nuclear Polyhedrosis Virus (NPV) on commercial scale is restricted to *in vivo* procedures in host larvae which are obtained by

- Field collection from cotton, pigeon pea and chickpea – *H. armigera*
- Mass culturing in the laboratory in semisynthetic diet – *H. armiger*

Some small scale producers use field – collected larvae for mass production of NPV in spite of the following constraints.

- Collection of a large number of larvae in optimum stage (late IV / early V instars) is time-consuming and can be expensive in terms of labour and transportation costs.
- Wild populations of insects may carry disease causing organisms like microsporidians, cytoplasmic polyhedrosis virus, stunt virus and fungal pathogens which will affect both virus production and quality.
- Introduction of wild strains of NPV resulting in quality control problems.
- Transportation of a large number of larvae with cannibalistic behaviour will be a difficult task.
- Parasitized larvae collected from the field will die prematurely yielding little virus.

Rearing of larvae in the natural host plant will involve frequent change of food at least once a day during the incubation period of 5-9 days increasing the handling time and hence the cost. In order to reduce the cost, field collected larvae are released into semi synthetic diet treated with virus inoculum. Mass culturing of insects in semi synthetic diet involves high level of expertise, hygiene and cleanliness.

**Production procedure**

The NPV of *H. armigera* is propagated in early fifth instar larvae. The virus is multiplied in a facility away from the host culture laboratory. The dose of the inoculum used is 5 x 105 polyhedral occlusion bodies (POB) in 10 ml suspension. The virus is applied on to the semisynthetic diet (lacking formaldehyde) dispensed previously in 5 ml glass vials. A blunt end polished glass rod (6 mm) is used to distribute the suspension containing the virus uniformly over the diet surface. Early fifth instar stage of larvae are released singly into the glass vials after inoculation and plugged with cotton and incubated at a constant temperature of 25oC in a laboratory incubator. When the larvae exhausted the feed, fresh untreated diet is provided. The larvae are observed for the development of virosis and the cadavers collected carefully from individual bottles starting from fifth day. Approximately, 200 cadavers are collected per sterile cheese cup (300 ml) and the contents are frozen immediately. Depending upon need, cadavers are removed from the refrigerator and thawed very rapidly by agitation in water.

**Processing of NPV**

The method of processing of NPV requires greater care to avoid losses during processing. The cadavers are brought to normal room temperature by repeatedly thawing the container with cadaver under running tap water. The cadavers are homogenized in sterile ice cold distilled water at the ratio 1: 2.5 (w/v) in a blender or precooled all glass pestle and mortar. The homogenate is filtered through double layered muslin and repeatedly washed with distilled water. The ratio of water to be used for this purpose is 1: 7.5-12.5 (w/v) for the original weight of the cadaver processed. The left over mat on the muslin is discarded and the filtrate can be semi-purified by differential centrifugation. The filtrate is centrifuged for 30-60 sec. at 500 rpm to remove debris. The supernatant is next centrifuged for 20 min at 5,000 rpm. Then the pellet containing the polyhedral occlusion bodies (POB) is suspended in sterile distilled water and washed three times by centrifuging the pellet in distilled water at low rpm followed by centrifugation at high rpm. The pellet finally collected is suspended in distilled water and made up to a known volume, which is necessary to calculate the strength of the POB in the purified suspension.