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RESEARCH ARTICLE

POTENTIAL AND USE OF BACULOVIRUSES AS INSECTICIDES

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ABSTRACT

At present crop protection is largely dependent on synthetic insecticides, with their associated resistance and environmental problems. So we require an integrated complex of control measures to manage crop pests. Biological control is one of the important components of integrated management system. Exploitation of insect pathogens in biological control is a rapidly growing aspect known as microbial pest control. Insect pathogens include bacteria, viruses, protozoa, fungi, nematodes, and other organisms that infect and kill insects. Naturally occurring as well as genetically modified insect pathogens that directly or indirectly reduce insect pest populations are known as microbial insecticides. Microbial insecticides are highly specific and safe to non-target microbes/animals including humans.

Key Words : Baculoviruses, Insecticides, IPM, NPV**.**

During the late nineteenth and early twentieth century, there was an increased interest in the use of fungi against insect pests but subsequently from 1920 to 1960, many bacteria, viruses and nematodes were tried out. Baculoviruses are widely used both as insect control agent and as protein expression vector. Baculoviruses have numerous unique features that have generated interest in their use as microbial insecticides *viz*., (i) host specificity, (ii) virulence in host insect (iii) no residual toxicity, (iv) environmental and mammalian safety (v) long shelf life, (vi) easily applied using conventional spray equipments, (vii) causing epizootic and (viii) compatibility with other control agents.

The majority of baculoviruses used as biological control agents are in the genus *Nucleopolyhedrovirus*, so “baculovirus” or “virus” will hereafter refer to nucleopolyhedroviruses. These viruses are excellent candidates for species-specific, narrow spectrum insecticidal applications. They have been shown to have no negative impacts on plants, mammals, birds, fish, or even on non-target insects. This is especially desirable when beneficial insects are being conserved to aid in an overall IPM programme, or when an ecologically sensitive area is being treated.

This article provides an over view of the baculovirus life cycle, production and use of baculoviruses as insecticide.

Entomopathogenic Viruses:

The virus is one of the simplest living form, composed of a protein shell (*capsid*) that surrounds the *nucleic acid*. The nucleic acid is infectious in nature while capsid provides the morphological properties. Each virus has one type of nucleic acid, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The nucleic acid may be double or single stranded. The nucleic acid together with capsid forms the *nucleocapsid or virion*. Virion may be further embedded singly or in groups (in some virus) in protective protein matrix known as occlusion body.

More than 20 groups of viruses are known to be pathogenic to insects.They have been placed in 16 viral families, but many remain unclassified. The family Baculoviridae is limited in its host range to arthropods whereas other families contain viruses associated with mammals or other non-arthropod groups, so are of less interest as potential insect control agents.

Baculoviruses :

Baculoviruses derived from the Latin word "Baculum" (rod or stick) are the rod shaped virus particles comprising of a lipoprotein envelope around a protein capsid containing DNA-protein core. Baculoviruses are a very diverse group of viruses with double-stranded, circular, supercoiled genomes, with sizes varying from about 80 to over 180 kb and that encode between 90 and 180 genes. The genome is packed in rod-shaped virions that are over 200 ìm in length and 30 ìm in diameter. Virions embedded singly or in groups in protective protein covering known as occlusion body.

The Baculoviridae contains two genera based on characteristic of presence of occlusion bodies called polyhedra for nucleopolyhedrovirus (NPVs) and granules for granulovirus (GVs). Baculovirus remain infectious eternally due to highly stable occlusion bodies. The occlusion body is composed of a protein called polyhedrin in NPVs and granulin in GVs. These two proteins are closely related, although they have different names.

Recent evidence from genome sequence analyses demands a substantial revision of the taxonomy and classification of the family *Baculoviridae*. Comparisons of 29 baculovirus genomes indicated that baculovirus phylogeny followed the classification of the hosts more closely than morphological traits that have previously been used for classification of this virus family. On this basis Jehle *et al*., 2006 proposed that member of the Baculoviridae be divided into four major phylogenetic group (Table1).

Table 1: Proposed genera of Baculoviridae

Sr. No.	Genus	Member
1.	Alphabaculovirus	Lepidopteran NPVs
2.	Betabaculovirus	Lepidopteran GVs
3.	Gammabaculovirus	Hymenopteran NPVs
4.	Deltabaculovirus	Dipteran NPVs

MATERIALS AND METHODS

Over the years baculoviruses are the best known of all the insect viruses, as they constitute one of the largest insect pathogenic viral groups and have been reported from a variety of different species of invertebrates. However, the only well-documented hosts are Diptera, Hymenoptera, and Lepidoptera. Baculoviruses were originally named by the first letter of the genus and species of their host such as AcMNPV, was named for its host, the alfalfa looper, *Autographa californica* (Ac). Now AcMNPV infects a wide variety of lepidopteran insects and its name originated because of its initial association. Similarly, a variety of other virus isolates e.g., *Galleria mellonella* (GmNPV), *Rachiplusia ou* (RoNPV), and *Plutella xylostella* (PlxyNPV), *Helicoverpa armigera* (HaNPV). However, as more and more viruses were discovered, some infected different insects having the names with the same first letters. This resulted in different viruses with the same description viz., NPV of *H. armigera* (HaNPV) and *H. assulta* (HaNPV). Consequently, the first two letters of the genus and species have become the convention, i.e., AcMNPV should really be

AucaMNPV and HaNPV be HearNPV. However, since AcMNPV, GmMNPV, HaNPV, SfNPV have been used so extensively, the original abbreviations have been retained.

Nuclopolyhedrosis Viruses (NPV)

The NPVs are the best known of all the viruses as they are primarily pathogens of agriculturally important lepidopteran pests. NPVs are virions embedded in a crystalline matrix of the protein, polyhedrin. The virions consist of an enveloped nucleocapsid that contains double-stranded circular DNA genome of about 130kb, singly (SNPV) or in multiple (MNPV). The occluded particles are known as polyhedral as they are typically polyhedral in shape. Polyhedra are large in size varying from 500 to 1500 nm and are formed in the nuclei where each occludes one or many virions (Fig.1).

Mode of action of NPV

NPVs are easily transmitted *per os.* On ingestion the polyhedra are rapidly dissolved in the highly alkaline digestive juices (pH 9.0 to 11.5) and acted upon by alkaline proteases to release the enveloped virions into the gut lumen. The virions released from the polyhedra fuse with the microvilli of midgut epithelial cells, migrate through the cytoplasm, and uncoat in the nucleus (Granados and Lawler, 1981). In the infected cells, progeny of virus particles seen in the nucleus may have two fates, either they may move out of the nucleus into the cytoplasm and bud through the plasma membrane (budded virus, BV) or they may be enveloped *de novo* in the nucleus and later occluded within polyhedrin (polyhedra derived virus, PDV). BV results in secondary infection of tissues throughout the insects. The PDV are released into the environment through cell lysis. Larvae infected with NPV lose their appetite but continue to feed at lower rate. After incubation period of some days, the larvae become sluggish pinkish in colour, swell slightly and then become limp and flaccid. Prior to death, larvae infected with NPV climb up the plant and hang by their prolegs upside down from an elevated position (Fig. 2). The cadaver eventually liberates polyhedra in to the environment.

NPV is highly species specific, However NPV of alfalfa looper, *Autographa californica* is capable of infecting many lepidopteran insect species.

Granulosis Virus (GV)

More than 200 isolates of GV are known from lepidopterous insects. They are closely related to NPVs but differ from latter in some important respects. They replicate in cell nucleus but unlike NPV continue to replicate in the cytoplasm of the cell after lysis of the nucleus. Virion size ranges from 30-60 x 260-360 nm, occluded singly in occlusion bodies (300-500 nm) known as granules (Fig. 3). The period between ingestion of virus and death of host ranges from 4-25 days. Initially there are no external symptoms but at the later stages,

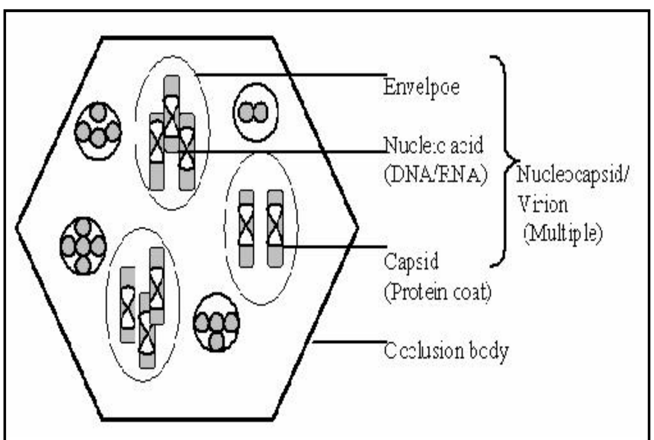

Fig 1 : Diagrammatic representation of NPV structure (Source : Kalia, 2009

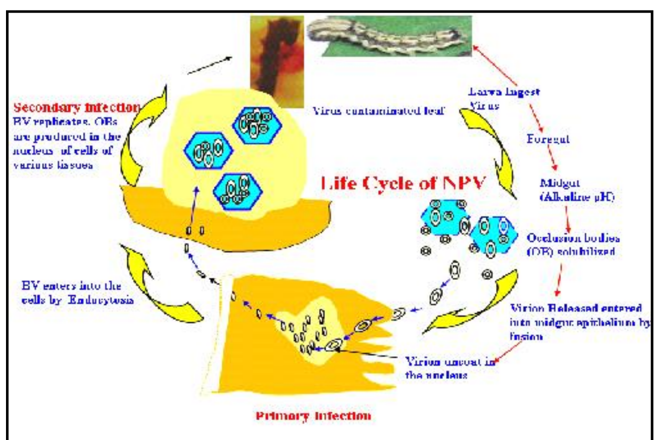

Fig 2 : Mode of action of NPV (Source Kalia, 2009) infected larvae develop a lighter colour.

Baculovirus as insecticide

The most commonly used viruses for development, as viral insecticides, are the NPVs although GVs are used in some countries for control of codling moth (*Cydia pomonella*) and cabbage worms (*Pieris* species). Use of NPV as an insect control agent was initially started against spruce sawfly, in forest and later for the control of *Helicoverpa zea* and *H. virescens* in agriculture (Black *et al.*, 1977; Szewezyk, 2006; Incoglu *et al.*, 2006). In 1975, the first viral insecticide Elcar ™ was registered in the United State for use on cotton. Indian standards for baculovirus specification are presented in Table 2 (www.cibrc.nic.in/revregubio2009apr.doc). The viruses that have been registered for use in agriculture and forestry in India Annexure-II (Table 3)]

Table 2 : Indian standards: baculovirus specification

Sr. No.	Baculovirus*	Viral Unit
1.	NPVs (Helicoverpa & Spodeptera)	$1x10^9$ OB/ml or g. (minimum)
2.	GV (Chilo, Plutella & Achea)	$5x10^9$ Capsules/ml or g. (minimum)

**Pathogenic contaminants such as gram negative bacteria Salmonella, Shigella, Vibrio etc should be absent

There have been many examples of the use of baculoviruses to control insect pests worldwide. One of the most successful has been the application of velvet bean caterpillar NPV to control the, *Anticarsia gemmatalis*, a major pest of soybean in Brazil (Moscardi and Sosa-Gomez, 1992). Fuxa (1990) lists 28 cases in which baculovirus have been used in attempts to suppress insect pests. Of these, 15 cases involves introduction of virus followed by its permanent establishment and 13 cases were based on augmentation use. Most of these cases involved NPV, four being GV and one non-occluded virus. Another major success has been the control of a coconut rhinocerous beetle, *Oryctes rhinoceros* of coconut palms, in the pacific region, which was suppressed by nonoccluded virus upto 3.5 year after introduction of virus (Zelazny *et al.*, 1990). The pine sawfly, *Neodiprion sertifer* is successfully controlled with NPV

Table 3 :

COMMODITY	INSECT PEST	VIRUS USED	VIRUS PRODUCT
Apple, pear, walnut and plum	Codling moth	Codling moth granulosis virus	Cyd-Xe
Cabbage, tomatoes, cotton,	Cabbage moth, American bollworm, diamondback moth, potato tuber moth, and grape berry moth	Cabbage army worm nuclear polyhedrosis virus	Mamestrin
Cotton, corn, tomatoes	Spodoptera littoralis	Spodoptera littoralis nuclear polyhedrosis virus	Spodopterin
Cotton and vegetables	Tobacco budworm Helicoverpa zea, and Cotton bollworm Heliothis virescens	Helicoverpa zea nuclear polyhedrosis virus	Gemstar LC, Biotrol, Elcar
Vegetable crops, greenhouse flowers	Beet armyworm (Spodoptera exigua)	Spodoptera exigua nuclear polyhedrosis virus	Spod-X
Vegetables	Celery looper (Anagrapha falcifera)	Anagrapha falcifera nuclear polyhedrosis virus	none at present
Alfalfa and other crops	Alfalfa looper (Autographa californica)	Autographa californica nuclear polyhedrosis virus	Gusano Biological Pesticide

(Doyle and Entwistle, 1990). In China, larger areas of cotton crops have been sprayed with the *H. zea* NPV (Guangyu, 1989).

Mass Production of NPV

Baculovirus is an important biological option of IPM available for many important pest viz., *H. armigera*, *Plutella xylostella*, *Spodoptera litura etc.*, management due to its well demonstrated proven efficacy on a number of crops all over the country (Kalia and Gujar, 2004). This option remains underutilized as the products are not yet available readily in the market mainly for the lack of organized efforts for its production. Its commercial production is limited since all viruses are obligate parasites so this necessitates a large and productive insect colony as a source of healthy larvae that can be inoculated with lethal amounts of POBs and later harvested as dead infected insects.

Mass production of NPV can be done by two methods:

– by using the live larvae or

– by inoculating the cell line culture of the pest.

The production of HaNPV/SlNPV on cell lines is not yet commercially viable. For the production in live larvae, either the field collected larvae of *H. armigera* or the laboratory-reared larvae can be used.

Application of NPV

On the other hand, limitations include like non-specificity, costly *in vivo* production, limited market size, and relatively low cost-effectiveness, therefore profitability and their efficacy. The slow speed of kill, vulnerable to solar UV light and target early instars due to maturation resistance limit the use of viral insecticides.

Quality control and Registration

Quality control is prerequisite for the ultimate success of the any biopesticides usage. Contamination during production, poor storage conditions, use of improper stage of insect or less effective strain of NPV, methodology used etc., effect the quality of the NPV product. As baculoviruses in the country have been included under Insecticide act, so all the commercial product of NPV / GV must follow the guidelines issued by central insecticide board u/s 9(3B) and 9(3) of the Insecticide Act, 1968.

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