Review Article

Baculoviruses: Lethal pathogens of lepidopteran caterpillars

SANJAY M. BANDI¹, G. SIVAKUMAR² and B. KIRAN GANDHI¹
¹ICAR-Indian Institute of Pulses Research (IIPR), Kanpur – 208024, Uttar Pradesh, India
²ICAR- National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru – 560024, Karnataka, India
Corresponding author E-mail: sanjaysmb@gmail.com

ABSTRACT: Baculoviruses, a diverse group of arthropod-specific viruses, have long been employed for the biological control of many economically significant insect pests on agricultural and forest crops all over the world. They are primarily pathogens of caterpillars and about 90 per cent are reported to cause diseases in members belonging to the order Lepidoptera. Baculoviruses have been attractive biological control agents because of their safety to vertebrates, other non-target fauna and high pathogenicity with host death being most likely outcome of an infection. They have an ability to persist outside the host insect by producing virions sequestered within the protein matrix and potential to trigger epizootics in insect population, thus being important factor in regulating the size of host insect population. Baculoviruses are relatively quick acting and lethal among the various insect pathogens infecting globally significant pest species. Considering the importance of baculoviruses in insect pest management, a review of the research work carried out is presented.

KEYWORDS: Baculoviruses, biological control, insect pathogenic viruses, lepidopteran pests
(Article chronicle: Received: 12-07-2019; Revised: 10-11-2019; Accepted: 25-11-2019)

INTRODUCTION

Insect pathogenic viruses from fifteen families were known to cause diseases in insects (Hu et al., 2002; Szewczyk et al., 2006). However, of all the insect viruses known to mankind, only members of family Baculoviridae have been considered as potential pathogen of insects. Baculoviruses were the most intensely studied and major group of arthropod-specific viruses (Herniou and Jehle, 2007), found ubiquitously in the environment. They were predominantly associated with members of arthropod species and reported non-pathogenic in members outside the phylum Arthropoda (Miller, 1997). Baculoviruses were employed in biological control of insect pests of forestry and agriculture since 1900s (Slack and Arif, 2007; Beas-Catena et al., 2014) with more than 600 host-insect species (Martignoni and Iwai, 1986) belonging to the orders Lepidoptera, Hymenoptera, Diptera (Herniou et al., 2012), Orthoptera, Coleoptera, Neuroptera, Thysanura, Trichoptera (Murphy et al., 1995) and crustacean order, Decapoda (Blissard et al., 2000). About 35 insect pathogenic viruses were reported in India (Rao et al., 2015) mostly from economically important pests like Helicoverpa armigera (Hubner), Spodoptera litura (Fab.), Spilosoma obliqua (Walk.), Amsacta albistriga (Walk.), Achaea janata, (L.) Chilo spp., etc., infesting cotton, pigeonpea, chickpea, tomato, groundnut, castor and sugarcane.

History

The historical accounts of baculovirus mainly the early discoveries were documented by Steinhaus (1949) and reviewed by Arif (2005). The earliest accounts of baculovirus infections were found in ancient Chinese literature describing the disease of silkworms as early as 16th century. The first description of baculovirus disease was credited to Marco Vida, an Italian poet in 1527 (Benz, 1986). Infected silkworm larvae had swollen body and appeared yellow shiny which eventually caused rupture of insect skin and exudation of body fluid. In 19th century, this disease of silkworm was referred as “jaundice” in America and “grasserie” in France (Steinhaus, 1949). Subsequently several investigators observed crystal like corpuscles in the tissues and body fluids of diseased larvae of silkworm. Maestri, A. and Cornalia, E. were among the first to describe about the refractive bodies in jaundiced larvae in 1856; later Bolle, J. demonstrated these refractive occlusion bodies as causative agents of infection. These causative agents were observed as tiny granules under dark-field microscope and named as Borrellina bombycis by Andre Paillot in honor of Professor Borrel of the Pasteur Institute.
The infectious nature of filtrate of diseased silkworm passed through several layers of filter papers was observed by Von Prowazek in 1907. Glaser, R.W. in 1918 documented the transmission of polyhedral disease in gypsy moth by using filtrates of infected larvae (Benz, 1986). Nearly a decade later, French scientist, Andre Paillot discovered disease of *Pieris brassicae* (L.) having etiology different from earlier descriptions and characterized by presence of numerous small granules and later he designated the pierid disease as “pseudo-grasserie”, the causative agent of which was subsequently termed as *Granulosis* by Steinhaus. Ishimori (1934) discovered another disease with Occlusion Bodies (OB) in the cytoplasm of infected host insect representing different replication site and etiology which was later referred as occlusionplasmic virus (reviewed by Arif, 2005). Bergold (1950) contributed to biochemical properties of virus in 1930s-40s and revealed the rod shaped virions within the polyhedra. Contributions of Steinhaus and other workers from 1950 to 1975 led to recognition of baculoviruses as biological control agent of many insect pests (reviewed by Miller, 1997). The first insect pathogenic baculovirus product, Elcar was registered as insecticide by Sandoz Inc., in the United States 1975 led to recognition of baculoviruses as biological control agent of many insect pests (reviewed by Miller, 1997). The first insect pathogenic baculovirus product, Elcar was registered as insecticide by Sandoz Inc., in the United States

Contributions of Steinhaus and other workers from 1950 to 1975 led to recognition of baculoviruses as biological control agent of many insect pests (reviewed by Miller, 1997). The first insect pathogenic baculovirus product, Elcar was registered as insecticide by Sandoz Inc., in the United States

In India, the existence of NPV in *H. armigera* was reported from Gujarat by Patel et al. (1968) subsequently, Jacob and Subramanian (1972) reported NPV from *H. armigera* and *A. albistriga* from Coimbatore. The occurrence of Granulosis Virus (GV) was reported on shoot borer, *Chilo infus catellus* Snell (Easwaramoorthy and David, 1979), internode borer, *Chilo sacchariphagus indicus* (Kapur) (Mehta and David, 1980) and top borer, *Scirpophaga exeruptalis* Walker (Singaravelu et al., 1999) in sugarcane. Gopinadhan et al. (1990) reported Cytoplasmic Polyhedrosis Virus (CPV) infection in all life stages of red palm weevil (*Rhynchophorus ferrugineus* Olivier) in coconut from Kerala.

**Baculovirus structure**

Baculoviruses are bacilliform, enveloped viruses with covalently closed circular double-stranded DNA genome (Blissard, 1996) ranging in size from 82 kb to 180 kb in size (Lauzon et al., 2006; Hayakawa et al., 1999) and predicted to encode for about 90 to 180 genes (Okhano et al., 2006). The genome packaged in bacillus-shaped nucleocapsids and hence the name “baculovirus” (Slack and Arif, 2007). Rod shaped nucleocapsid (average 30-60 nm diameter and 250-300 nm length) (Jehle et al., 2006) surrounded by lipoprotein envelope and referred as virion (Occlusion Derived Virus). Two phenotypically distinct but genetically identical forms, Occlusion-Derived Virion (ODV) and Budded Virion (BV) were recognized in baculoviruses (Blissard and Theilmann, 2018). BV differed from ODV in the structure of their envelope (Okano et al., 2006). Virions were occluded either within polyhedral or granular OB’s. Polyhedral-shaped Occlusion Bodies (POB’s) of NPV’s composed of matrix protein called polyhedron (Murphy et al., 1995; Hooft van Iddekinge et al., 1983), while GVs produced smaller ovi-cylindrical occlusions (Williams and Faulkner, 1997) composed of a major matrix protein called granulin (Funk et al., 1997; Winstanley and O’Reilly, 1999). Proteinaceous occlusion bodies contributed to stability and persistence of virus outside the host insect in the environment (Slack and Arif, 2007).

**Baculovirus classification**

Francis O. Holmes published an account of virus classification titled “Order Virales, The Filterable Viruses” in sixth edition of *Bergey’s Manual of Determinative Bacteriology* Holmes group categorized the insect pathogenic viruses into two genera, *Borrelina* and *Morator* in the family Borrelinaceae, suborder Zoophagineae and order Virales (Holmes, 1948). The genus *Borrelina* included viruses inducing polyhedral, wilt and other diseases of Lepidoptera. Genus *Morator* had only one virus species inducing sacbrood disease in honeybee. Steinhaus (1949) revised the Holmes classification and recognized four genera based on the type of inclusion bodies formed by viruses. Genera, *Borrelina* and *Bergoldia* were characterized by the polyhedral and granular inclusions, respectively, in the infected cells of host-insect. Genus, *Paillotella* showed refringent, polymorphic inclusions of very irregular shape and size. Genus, *Morator* was characterized by no visible pathological inclusions.

Subsequently arthropod specific viruses were classified as baculoviruses, which had three subgroups; subgroup A (NPV), subgroup B (GV) and subgroup C (Non-occluded Viruses) (Moore et al., 1987). Until 1995, family Baculoviridae was subdivided into two subfamilies, *Eubaculovirinae* comprising occluded viruses and *Nudibaculovirinae* with non-occluded viruses (Francki et al., 1991). *Eubaculovirinae* included two genera, NPV and GV. Two subgenera were established within the NPV genus, one comprising viruses with multiple nucleocapsids

---

308

---
per envelope (MNPV) and other comprising viruses with a single nucleocapsid per envelope (SNPV). A genus Non-Occluded Baculoviruses (NOB) had been established within the subfamily Nudibaculovirinae. Following reclassification, family Baculoviridae was subdivided into two genera, Nucleopolyhedroviruses (NPVs) and Granuloviruses (GVs) (Murphy et al., 1995; Rohrmann, 1999; Winstanley and O’Reilly, 1999) mainly based on the morphology of occlusion bodies formed in the infected cells. Both genera showed considerable similarities in genome structure, infection cycle, morphology and structure of nucleocapsids (Ikeda et al., 2015), but were clearly distinguished by the type of occlusion bodies produced. Granuloviruses were composed of one nucleocapsid per envelope, while NPVs were packaged either as one nucleocapsid per envelope (SNPVs) or one through many nucleocapsids per envelope (MNPVs) (Adams and McClintock, 1991). The GVs were reported solely from lepidopteran hosts (Murphy et al., 1995), whereas NPVs were isolated from a wider range of insects. About 90 per cent of baculoviruses were reported from 34 different families of Lepidoptera (Lange et al., 2004).

Jehle et al. (2006) proposed the revision of baculovirus classification on the basis of genome sequence analysis and associated arthropod host, and recognized four genera, Alphabaculovirus, Betabaculovirus, Gammabaculovirus and Deltabaculovirus within the family Baculoviridae. Genera, Alphabaculovirus, Gammabaculovirus and Deltabaculovirus included NPVs that were specific for members belonging to orders Lepidoptera, Hymenoptera and Diptera, respectively, whereas Betabaculovirus included lepidopteran specific GV’s (Herniou et al., 2012). The lepidopteran specific NPVs (Alphabaculovirus), on the basis of phylogenetic analyses, were subdivided into Group I and II NPVs (Bulachet al., 1999; Zonatto et al., 1993; Herniou et al., 2001). Group I and II NPVs had GP64 and F-proteins, respectively, as envelope fusion proteins of Budded Viruses (BV), which were essential for cell attachment, membrane fusion during viral entry through endocytosis, and efficient budding of progeny BV’s from infected cells (Garcia-Maruniak et al., 2004; Lauzon et al., 2006; Ikeda et al., 2015).

Baculovirus nomenclature

Baculovirus nomenclature was based on the host-insect species from which they were first isolated (Tinsley and Kelly, 1985) thus species name consisted the name of the host insect followed by the abbreviations GV or NPV indicating the type of OB formed. The Nucleopolyhedrovirus isolated from insect host, H. armigera was originally named as HaNPV. However, this kind of nomenclature occasionally created confusion for baculoviruses that had relatively wide host range. Some baculoviruses were completely specific to their host. e.g., SeMNVP infected only Spodoptera exigua (Hubner) (Williams et al., 2016), whereas AcMNPV reported to infect 30 species from about 10 insect families belonging to order Lepidoptera (Groner, 1986). Presently insect pathogenic viruses were named using first two letters of the genus and species of their host, hence NPV species isolated from Helicoverpa armigera abbreviated as HeNPV.

Life cycle and pathogenesis of baculoviruses

Baculoviruses are obligate pathogens (Steinhaus, 1949) and require host cells for their replication. Baculovirus infection involved bi-phasic process, life cycle was characterized by production of two genotypically identical but structurally distinct (reviewed by Blissard, 1996; Rohrmann, 1999) virus phenotypes, each playing specific role during baculovirus life cycle. The BV phenotype (also referred as nonoccluded virus/ extra-cellular virus) produced during early stage of infection which was responsible for systemic cell-to-cell propagation of virus within the host insect (Rohrmann, 1999). In contrast, ODV was produced during the terminal stage of virus infection affecting host-to-host transmission of disease by persisting outside the host insect. The infection of NPVs in Diptera (Deltabaculoviruses) and Hymenoptera (Gammabaculoviruses) reported to be restricted to gut cells (Federici, 1997; Moser et al., 2001; Young et al., 1972) whereas most of the Lepidopteran specific NPVs (Alphabaculoviruses and some Betabaculoviruses), infection had spread from the midgut cells to a variety of other tissues via BV’s (Okano et al., 2006).

Baculovirus infection in insects was initiated by per os (Slack and Arif, 2007) when susceptible host-insect larvae inadvertently consumed the OB contaminated foliage. Infectious units of baculovirus, OBs along with food particle passed through foregut of the insect to midgut. The combined action of gut protease (Granados, 1978; Harrap, 1970) and alkaline nature of caterpillar midgut triggered the dissolution of OBs (Federici, 1997) and ODVs reached midgut lumen where they passed through peritrophic membrane and entered the midgut cells, a primary site of infection. In midgut cells, ODV’s fused with plasma membrane surrounding microvilli on the apical brush border of columnar epithelial cells (Granados, 1973). The DNA-containing nucleocapsids were transported to the nucleus to initiate infection (Okaoka et al., 2010) wherein, viral DNA was uncoated followed by transcription and genome replication (reviewed by Slack and Arif, 2007). The newly synthesized genomes were packaged and transported to the basal site of infected midgut cell to produce BVs, which initiated the infection from cell to cell inside the insect body, but in the very late stage of infection, infected cells switched to ODV production and OB formation (Williams et al., 2016). As infection advanced, hundreds of millions of polyhedra were produced and released into the environment upon liquefaction of infected host.
The progression and signs of diseases caused by baculoviruses are influenced by numerous factors including the stage (instar) and physical attributes of the host insect, temperature, inoculum dose and virulence of virus. Ingestion of numerous polyhedra caused the death of early instars (1, 2 and 3) within 72 hours (Federici, 1997). However, the host infected with same amount of virus inoculum during later instars (4th or early 5th) developed the disease between 5-10 days at temperatures of 25-30 °C.

Near the end of infection cycle, the food consumption in NPV infected insects ceased (Aizawa, 1963), larvae turned sluggish and swollen. Cuticle appeared glossy due to accumulation of polyhedra in epidermal and fat body nuclei. The hemolymph of infected host larvae was cloudy owing to the circulation of large numbers of infected hemocytes and polyhedra into the haemolymph upon cell lysis at an advanced stage of disease (Granados and Lawler, 1981). Just prior to death, diseased larvae of many lepidopteran species often found attached themselves to top of the vegetation by their prolegs with the head and rear portion of the abdomen facing downward in an inverted ‘V’ shape. This characteristic posture of larvae in case of nun-moth caterpillar (*Lymantria monacha* L.) to the tops (*Wipfeln*) of tree was described as ‘wipfelkrankheit’ in German or tree top disease (Hofmann, 1891; Goulson, 1997). Death of the host insect was accompanied by liquefaction of the internal anatomy, weakening of the cuticle and liberation of progeny OBs into the environment which acted as source of inoculums.

**Host range**

Baculovirus infection was limited exclusively to arthropods, predominantly holometabolous insects (those undergoing complete metamorphosis). Baculoviruses have been reported to have evolved with their holometabolous insect-hosts beginning around 310 million years ago way back in the Paleozoic Era in the Carboniferous Period (Theze et al., 2011). The host range of GVs was relatively narrow, being pathogenic only to the members of order Lepidoptera, whereas NPVs were reported from Lepidoptera, Diptera, Hymenoptera (Herniou et al., 1999). Although, 90 per cent of NPVs had been isolated from members of Lepidoptera family, most species of baculoviruses were found within the genus *Alphabaculovirus* and *Betabaculovirus*. The NPV species isolated from the family Lymantridae tend to be very narrow in host range (Barber et al., 1993; Grzywacz, 2016), while some of the species isolated from Noctuidae showed extensive host ranges (Vail et al., 1999). Among NPVs, SNPV reported to have fewer hosts than MNPVs (Groner, 1986). The host range of *Autographa californica* MNPV (AcMNPV) was extremely wide and reported to infect at least 32 species of Lepidoptera within 12 families (Volkman, 1997; Vail et al., 1999). The NPVs of *Anagapha falcifera* (Kirby) and *Mamestra brassicae* (L.) were also reported to have broader host range (Hostetter and Putler, 1991; Groner, 1986). The host range of NPV has been reported to about 300 species of Lepidoptera, 20 species each of Diptera and Hymenoptera while GVs reportedly infected about 150 species of Lepidoptera. (Rohrmann, 1999). Globally several insect pathogenic viruses belonging to the genus *Alphabaculovirus* and *Betabaculovirus* of baculoviridae have either been employed or shown enormous potential in the biological suppression of economically significant caterpillar pests of agriculture and forestry (Table 1).

**CHARACTERIZATION OF BACULOVIRUSES**

**Morphological properties**

The initial differentiation of virus groups was largely based on light microscopy with phase contrast or bright-field microscope. Baculoviruses were large rod shaped or bacilliform viruses (Blissard, 1996) whose infection was characterized by production of two morphologically distinct virion phenotypes, BV and ODV (Blissard and Theilmann, 2018). Virions of the genera *Alphabaculovirus*, *Gammabaculovirus*, *Deltabaculovirus* involved in infection process composed of crystalline protein matrix (polyhedrin) and polyhedral in shape which measured about 0.5 to 5 µm in diameter. In *Betabaculovirus*, these virions were ovicylindrical composed of granulin protein matrix with a diameter of

**Table 1. Baculovirus species associated with economically important lepidopteran caterpillar pests**

| Sl No | Baculoviruses | Host insect | Crop | References |
|-------|---------------|-------------|------|------------|
| 01    | HearNPV       | *Helicoverpa armigera* | Cotton, Pigeonpea, Chickpea, Vegetables | Rabindra and Jayaraj, 1988 and 1995 |
| 02    | HzNPV         | *Heliothis zea* | Corn, Cotton | Ignoffo, 1973, 1981 and 1999 |
| 03    | SeMNPV        | *Spodoptera exigua* | Wide variety of vegetables | Kolodny-Hirsch et al., 1993 and 1997 |
| 04    | SpltNPV       | *S. litura* | Vegetables | Sun and Peng, 2007 |
about 0.3×0.5 µm (Herniou et al., 2012). Occlusion-derived virions consisted of one or more rod-shaped nucleocapsids and enclosed within an envelope. In contrast, virions of the second phenotype, BV - typically had a single nucleocapsid (Jehle et al., 2006). Nucleocapsids measured about 30-60 nm diameter and 250-300 nm in length. (Theilmann et al., 2005).

### Biological activity

The biological activity of virus was expressed in terms of pathogenicity and virulence. Virulence of insect pathogenic virus measured in terms of dose-response or time-response relationships through bioassay technique using its host insect (Eberle et al., 2012) and expressed as median lethal concentration (LC) and median lethal time (LT) or median survival time (ST). Several workers assessed the biological activity of virus isolates from members of same host species collected from different geographic locations. The results indicated significant variation in LC values with overlapping fiducial limits; hence use of native isolate was advocated for the biological suppression of respective insect pests (Rao et al., 2015).

### Baculovirus genome

Baculovirus genome consisted double-stranded, covalently closed circular DNA molecules of 82 kbp to 180 kbp in size and encodes between 90 to180 putative genes (Lauzon et al., 2006; Hayakawa et al., 1999). Autographa californica MNPV was the first baculovirus with whole genome sequenced and designated as the type species of the genus Alphabaculovirus (Herniou et al., 2012; Chateigner et al., 2015). To date, about 85 baculovirus whole genome sequences had been reported according to National Centre for Biotechnology Information (NCBI, 2019).

Polyhedrin gene was the first baculovirus gene ever sequenced (van Oers and Vlak, 2007) and was one of the most conserved genes of lepidopteran-specific baculoviruses (Lange et al., 2004).

Available whole genome sequence analysis had shown that about 38 genes were conserved among all baculoviruses sequenced and they have been assigned as core genes (Garavaglia et al., 2012; Williams et al., 2016; Javed et al., 2017; Blissard and Theilmann, 2018; Herniou et al., 2012). The core genes encode factors that are crucial for the life cycle such as viral DNA replication, viral gene transcription, production of structural proteins, DNA packaging, virion assembly, virion architecture and per os infection (Garavaglia et al., 2012; Herniou et al., 2003; Herniou et al., 2012; van Oers and Vlak, 2007) and for uniqueness of baculoviruses (Ikeda et al., 2015). These baculovirus core genes were grouped into five functional categories: RNA transcription, DNA replication, structural proteins, auxiliary proteins and proteins of unknown function (Herniou et al., 2003). Whole genome sequences provided a wealth of data about

### Table: Baculovirus isolates

| No | Strain | Host | Host Family | Reference |
|----|--------|------|-------------|-----------|
| 05 | SfMNPV | *S. frugiperda* (J.E. Smith) | Maize, Sorghum | Escribano et al., 1999 |
| 06 | SpobNPV | *S. obliqua* | Pulses, Vegetables | Kumar et al., 2015 |
| 07 | AgMNPV | *Anticarsia gemmatalis* (Hubner) | Soybean | Moscardi, 1999 |
| 08 | AcMNPV | *A. californica* | Cotton, Cabbage | Vail et al., 1999 |
| 09 | LdMNPV | *Lymantria dispar* L. | Forest trees | Podgwaite et al., 1991; Webb et al., 1999 |
| 10 | HpNPV | *Hyblaea puera* (Cramer) | Teak | Nair et al., 1996 |
| 11 | TnSNPV | *Trichoplusia ni* (Hubner) | Cabbage | Vail et al., 1999; Ignoffo, 1964 |
| 12 | CPGV | *Cydia pomonella* L. | Apple, Pear | Vincent et al., 2007; Moscardi, 1999 |
| 13 | PlxyGV | *Plutella xylostella* (L.) | Cabbage | Grzywacz et al., 2004 |
| 14 | PbGV | *Pieris brassicae* | Cabbage | Hostetter et al., 1973; Tatchell and Paynee, 1984 |
| 15 | PhopGV | *Phthorimaea operculella* (Zeller) | Potato | Salah and Aalbu, 1992; Lacey et al., 2010 |
| 16 | PiGV | *Plodia interpunctella* (Hub.) | Stored products | Vail et al., 1991, Hunter et al., 1979 |
| 17 | AmalNPV | *Amsacta albistriga* | Groundnut | Chandramohan and Kumaraswami, 1979; Rabindra and Balasubramanian, 1980; Veenakumari et al., 2005 |
| 18 | AjGV | *Achaea janata* | Castor | Singaravelu and Ramakrishnan, 1998 |
| 19 | Gv | *Chilo spp.* | Sugarcane | Easwaramoorthy and David, 1979; Mehta and David, 1980 |
Baculoviruses: Lethal pathogens of lepidopteran caterpillars

Baculoviruses: Lethal pathogens of lepidopteran caterpillars

BeIt (Jehle et al., 2006). Baculoviruses are insect-specific viruses belonging to the family Baculoviridae. They are transmitted through infected host insects and are known to infect and kill lepidopteran larvae, which are economically important pests. Baculoviruses encode a variety of genes, including the carrier protein polyhedrin (polh) and late expression factor 8 (lef-8), which are essential for the virus's infectivity and pathogenicity. These proteins contribute to the virus's ability to replicate and spread within the host insect.

Polyhedrin/granulin (polh/ gran), late expression factor-8 (lef-8) and late expression factor-9 (lef-9) had been largely used to characterize several baculoviruses (Harrison et al., 2012; Lange et al., 2004); and that led to the proposition of criteria for species demarcation and new classification of baculovirus (Jehle et al., 2006).

Genetic improvement of baculoviruses

Despite the high degree of host specificity and pathogenicity of baculoviruses, there were also some limitations for their successful use as biological control agents. The use of baculoviruses for pest control was generally limiting due to their slow speed of killing the host insect and partially due to their relatively narrow host specificity (i.e., more target specificity). Slower acting attribute allows the baculovirus to replicate and multiply in large numbers while allowing the host insect to survive for several days, however this ‘slow acting’ attribute had severe limitation for successful use of baculoviruses in insect pest management. These drawbacks were addressed by genetic modification of baculovirus using recombinant DNA technology and activity of baculoviruses against their natural hosts had been enhanced. The approaches to improve the speed of kill and activity of baculoviruses included introduction of foreign gene into the baculovirus genome to alters target host-insect physiology, deletion of an endogenous gene from the baculovirus genome, etc. (Kamita et al., 2017). Recombinant baculoviruses could express host-insect specific toxins, hormones or enzymes (Inceoglu et al., 2006; Elvira et al., 2010; Gramkow et al., 2010) affecting paralysis/death, or disturbing the physiology and behaviour of target insects (Ikeda et al., 2015).

Carbonell et al. (1988) reported recombinant baculovirus expressing biologically active insectotoxin-1 (Belt) of scorpion, Buthus eupeus. Belt was an insect-specific paralytic neurotoxin, however, paralytic activity was not detected during bioassay on insects. A neurotoxin, AaIT from the venom of North African (Algerian) scorpion, Androctonus australis Hector which selectively targets the insect sodium channel (Maeda et al., 1991) was incorporated into Autographa california NPV (AcNPV) genome (McCutchen et al., 1991) and recombinant baculovirus showed the reduction in time required to kill the host insect (Stewart et al., 1991). AaIT gene was successfully engineered in Helicoverpa armigera single-nucleocapsid NPV (HearNPV) which reduced the foliage damage and improved the speed of kill compared to wild type virus (Chen et al., 2000; Sun et al., 2002). Tomalski and Miller (1991) reported recombinant baculovirus expressing tox34 gene encoding a neurotoxin (TxP-I) associated with venom apparatus of male predatory straw itch mite, Pyemotes tritici that induced the rapid muscle-contractive paralysis in the larvae of greater wax moth Galleria mellonella (Tomalski and Miller, 1992). Insecticidal crystal protein gene, cyaIA(b) derived from Bacillus thuringiensis was engineered into AcNPV (Martens et al., 1995), however, the median lethal time for recombinant viruses did not significantly reduced than wild-type virus (Ribeiro and Crook, 1993). Genes encoding insect Juvenile Hormone Esterase (Hammock et al., 1990), Eclosion Hormone (Eldridge et al., 1992) and Diuretic Hormone (Maeda, 1989) were expressed in recombinant baculoviruses with an aim of enhancing their efficacy. An egt deletion mutant of AcMNPV exhibited reduced feeding and earlier mortality of infected larvae than wild type AcMNPV (O’Reilly and Miller, 1991), hence deletion of egt gene that encodes the enzyme, ecdysteroid UDP-glucosyl-transferase reported as a simple approach to improve the activity of baculoviruses. Thus, limiting traits of baculoviruses like slow acting and narrow host range could be addressed by genetically modifying the baculoviruses through recombinant DNA technology.

Baculovirus production

Production of baculoviruses at commercial-scale was done exclusively in-vivo by culturing large number of larvae of host insect and subsequently feeding them with semi-synthetic diet contaminated with virus inoculums in laboratory. Mass culturing of host insect under optimal rearing conditions in a synchronized manner for in-vivo virus production is found to be labour intensive which accounted for high production cost and potential contamination of viral preparations by microorganisms (Rao et al., 2015). Baculovirus production in-vitro by culturing insect cells in bioreactors was a substitute for labour intensive maintenance of the massive host-insect colony. This approach is more promising in terms of process scalability, however, the success of the baculovirus in-vitro production strongly influenced by cost of growth medium, media composition...
(Huynh et al., 2012) and passage effect. Passage effect or repeated serial passage of baculovirus in-vitro resulted in loss of virulence of POB due to the lack of selection pressure for virus in tissue culture to produce infectious polyhedra (Krell, 1996). The process of baculovirus production adopted should be efficient, inexpensive and resulting in production of highly pathogenic virus.

Baculovirus formulation

The baculoviruses were formulated in the same way as other biopesticides to enhance its shelf life, delivery, efficacy and persistence under field condition. In India, several products based on insect pathogenic viruses belonging to the genus *Alphabaculovirus* and *Betabaculovirus* have been developed against economically significant caterpillar pests (DPPQS, 2019) (Table 2). The most common preparation of a baculovirus was liquid formulation, invariably as a suspension concentrate. Dry formulations were generally prepared through air-drying, freeze-drying or lyophilisation, spray drying, co-precipitation with lactose, or microencapsulation (Grzywacz and Moore, 2017). Dust formulations were uncommon but generally employed for post-harvest management of potato tuber moth (*Phthorimaea operculella* Zeller) on stored potatoes with PhopGV (Lagnaoui et al., 1997). Liquid formulations (suspension concentrates) were preferred due to their greater ease of application. Survival or virulence of virus largely influenced by virus isolate, duration and conditions of storage, temperature, additives and pH of the formulation. The most detrimental effect on virus activity was attributed to its sensitivity to solar radiation under field condition. Several additives including spreaders, wetting agents, surfactants, phagostimulants, UV protectants, etc. had been used in the formulations to enhance the efficacy of baculovirus under field conditions. Optical brighteners were used to protect the insect viruses against sunlight exposures (Shapiro, 1992). The synergistic activity of boric acid was demonstrated on *Hear*NPV (Bijjur et al., 1991), *Spl*NPV (Chaudhari, 1992), *Ld*NPV (Shapiro and Bell, 1982) and *Sy*MNPV (Cisneros et al., 2002). Recently microencapsulation of insect pathogenic viruses using polymers like lignin, sodium alginate (Gifani et al., 2015), starch (Ignoffo et al., 1991), etc. have been attempted to protect the infectivity of viruses under natural conditions.

**Conclusion**

Baculoviruses have long been recognized as potential and attractive candidates for biological control of several economically important pests due to their high pathogenicity, host specificity, safety to non-target arthropod fauna and ability to persist outside the host for a considerable period of time and induce epizootics in insect population. The potential of baculovirus for biological suppression of economically significant lepidopteran pests has been demonstrated worldwide. In India, under specific instances like pest outbreak, use of baculoviruses appears to be better biological control option to bring down the pests population to the level that would not cause economic damage to the crops. Large scale adoption of baculoviruses as commercial insecticides is, however, hindered by their slow killing action, narrow spectrum as compared to most of the synthetic insecticides and technical difficulties associated with large scale commercial and in-vitro production. The success and reliability of insect pathogenic viral products depends on efficacy under field condition, ease and cost of commercial production and quality. The stability of baculovirus formulation is influenced by temperature, moisture, presence of additives, plant surface chemistry and pH but ultraviolet light is probably most detrimental factor affecting the survival of virus particle under field condition. The additives like surfactants, spreaders, optical brighteners, feeding stimulants, etc., are used to improve the efficacy of final product. Notwithstanding these factors, baculoviruses are regarded as one of the lethal microbial biocontrol agents of lepidopteran caterpillars devastating many economically significant agricultural and horticultural crops of the world.

**Table 2. Baculovirus based commercial formulations/products developed in India**

| Sl. No. | Baculovirus species | Product | Formulation | Agency/company | Target pest |
|---------|---------------------|---------|-------------|----------------|-------------|
| 01      | HearNPV             | Helicide| Aqueous Suspension 0.50 % | Pest Control India Ltd. | *Helicoverpa armigera* |
| 02      | HearNPV             | Helimar | Aqueous Suspension 0.50%  | Multiplex group of companies | *H. armigera* |
| 03      | HearNPV             | Biovirus-H | Aqueous Suspension  | Biotech International Ltd. | *H. armigera* |
| 04      | HearNPV             | Biokill-H | Aqueous Suspension 2 % | Ganesh Bio Control System | *H. armigera* |
| 05      | HearNPV             | Helicop  | Aqueous Suspension 2 % | Indore Biotech Inputs and Research Pvt Ltd | *H. armigera* |
| 06      | HearNPV             | Helistop | Aqueous Suspension 0.50 % | Harit Bio-control Labs | *H. armigera* |
Baculoviruses: Lethal pathogens of lepidopteran caterpillars

|    | SpiltNPV | Aqueous Suspension 0.50 %       | Pest control India Ltd.          | S. litura |
|----|----------|---------------------------------|----------------------------------|----------|
| 07 | SpiltNPV | Spodocide Aqueous Suspension 0.90 % | Multiplex group of companies S. litura |
| 08 | SpiltNPV | Spodomar Aqueous Suspension      | Biotech International Ltd S. litura |
| 09 | SpiltNPV | Biovirus-S Aqueous Suspension    | Biotech International Ltd S. litura |
| 10 | SpiltNPV | Biokill-S Aqueous Suspension 2 % | Ganesh Bio Control System S. litura |

References

Adams JR, McClintock JT. 1991. Baculoviridae. Nuclear polyhedrosis viruses, Part 1. Nuclear polyhedrosis viruses of insects. In: Adams JR, Bonami JR. (Eds.) *Atlas of Invertebrate Viruses*, CRC Press, Florida, pp. 89-180.

Aizawa K. 1963. The nature of infections caused by nuclear-polyhedrosis viruses. In: Steinhaus, EA. (Ed.). *Insect Pathology: An Advanced Treatise*, Vol. 1. Academic Press, New York, pp. 381-412. https://doi.org/10.1016/B978-0-12-395602-6.50016-0

Arif BM. 2005. A brief journey with insect viruses with emphasis on baculoviruses. *J Invertebr Pathol*. **89**: 39-45. https://doi.org/10.1016/j.jip.2005.05.011 PMid:16039304

Ayres MD, Howard SC, Kuzio J, Lopez-Ferber M, Possee RD. 1994. The complete DNA sequence of *Autographa californica* Nuclear Polyhedrosis Virus. *Virology*. **202**: 586-605. https://doi.org/10.1006/viro.1994.1380 PMid:8030224

Barber KN, Kaupp WJ, Holmes SB. 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). *Can Entomol*. **125**: 1055-1066. https://doi.org/10.4039/Ent1251055-6

Beas-Catena A, Sánchez-Mirón A, García-Camacho F, Contreras-Gómez A, Molina-Grima E. 2014. Baculovirus biopesticides: An overview. *J Anim Plant Sci*. **24**(2): 362-373.

Benz GA. 1986. Introduction: historical perspective. In: Granados RR, Federici BA. (Eds.), *The Biology of Baculoviruses* Vol. 1. CRC Press, Boca Raton, FL, pp. 1-36.

Bergold GH. 1950. The multiplication of insect viruses in organisms. *Can J Res*. **28**: 5-11. https://doi.org/10.1139/cjr50e-002

Bijjur S, Kulkarni KA, Lingappa S. 1991. Evaluation of nuclear polyhedrosis with certain adjuvants for the control of *Heliothis armigera* (Hübner). *Ind J Entomol*. **53**: 479-483.

Blissard GW, Black B, Crook N, Keddie BA, Possee R. 2000. Family Baculoviridae. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK. (Eds.) *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, San Diego: Academic, pp. 195-202.

Blissard GW, Theilmann DA. 2018. Baculovirus entry and egress from insect cells. *Annu Rev Virol*. **5**: 113-139. (First published as a Review in Advance on July 13, 2018) https://doi.org/10.1146/annurev-virology-092917-043356 PMid:3004832

Blissard GW. 1996. Baculovirus-insect cell interactions. *Cytotechnology*. **20**: 73-93. https://doi.org/10.1007/BF00350390 PMid:8987578

Bulach DM, Kumar CA, Zaia A, Liang B, Tribe DE. 1999. Group II nucleopolyhedrovirus subgroups revealed by phylogenetic analysis of polyhedron and DNA polymerase gene sequences. *J Invertebr Pathol*. **73**: 59-73. https://doi.org/10.1006/jipa.1998.4797 PMid:9878291

Carbonell LF, Hodge MR, Tomalski MD, Miller LK. 1988. Synthesis of a gene coding for an insect specific scorpion neurotoxin and attempts to express it using baculovirus vectors. *Gene*. **73**(02): 409-418. https://doi.org/10.1016/0378-1119(88)90505-7

Chandramohan N, Kumaraswami T. 1979. Comparative efficacy of chemical and nuclear polyhedrosis virus in the control of groundnut red hairy caterpillar, *Amsacta albistriga* (Walker). *Sci Cult*. **45**: 202-204.

Chateignier A, Bézier A, Labrousse C, Jiole D, Barbe V, Herniou EA. 2015. Ultra deep sequencing of a Baculovirus population reveals widespread genomic variations. *Viruses*. **7**: 3625-3646. https://doi.org/10.3390/v7072788 PMid:26198241 PMid:PMC4517117

Chaudhari S. 1992. Formulation of nuclear polyhedrosis virus of *Spodoptera litura* with boric acid. *Ind J Entomol*. **54**: 202-206.
Chen X, Sun X, Hu Z, Li M, O’Reilly DR, Zuidema D, Vlak JM. 2000. Genetic engineering of Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus as an improved pesticide. *J Invertebr Pathol.* 76(02): 140-146. https://doi.org/10.1006/jipa.2000.4963 PMid:11023738

Cisneros J, Angel-Perez J, Penagos DI, Ruiz VJ, Goulson D, Caballero P, Cave RD, Williams T. 2002. Formulation of a nucleopolyhedrovirus with boric acid for control of Spodoptera frugiperda (Lepidoptera: Noctuidae) in maize. *Biol Control.* 23: 87-95. https://doi.org/10.1006/bcon.2001.0985

Condraey JP, Ames RS, Hassan NJ, Kost TA, Merrihew RV, Mossakowska DE, Pountney DJ, Romanos MA. 2006. Baculoviruses and mammalian cell-based assays for drug screening. *Adv Virus Res.* 68: 255-286. https://doi.org/10.1016/S0065-3527(06)68007-X

DPPQS. 2019. Bio-pesticide Registrant. Retrieved from: http://ppqs.gov.in/divisions/cib-rc/bio-pesticide-registrant

Easwaramoorthy S, David H. 1979. A Granulosis virus of sugarcane shoot borer, Chilo infuscatae Snell. (Lepidoptera: Crambidae). *Curr Sci.* 48(15): 685-686.

Eberle KE, Wennmann JT, Kleespies RG, Jehle, JA. 2012. Basic techniques in insect virology. In: Lacey, LA. (Eds.) *Manual of techniques in invertebrate pathology.* pp. 15-74. Academic Press. https://doi.org/10.1016/B978-0-12-386899-2.00002-6 PMid:22677600

Eldridge R, O’Reilly DR, Miller K. 1992. Efficacy of a baculovirus pesticide expressing an eclosion hormone gene. *Biol Control* 102(02): 104-110. https://doi.org/10.1016/1049-9644(92)90033-A

Elvira S, Williams T, Caballero P. 2010. Juvenile hormone analog technology: Effects on larval cannibalism and the production of *Spodoptera exigua* (Lepidoptera: Noctuidae) nucleo polyhedron virus. *J Econ Entomol.* 103: 577-582. https://doi.org/10.1603/EC09325 PMid:20568601

Escribano A, Williams T, Goulson D, Cave RD, Chapman JW, Caballero P. 1999. Selection of a nucleopolyhedrovirus for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): Structural, genetic and biological comparison of four isolates from the America. *J Econ Entomol.* 92(5): 1079-1085. https://doi.org/10.1093/jee/92.5.1079 PMid:10582046

Federici BA. 1997. Baculovirus pathogenesis. In: Miller, L.K. (Ed.). *The Baculoviruses.* Plenum, New York, pp. 33-59. https://doi.org/10.1007/978-1-4899-1834-5_3

Francki RIB, Fauquet CM, Knudson DL, Brown F. (eds.) 1991. Classification and nomenclature of viruses: Fifth report of the International Committee on Taxonomy of Viruses. *Arch Virol.* Supplementum-2. 117(2): 117-123. https://doi.org/10.1007/978-3-7091-9163-7

Funk CJ, Braunagel SC, Rohrmann GF. 1997. Baculovirus structure. In: Miller LK. (Ed.) *The baculoviruses.* pp. 7-32. Plenum, New York. https://doi.org/10.1007/978-1-4899-1834-5_2

Garavaglia MJ, Miele SAB, Iserte JA, Belaich MN, Ghiringhelli PD. 2012. The ac53, ac78, ac101, and ac103 genes are newly discovered core genes in the family Baculoviridae. *J Virol.* 86(22): 12069-12079 https://doi.org/10.1128/JVI.01873-12 PMid:22933288 PMCid:PMC3486445

Garcia-Maruniak A, Maruniak JE, Zanotto PM, Doumbouya AE, Liu JC, Merritt TM, Lanoie JS. 2004. Sequence analysis of the genome of the *Neodiprionsetifer* nucleo polyhedron virus. *J Virol.* 78: 7036-7051. https://doi.org/10.1128/JVI.78.13.7036-7051.2004 PMid:15194780 PMCid:PMC421636

Gifani A, Marzban R, Safekordi A, Ardjmand M, Dezianian A. 2015. Ultraviolet protection of nucleopolyhedrovirus through microencapsulation with different polymers. *Biocontrol Sci Technol.* 25(7): 814-827. https://doi.org/10.1080/09583157.2015.1018814

Gopinadhan PB, Mohandas N, Vasudevan Nair KP. 1990. Cytoplasmic Polyhedrosis virus infecting redpalm weevil of coconut. *Curr Sci.* 59(11): 577-580.

Goulson D. 1997. Wipfelkrankheit: Modification of host behaviour during baculoviral infection. *Oecologia* 109: 219-228. https://doi.org/10.1007/s004420050076 PMid:28307172

Gramkow AW, Perecmanis S, Sousa RL, Noronha EF, Felix CR, Nagata T, Ribeiro BM. 2010. Insecticidal activity of two proteases against *Spodopterafrugiperda* larvae infected with recombinant baculoviruses. *J Virol.* 7: 7-143. https://doi.org/10.1186/1743-422X-7-143 PMid:20587066 PMCid:PMC2903562

Granados RR. 1973. Entry of an insect poxvirus by fusion of the virus envelope with the host cell membrane. *Virology* 52(1): 305-309. https://doi.org/10.1016/0042-6822(73)90422-4
Granados RR. 1978. Early events in the infection of Heliothis zea midgut cells by a baculovirus. Virology 90: 170. https://doi.org/10.1016/0042-6822(78)90347-1

Granados RR and Lawler KA. 1981. In vivo pathway of Autographa californica baculovirus invasion and infection. Virology 108: 297. https://doi.org/10.1016/0042-6822(81)90343-4

Groner A. 1986. Specificity and safety of baculoviruses. pp. 177-202. In: Granados RR, Federici BA. (Eds.) The biology of Baculoviruses: Biological properties and molecular Biology. Vol. I. CRC Press, Boca Raton.

Grzywacz D, Moore S. 2017. Production, Formulation and Bioassay of Baculoviruses for Pest Control. pp. 461. In: Lacey L. (Ed.). Microbial Control of Insect and Mite Pests from Theory to Practice. Academic Press. https://doi.org/10.1016/B978-0-12-803527-6.00007-X

Grzywacz D, Parnell D, Kubata G, Odour G, Ogutu OO, Miano D, Winstanley D. 2004. The development of endemic baculoviruses of Plutella xylostella (diamondback moth, DBM) for control of DBM in East Africa. pp. 197-206. In: Endersby N, Ridland PM. (Eds.). The Management of Diamondback Moth and Other Crucifer Pests. Proceedings of the 4th International Workshop, 26-29 Nov 2001. Melbourne, Victoria, Australia.

Grzywacz D. 2016. Basic and applied research: baculovirus. pp. 482. In: Lacey L. (Ed.). Microbial Control of Insect and Mite Pests from Theory to Practice, Academic Press. https://doi.org/10.1016/B978-0-12-803527-6.00003-2

Hammock BD, Bonning BC, Possee RD, Hanzlik TN, Maeda S. 1990. Expression and effects of the juvenile hormone esterase in a baculovirus vector. Nature 344: 458-461. https://doi.org/10.1038/344458a0

Harrap KA. 1970. Cell infection by a nuclear polyhedrosis virus. Virology 42: 311. https://doi.org/10.1016/0042-6822(70)90275-8.

Harrison RL, Popham HJR, Breitenbach JE, Rowley DL. 2012. Genetic variation and virulence of Autographa californica multiple nucleopolyhedrovirus and Trichoplusia ni single nucleopolyhedrovirus isolates. J Invertebr Pathol. 110: 33-47. https://doi.org/10.1016/j.jip.2012.02.002 PMid:22349146

Hayakawa T, Ko T, Okano K, Seong S, Goto C, Maeda S. 1999. Sequence analysis of the Xestia c-nigrumgranulovirus genome. Virology 262: 277-297. https://doi.org/10.1006/viro.1999.9894 PMid:10502508

Herniou EA, Arif BM, Becnel JJ, Blissard GW, Bonning B, Harrison R, Jehle JA, Theilmann DA, Vlak JM. 2012. Family: Baculoviridae. pp. 163-173. In: Andrew MQ, King Elliot Lefkowitz, Michael JA, Eric BC (Eds.). Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses.

Herniou EA, Jehle JA. 2007. Baculovirus Phylogeny and Evolution. Curr Drug Targets 8: 1043-1050. https://doi.org/10.2174/138945007782151306 PMid:17979664

Herniou EA, Luque T, Chen X, Vlak JM, Winstanley D, Cory JS, O’Reilly DR. 2001. Use of whole genome sequence data to infer baculovirus phylogeny. J Virol. 75: 8117-8126. https://doi.org/10.1128/JVI.75.17.8117-8126.2001 PMid:11483757 PMCid:PMC115056

Hofmann O. 1891. Die Schlaffsucht (Flacherie) der Nonne (Liparis monacha) nebst einem Anhang. Insektentötende Pilze mit besonderer Berücksichtigung der Nonne. P Weber, Frankfurt.

Holmes FO. 1948. Order Virales, the filterable viruses. Bergey’s manual of determinative bacteriology. 6th Edition. Williams and Wilkins, Baltimore. 1225-1228.

Hooft van Iddekinge BJL, Smith GE, Summers MD. 1983. Nucleotide sequence of the polyhedrin gene of Autographa californica nuclear polyhedrosis virus. Virology 131: 561-565. https://doi.org/10.1016/0042-6822(83)90522-6

Hostetter DL, Pinnell RE, Greer PA, Ignoffo CM. 1973. A granulosis virus of Pieris rapae as a microbial control agent on cabbage in Missouri. Environ Entomol. 2(6):1109-1112. https://doi.org/10.1093/ee/2.6.1109

Hostetter DL, Puttler B. 1991. A New Broad Host Spectrum Nuclear Polyhedrosis Virus Isolated from a Celery Looper, Anagrapha falcifera (Kirby), (Lepidoptera: Noctuidae). Environ Entomol. 20(5): 1480-1488. https://doi.org/10.1093/ee/20.5.1480

Hu YC. 2006. Baculovirus vectors for gene therapy. Adv Virus Res. 68: 287-320. https://doi.org/10.1016/S0065-3527(06)80080-8

Hu Z, Chen X, Sun X. 2002. Molecular Biology of Insect Viruses. pp. 83-108. In: Upadhay RK. (Ed.). Advances in Microbial Control of Insect Pests. Kluwer Academic / Plenum Publishers, New York. https://doi.org/10.1007/978-1-4757-4437-8_5

Hunter DK, Collier SS, Hoffmann DF. 1979. The effect of a granulosis virus on Plodia interpunctella (Hubner)
(Lepidoptera: Pyralidae) infestations occurring in stored raisins. *J Stored Products Res.* 15: 65-69. https://doi.org/10.1016/0022-474X(79)90014-6

Huynh HT, Chan LCL, Tran TTB, Nielsen LK, Reid S. 2012. Improving the robustness of a low-cost insect cell medium for baculovirus biopesticides production, via hydrolysate streamlining using a tube bioreactor-based statistical optimization routine. *Biotechnol Prog.* 28(3): 788-802. https://doi.org/10.1002/btpr.1529 PMid:22323401

Ignoffo CM, Shasha BS, Shapiro M. 1991. Sunlight ultraviolet protection of the Heliothis Nuclear Polyhedrosis Virus through starch-encapsulation technology. *J Invertebr Pathol.* 57(1): 134-136. https://doi.org/10.1016/0022-2011(91)90053-S

Ignoffo CM. 1964. Production and virulence of a nuclear-polyhedrons virus from larvae of *Trichoplusia ni* (Hubner) reared on a semisynthetic diet. *J Invertebr Pathol.* 7: 318-326.

Ignoffo CM. 1973. Development of a viral insecticide: Concept to Commercialization. *Exp Parasitol.* 33: 380-406. https://doi.org/10.1016/0014-4894(73)90041-6

Ignoffo CM. 1981. Living microbial insecticides. pp. 2-31. In: Norris JR, Richmond MH. (Eds.). *Essays in Applied Microbiology.* Vol. 7. John Wiley, New York.

Ignoffo CM. 1999. The first viral pesticide: past present and future. *J Ind Microbiol Biotechnol.* 22: 407-417. https://doi.org/10.1038/sj.jim.2900654

Ikeda M, Hamajima R, Kobayashi M. 2015. Baculoviruses: Diversity, evolution and manipulation of insects. *Entomol Sci.* 18:1-20. https://doi.org/10.1111/ens.12105

Inceoglu AB, Kamita SG, Hammock BD. 2006. Genetically modified baculoviruses: A historical overview and future outlook. *Adv Virus Res.* 68: 323-360. https://doi.org/10.1016/S0065-3527(06)68009-3

Jacob A, Subramanian TR. 1972. Nuclear Polyhedrosis on some Lepidoptera. *Curr Sci.* 4: 536.

Javed MA, Biswas S, Willis LG, Harris S, Pritchard C, van Oers MM, Donly BC, Erlandson MA, Hegedus DD, Theilmann DA. 2017. *Autographa californica* multiple nucleopolyhedrovirus AC83 is a Peros Infectivity Factor (PIF) protein required for Occlusion-Derived Virus (ODV) and budded virus nucleocapsid assembly as well as assembly of the PIF complex in ODV envelopes.
Baculoviruses: Lethal pathogens of lepidopteran caterpillars. *Virology* **325**: 36-47. https://doi.org/10.1016/j.virol.2004.04.023 PMid:15231384

Lauzon HAM, Garcia-Maruniak A, Zanotto PMA, Clemente JC, Herniou EA, Lucarotti CJ, Arif BM, Maruniak JE. 2006. Genomic comparison of *Neodiprionsertifer* and *Neodiprionlecontei* nucleo polyhedron viruses and identification of potential hymenopteran baculovirus-specific open reading frames. *J Gen Virol.* **87**:1477-1489. https://doi.org/10.1099/vir.0.81727-0 PMid:16690912

Maeda S, Volrath SL, Hanzlik TN, Harper SA, Majima K, Maddox DW, Hammock BD, Fowler E. 1991. Insecticidal effects of an insect-specific neurotoxin expressed by a recombinant baculovirus. *Virology* **184**(02): 777-780. https://doi.org/10.1016/0042-6822(91)90451-G

Martens J, Knoester M, Weijts F, Groffen S. 1995. Characterization of baculovirus insecticides expressing tailored *Bacillus thuringiensis* Cry1a(B) crystal proteins. *J Invertebr Pathol.* **66**(03): 249-257. https://doi.org/10.1006/jipa.1995.1097 PMid:8568280

NCBI. 2019. Baculoviridae- Complete genome. Retrieved from: https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=10442

O'Reilly DR, Miller LK. 1991. Improvement of a baculovirus pesticide by deletion of the EGT gene. *Bio/Technol.* **09**:1086-1089. https://doi.org/10.1038/nbt1191-1086

Ohkawa T, Volkman LE, Welch MD. 2010. Actin-based motility drives baculovirus transit to the nucleus and cell surface. *J Cell Biol.* **190**: 187-195. https://doi.org/10.1083/jcb.201001162 PMid:20660627 PMCid:PMC2930276

Okano K, Vanarsdall AL, Mikhailov VS, Rohrmann GF. 2006. Conserved molecular systems of the Baculoviridae. *Virology* **344**: 77-87. https://doi.org/10.1016/j.virol.2005.09.019 PMid:16364739

Patel RC, Singh R, Patel PB. 1968. Nuclear polyhedrosis virus of gram pod borer, *Heliothis armigera*. *J Econ Entomol.* **61**: 191-193. https://doi.org/10.1093/jee/61.1.191

Podgwaite JD, Reardon RC, Kolodny-Hirsch DM, Walton GS. 1991. Efficacy of Ground Application of the Gypsy Moth (Lepidoptera: Lymantriidae) Nucleo polyhedrosis Virus Product, Gypchek. *J Econ Entomol.* **84**(2): 440-444. https://doi.org/10.1093/jee/84.2.440
Rabindra RJ, Balasubramanian M. 1980. Observations on an induced epizootic of nuclear polyhedrosis of *Amsacta albistriiga* Walker. *Curr Sci.* 49(07): 279.

Rabindra RJ, Jayaraj S. 1980. Observations on an induced epizootic of nuclear polyhedrosis of *Amsacta albistriiga* Walker. *Curr Sci.* 49(07): 279. https://doi.org/10.1080/09670878809371298

Rabindra RJ, Jayaraj S. 1988. Efficacy of nuclear polyhedrosis virus with adjuvants as high volume and ultra-low volume applications against *Heliothis armigera* Hbn. on chickpea. *Trop Pest Manag.* 34(4): 441-444. https://doi.org/10.1007/s12912-016-0011-8

Rabindra RJ, Jayaraj S. 1995. Management of *Helicoverpa armigera* with nuclear polyhedrosis virus on cotton using different spray equipment and adjuvants. *J Biol Control* 9: 34-36.

Rao GVR, Kumar Ch. S, Sireesha K, Kumar PL. 2015. Role of Nucleo Polyhedron Viruses (NPVs) in the Management of Lepidopteran Pests in Asia. pp. 11-52. In: Sree KS, Varma A. (Eds.). *Biocontrol of Lepidopteran Pests.* Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-319-14499-3_2

Ribeiro BM, Crook NE. 1993. Expression of full-length and truncated forms of crystal protein genes from *Bacillus thuringiensis* subsp. *kurstaki* in a baculovirus and pathogenicity of the recombinant viruses. *J Invertebr Pathol.* 62(02): 121-130. https://doi.org/10.1006/jipa.1993.1087 PMid:8228317

Rohrmann GF. 1999. Nuclear polyhedrosis viruses. pp. 146-152. In: Webster RG, Granoff A. (Eds.). *Encyclopedia of Virology*, 2nd Ed, Academic Press, London. https://doi.org/10.1006/rwvi.1999.0031

Rowley DL, Popham HJR, Harrison RL. 2011. Genetic variation and virulence of nucleo polyhedrosis viruses isolated worldwide from the heliothine pests *Helicoverpa armigera*, *Helicoverpa zea* and *Heliothis virescens*. *J Invertebr Pathol.* 107: 112-126. https://doi.org/10.1016/j.jip.2011.03.007 PMid:21439295

Salah HB, Aalbu R. 1992. Field use of granulosis virus to reduce initial storage infestation of the potato tuber moth, *Phthorimaea operculella* (Zeller) in North Africa. *Agric Ecosyst Environ.* 38: 119-126. https://doi.org/10.1016/0167-8809(92)90137-Z

Shapiro M, Bell RA. 1982. Enhanced effectiveness of *Lymantria dispar* (Lepidoptera: Lymantridae) nucleopolyhedrosis virus formulated with boric acid. *Ann Entomol Soc Am.* 75: 346-349. https://doi.org/10.1093/aes/75.3.346

Shapiro M. 1992. Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera, Lymantridae) nuclear polyhedrosis virus. *J Econ Entomol.* 85: 1682-1686. https://doi.org/10.1093/jee/85.5.1682

Singaravelu B, Nirmala R, Easwaramoorthy S. 1999. A new record of granulovirus on sugarcane top borer, *Scirpophaga excerptalis* Walker (Lepidoptera: Pyralidae). *J Biol Control* 13:133-135.

Singaravelu B, Ramakrishnan N. 1998. Characterization of a Granulovirus Virus from the Castor Semilooper, *Achaea janata* L. *J Invertebr Pathol.* 71(3): 227-235. https://doi.org/10.1006/jipa.1997.4735 PMid:9538027

Slack J, Arif B. 2007. The Baculoviruses occlusion derived virus: Virion structure and function. *Adv Virus Res.* 69: 99-165. https://doi.org/10.1016/S0065-3527(06)69003-9

Steinhaus EA. 1949. *Principles of Insect Pathology* (first edition). McGraw-Hill Book Company Inc., New York. 757 pp.

Stewart LMD, Hirst M, Ferber ML, Merryweather AT, Carley PJ, Possee RD. 1991. Construction of an improved baculovirus insecticide containing an insect-specific toxin gene. *Nature* 352: 85-88. https://doi.org/10.1038/352085a0 PMid:2062383

Sun X, Chen X, Wang H, Bianchi FJ, Peng H, Vlak JM, Hu Z. 2002. Bollworm responses to release of genetically modified *Helicoverpa armigera* nucleopolyhedroviruses in cotton. *J Invertebr Pathol.* 81(02): 63-69. https://doi.org/10.1007/S1022-2011(02)00144-1

Sun X, Peng H. 2007. Recent advances in control of insect pests by using viruses in China. *Virol Sin.* 22: 158-162. https://doi.org/10.1007/s12250-007-0017-0

Szewczyk B, Hoyos-Carvajal L, Paluszek M, Skrzecz I, Lobo de Souza M. 2006. Baculoviruses- re-emerging biopesticides. *Biotechnol Adv.* 24: 143-160. https://doi.org/10.1016/j.biotechadv.2005.09.001 PMid:16257169

Tatchell GM, Payne CC. 1984. Field evaluation of a granulosis virus for control of *Pieris rapae* (Lep.:Pieridae) in the United Kingdom. *Entomophaga* 29(2): 133-144. https://doi.org/10.1007/BF02372103

Theilmann DA, Blissard GW, Bonning B, Jehle J, O’Reilly DR, Rohrmann GF, Thiem S, Vlak JM. 2005. Family: Baculoviridae. pp. 177-186. In: Fauquet C, Mayo MA, Maniloff J, Desselberger U, Ball LA. (Eds.). *Virus taxonomy*. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press.
Baculoviruses: Lethal pathogens of lepidopteran caterpillars

Theze J, Bezier A, Periquet G, Drezen JM, Herniou EA. 2011. Paleozoic origin of insect large dsDNA viruses. *PNAS.* 108(38): 15931-15935. https://doi.org/10.1073/pnas.1105580108 PMid:21911395 PMCid:PMC3179036

Tinsley TW, Kelly DC. 1985. Taxonomy and nomenclature of insect pathogenic viruses. pp. 1-7. In: Maramorosch K, Sherman KE. (Eds.). *Virus Insecticides for biological Control,* Academic Press, New York. https://doi.org/10.1016/B978-0-12-470295-0.50006-9

Tomalski MD, Miller LK. 1991. Insect paralysis by baculovirus-mediated expression of a mite neurotoxin gene. *Nature* 352: 82-85. https://doi.org/10.1038/352082a0 PMid:1840646

Tomalski MD, Miller LK. 1992. Expression of a paralytic neurotoxin gene to improve insect Baculoviruses as biopesticides. *Biotechnology* 10: 545-549. https://doi.org/10.1038/nbt0592-545

Vail PV, Hostetter DL, Hoffmann DF. 1999. Development of the Multi Nucleocapsid Nucleo Polyhedro Viruses (MNPVs) infectious to loopers (Lepidoptera: Noctuidae: Plusiinae) as microbial control agents. *Integrated Pest Manag Rev.* 4: 231-257. https://doi.org/10.1023/A:1009601212375

Vail PV, Tebbets JS, Cowan DC, Jenner KE. 1991. Efficacy and persistence of a granulosis virus against infestations of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) on raisins. *J Stored Products Res.* 27(2): 103-107. https://doi.org/10.1016/0022-474X(91)90019-9

van Oers MM, Vlak JM. 2007. Baculovirus Genomics. *Curr Drug Targets* 8: 1051-1068. https://doi.org/10.2174/138945007782151333 PMid:17979665

van Oers MM. 2006. Vaccines for viral and parasitic diseases produced with baculovirus vectors. *Adv in Virus Res.* 68: 193-253. https://doi.org/10.1016/S0065-3527(06)68006-8

Veenakumari K, Rabindra RJ, Srinivas Naik CD, Shubha MR. 2005. Field efficacy of nuclear polyhedrosis virus against the red hairy caterpillar, *Amsacta albistriga* (Walker) (Lepidoptera: Arctiidae) on groundnut in Karnataka *J Biol Control* 19(2): 141-144.

Vincent C, Andermatt M, Valero J. 2007. Madex® and VirosoftCP4®, viral biopesticides for coddling moth control. pp. 336-343. In: Vincent C, Goettel MS, Lazarovits G. (Eds.). *Biological Control: a Global Perspective.* CAB International. https://doi.org/10.1079/9781845932657.0336 PMid:17717899

Volkman LE. 1997. Nucleopolyhedrovirus interactions with their insect hosts. *Adv Virus Res.* 48: 313-348. https://doi.org/10.1016/S0065-3527(08)60291-2

Webb RE, Thorpe KW, Podgwaite JD, Reardon RC, White GB, Talleya SE. 1999. Field evaluation of an improved formulation of Gypchek (a nuclear polyhedrosis virus product) against gypsy moth (Lepidoptera: Lymantriidae). *J Entomol Sci.* 34(1): 72-83. https://doi.org/10.18474/0749-8004-34.1.72

Williams GV, Faulkner P. 1997. Cytological changes and viral morphogenesis during baculovirus infection. pp. 61-107. In: Miller LK, (Ed.) *The baculoviruses,* Plenum, New York. https://doi.org/10.1006/rwvi.1999.0030

Williams T, Bergoin M, Van Oers MM. 2016. Diversity of large DNA viruses of invertebrates. *J Invertebr Pathol.* 147: 4-22. https://doi.org/10.1016/j.jip.2016.08.001 PMid:27592378

Winstanley D, O’Reilly D. 1999. Granuloviruses. pp. 140-146. In: Webster RG, Granoff A. (Eds.) *Encyclopedia of Virology.* 2nd Edition, Academic Press, London. https://doi.org/10.1006/rwvi.1999.0030

Young SY, Livingston JM, McMasters JA, Yearian WC. 1972. A nuclear polyhedrosis virus of the loblolly pine sawfly, *Neodiprion taedaelinaris* Ross. *J Invertebr Pathol.* 20(2): 220-221. https://doi.org/10.1016/0022-2011(72)90142-5

Zanotto PMD, Kessing BD, Maruniak JE. 1993. Phylogenetic interrelationships among baculoviruses: Evolutionary rates and host associations. *J Invertebr Pathol.* 62: 147-164. https://doi.org/10.1006/jipa.1993.1090 PMid:8228320