

Review Article

doi: <http://dx.doi.org/10.20546/ijcrbp.2016.308.016>

An Overview on Nuclear Polyhedrosis Virus (NPV) as a Valuable Biopesticide in Enhancing Ecofriendly Management of Insect Pests

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Abstract

The various impact of using chemical insecticides in agriculture has been experienced with several facts like contamination of environment; effects in human health and impact in relation to insect pests consist of effect on non target pests, development of insecticide resistance to insect pests, secondary pest's outbreak and resurgence of pest populations. So, development of alternatives to chemical insecticides has been an important action in managing pests for promoting a sustainable agriculture. Microbial biopesticides acts as a solution as they are environmentally safe, self perpetuating in nature, specific to target pests etc. Among the microbial biopesticides, viruses after bacterial and fungal products occupy a special space in managing several pests. NPV forming polyhedra like occlusion bodies kills most important crop pests such as *Helicoverpa armigera* and *Spodoptera litura*. NPV can be implemented as one of major component in IPM programme. So, reduction of application of broad-spectrum pesticides increases the potentiality of implementing NPV in IPM programme and allow to the build-up of natural enemies in crop ecosystem.

Article Info

Accepted: 23 July 2016

Available Online: 06 August 2016

Keywords

Baculovirus
Biopesticides
Budded virus (BV)
Nuclear polyhedrosis virus (NPV)
Occlusion derived virus (ODV)

Introduction

The global population has been increasing exponentially along with these a million people suffered from hunger and live without secure access of food. Developing countries produces less food production leading to increase in the price of food and it ought to be responsible for current rates of food scarcity and issue of global food security. Besides, there are several limiting factors including biotic and abiotic factors that limit food production. Biotic factors consist of several diseases of plant caused by fungal, bacterial, viral diseases and problem of insect pests along with the various effects of weeds. Among these biotic factors, insect pests of crop plants were affecting agriculture in an immense ways. Insect pests act as major constraints in reducing the productivity in agriculture and losses

estimated approximately of 10.1% for crops like rice, wheat, maize, barley, potatoes, soybeans, cotton and sugar beet due to pests (Oerke and Dehne, 2004). To manage the problem of insect's damage, several insecticides were used from the very ancient times till today's status of agriculture. Global pesticide production has increased for several decades and is predicted to more than double by 2050 to around 10 million metric tonnes (Tilman et al., 2001; Wanger et al., 2010). On the other hand, there are several risks associated with continuous use of chemical pesticides in agriculture which consist of undesirable effects on humans and natural environments, eliminated natural enemies from crop ecosystems and estimates proved that only a small portion of pesticides applied to the crop reaches the target while the major portion reaches to the non-target (Erayya et al., 2013). The

environmental impacts of pesticide include mostly the contamination of surface and ground water that are used for drinking, irrigation and soil contamination (Fernandes and Sarcinelli, 2009; Pedlowski et al., 2012). Along with these the development of pesticide resistance to insect pests, secondary outbreak of pests and resurgence of pest populations have been reported in relation to impact of pesticides on insect pests (Desneux et al., 2007) which render the agriculture system unsustainable (Pawar, 2002).

Resistance development of widely used insecticides pyrethroids has been reported in *Spodoptera litura* (Ahmad et al., 2007), in *Helicoverpa armigera* to many a number of insecticides have been verified globally (Nguyen et al., 2007). Many concerns about the deleterious effects of synthetic pesticides have driven a significant research and development effort directed towards alternative pest control strategies (Skovmand 2007).

Focusing on alternatives control measures like biological control, including the use of microbial control agents like bacteria, fungi, nematodes and virus etc. acts as an ecofriendly management approach of insect pests. The microbial pesticides occupy around 1.3% of the world's total pesticide market and out of which, 90 per cent are used as insecticides (Menn et al., 2001). Among the microbial pathogens, 60% share has been occupied by bacterial pathogens followed by fungal and viral pathogens globally as presented in Fig. 1 (Jayanth, 2002). The importance and exploitation of viruses as microbial biopesticides will be considered in details by referring various research papers.

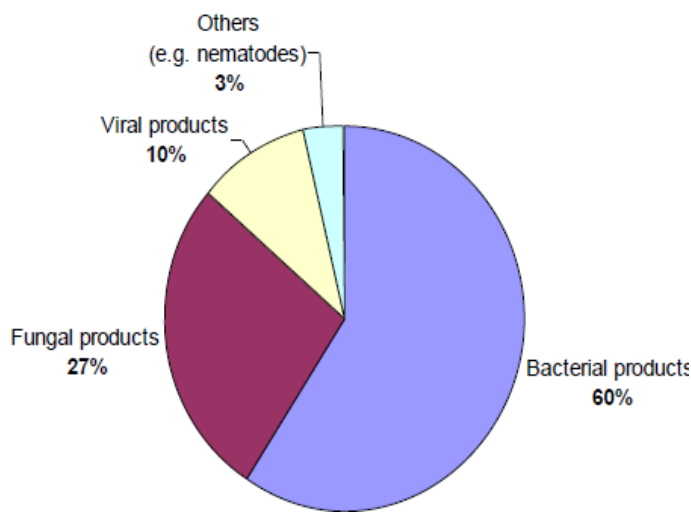


Fig. 1: Global share of microbial biopesticides products.

Baculoviruses as microbial pesticides

Viruses are sub microscopic, intracellular and obligate pathogenic entities with nucleic acid and protein. These viruses are often genus or species specific and often highly virulent to their hosts. Several groups of viruses like Baculoviruses, Cytoplasmic Polyhedrosis Viruses, Entomopox Viruses, Iridoviruses, Densoviruses and Small RNA Viruses have been also reported having a potential in controlling many pests. Among these groups of viruses, baculoviruses are rod shaped enveloped viruses with a circular double stranded DNA genome of 80-180kb under the family baculoviridae were exploited widely as a microbial control (Herniou et al., 2012). The family baculoviridae has two subfamilies, Eubaculovirinae and Nudibaculovirinae (Francki et al., 1991).

Based on the type of virion occlusion Eubaculovirinae consist of Nuclear Polyhedrosis Virus (NPV) and Granulosis Virus (GV). The occlusion body consists of a crystalline matrix composed of a protein called polyhedrin in NPVs and granulin in GVs. Nudibaculovirinae consist of only non occluded virus (NOB) without a occlusion body formation (Burand 1991).

Later, International Committee on Taxonomy of Viruses (ICTV) have revised the classification that baculoviruses consist of only two genera i.e. nucleopolyhedrosis virus and granulovirus (Murphy et al., 1995). The family Baculoviridae consisting of four Genera: Alpha baculovirus (Lepidopteran specific NPVs), Betabaculoviruses (Lepidopteran specific GVs), Gammabaculoviruses (Hymenopteran specific NPVs) and Deltabaculoviruses (Dipteran specific NPVs) (Jehle et al., 2006). A prominent feature of the nucleocapsids within polyhedra is their organization into either single or multiple aggregates of nucleocapsids within an envelope (Erlandson, 2008).

Baculovirus species are traditionally named after the insect species from which they were first isolated, such that *Autographa californica* MNPV (AcMNPV), *Bombyx mori* NPV (BmNPV) and *Lymantria dispar* MNPV (LdMNPV) were first isolated from the alfalfa looper (*Autographa californica*), silkworm (*Bombyx mori*), and gypsy moth (*Lymantria dispar*), respectively. Among 633 potential species of Baculovirus has been reported by ICTV and among them 15 NPV were categorised as assigned species and 483 were defined as tentative species (Table 1).

Table 1. List of assigned species of NPV.

Sl. No.	Nucleopolyhedrosis virus	NPV
1	<i>Autografa californica</i> MNPV	AcMNPV
2	<i>Anticarsia gemmatalis</i> MNPV	AgMNPV
3	<i>Bombyx mori</i> MNPV	BmMNPV
4	<i>Choristonerra fumiferana</i> MNPV	CfMNPV
5	<i>Galleria mellonella</i> MNPV	GmMNPV
6	<i>Helicoverpa zea</i> SNPV	HzMNPV
7	<i>Lymantria dispar</i> MNPV	LdMNPV
8	<i>Manestra brassicae</i> MNPV	MbMNPV
9	<i>Orgyia pseudotsugata</i> MNPV	OpMNPV
10	<i>Orgyia pseudotsugata</i> SNPV	OpSNPV
11	<i>Rachiplusia ou</i> MNPV	RoMNPV
12	<i>Spodoptera exigua</i> MNPV	SeMNPV
13	<i>Spodoptera litura</i> MNPV	SIMNPV
14	<i>Trichoplusia ni</i> MNPV	TnMNPV
15	<i>Trichoplusia ni</i> SNPV	TnSNPV

Two forms of virions are produced during the course of infection: BVs and OB-derived virions (ODVs), which have identical genotypes, but distinct phenotypes (Fig. 2); same nucleocapsid but differs in their envelopes. The envelopes of former are derived from modified plasma membranes through nucleocapsid budding, whereas the envelopes of latter are formed from membranes assembled within the nucleus (Ikeda et al., 2015). BVs consist of only a single nucleocapsid surrounded by an envelope whereas ODVs embedded within OBs contain either single or multiple nucleocapsids and are referred to as single (SNPVs) or multiple NPVs (MNPVs) respectively.

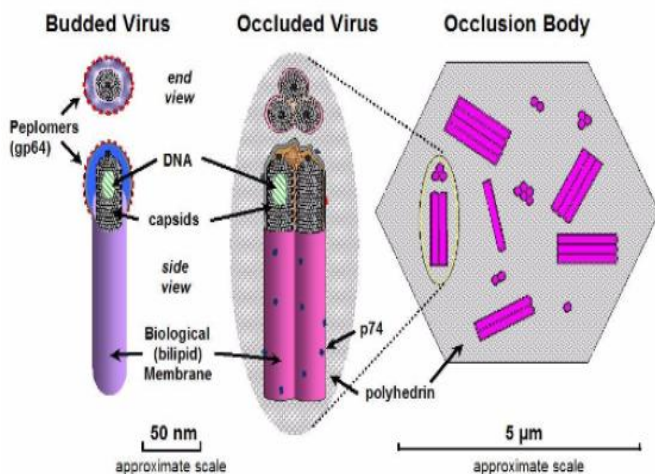


Fig. 2: Two distinct NPV phenotypes produced during lepidopteran NPV infection, Budded virus (BV) and Occlusion derived virus (ODV).

NPV and GV attracted the attention of many researchers looking for an alternative approach to

hazardous chemical pesticides. NPV infects and kills some of the most important crop pests such as *Helicoverpa armigera* and *Spodoptera litura*. NPV form polyhedra like occlusion bodies of 0.015-15um in size and encoded with polyhedrin matrix protein. Occlusion bodies (OBs) are responsible for the survival of the virus in the environment and the transmission of the virus from insect to insect. The occlusion bodies contain many nucleocapsids surrounded by a matrix composed mainly of polyhedrin, a major structural protein (Braunagel et al., 2003).

The baculoviruses currently used in India includes *Helicoverpa armigera* NPV (HaNPV) and *Spodoptera litura* NPV (SINPV) on crops like cotton, tomato, chickpea, groundnut and field bean, while other baculoviruses that have the potential to be developed commercially for pest management consist of *Amsacta albistriga* NPV, *Plutella xylostella* granulovirus (GV) and *Chilo infuscatellus* GV (Jayanth, 2002; Rabindra, 2002).

Mode of action

NPV has a biphasic infection cycle, during which the two distinct virion phenotypes are generated through a number of distinct and sequential steps. Primary infection begins when susceptible larvae ingest foods contaminated with OBs containing infectious ODVs. In the presence of alkaline digestive juice within the midgut lumen, the ingested OBs get dissolved and release numerous ODVs that adsorb to brush border microvilli of midgut columnar cells. The ODV envelopes fuse directly with the plasma membranes facilitating penetration of the nucleocapsid into the cytoplasm. The nucleocapsids migrate into the nucleus through actin-based motility (Volkman, 2007; Ohkawa et al., 2010), where the capsid is uncoated, releasing the naked viral DNA. The expression of early genes, replication of genomic DNA, and expression of late genes to produce structural proteins proceed sequentially in the stroma within the infected nucleus (Kawasaki et al., 2004; Nagamine et al., 2011) and form progeny viruses after morphogenesis of nucleocapsids. In infected midgut epithelial cells, newly formed enter the cytoplasm and subsequently bud from the basal plasma membranes that have been modified by baculovirus-encoded envelope fusion proteins to form enveloped BVs (Blissard and Rohrmann, 1990). Progeny BVs penetrate into the haemocoel either by directly crossing the basal lamina of the midgut or using tracheal cells as a conduit (Passarelli, 2011).

Two forms of progeny viruses are produced which have structurally and functionally distinct after secondary infection of haemocoel tissues started. Nucleocapsids are enveloped within the nucleus to form ODVs, which are then embedded within OBs. The OBs containing ODVs remain in the nucleus and are released into the environment following the death of infected larvae by liquefaction. Therefore, ODVs embedded in OBs play a role in horizontal viral transmission from insects to insects, whereas BVs are responsible for cell-to-cell viral transmission that helps in spreading of the virus within an infected insect. In contrast to ODVs, BVs enter cells through adsorptive endocytosis (Long et al., 2006; Katou et al., 2010) where proteins GP64 and F-proteins interact with plasma membrane receptors in the host cells (Zhou and Blissard, 2008), mediating the fusion between the viral membrane and the cell plasma membrane and creating a low pH environment that triggers the membrane penetration of the nucleocapsid (Ijkel et al., 2000). Upon binding to the cell surface, BVs are internalized by endocytotic vesicles called endosomes, which then migrate to the nucleus, in presence of low pH triggering membrane fusion between the BV envelope and endosomal membrane and allows in releasing nucleocapsids into the cytoplasm. After entering the nucleus, nucleocapsids are uncoated to release the viral genome and commence replication, as occurs for ODVs.

Symptoms

Insect larvae infected with baculoviruses usually die from 3 to 12 days after infection depending on viral dose, temperature and the larval instars at the time of infection. Insects killed by baculoviruses have a characteristic shiny-oily appearance and often crawl to the tops of plants where they die and decompose. The infected larvae remain extremely fragile to touch, rupturing to release fluid filled with infective virus particles (Ramanujam et al., 2014). The characteristic symptoms are shown in the Fig. 3. Infected larvae shows characteristic symptoms of sluggishness, discolouration of skin, regurgitation of fluids, wet droppings and ultimately larvae show hanging upside down in the top of the plant so called tree top disease as shown in the photograph (Sharma and Srivastava, 2013). The survival time after infection ranges from 2–5 days to 2–3 weeks suggested their slow in action but host survival time can be shortened by genetic engineering of NPVs (Kunimi et al., 1997). Infection of other insects will occur if they eat foliage that has been contaminated by virus-killed larvae (Ramanujam et al., 2014).



Fig. 3: Symptoms of baculovirus infected larvae.

In vitro and *vivo* production of NPV

Entomopathogenic viruses are obligate pathogens, can be replicate only in its host larvae (Ignoffo and Anderson, 1979). Baculoviruses can be produced *in vitro* in the infected cells cultivated in the bioreactors. Many insect cell lines have been developed for *in vitro* study of the mode of action and multiplication of NPV (Smagghe et al., 2009). Sundeep et al. (2005) developed two cell lines from the larval haemocyte and embryonic tissues of *Helicoverpa armigera* and designated as NIV-HA-1195 and NIV-HA-197. Other four cell lines like NTU LY-1 to 4 were established from the pupal tissues of *Lymantria xyliana* (Chih and Wang, 2006). New cell lines from embryos of *Heliothis virescens* were developed and characterization and their susceptibility to Baculoviruses were examined by inoculating all the strains with various baculoviruses, including *Autographa californica* nucleopolyhedrovirus (NPV), *Anagrapha falcifera* NPV, *Anticarsa gemmatalis* NPV, *Rfachoplusia ou* NPV, *Lymantria dispar* NPV (LdMNPV), *Orgyia pseudotsugata* NPV (OpSNPV), *Orgyia leucostigma* NPV (OIMNPV), and *Helicoverpa zea* NPV (HzSNPV). It was observed that no cytopathology in any cells inoculated with OIMNPV or LdMNPV suggesting that these new strains can be useful for the possibly large scale production of baculoviruses for which no effective cell systems are available and for comparative studies on multiple virus species (Lynn et al., 1998).

Mass production of baculoviruses by *in vivo* method is being influenced by several factors like larval age at virus treatment, virus concentration and the incubation

temperature. The larval age at virus treatment and virus concentration should be synchronized to result in insect death at a fully grown larval stage to maximize the productivity. The use of viruses as microbial insecticides depends on the development of large scale virus production methods. The *in vivo* production of viruses experienced considerable progress with the improvement of insect rearing techniques (Shapiro, 1986), allowing the commercialization of viral insecticides, such as the NPV of *Helicoverpa* spp. (Ignoffo and Couch, 1981), the NPV of *Lymantria dispar* L. (Huber, 1986). The *in vivo* production of nucleopolyhedrovirus of the Egyptian cotton leafworm *Spodoptera littoralis* was studied experimentally (Grazywacz et al., 1998). But *in vivo* production are still the only viable economic method and in industrialised countries the cost of labour associated with this method of production has made it difficult for virus products to compete in the marketplace. The other major problem associated with *in vivo* systems is contamination by other microbes (Grazywacz et al., 1997). Insects killed by viruses are often found to contain high levels of bacterial contamination (McKinley et al., 1989). The Optimization of *in vivo* NPV production in *Spodoptera exempta* (Walker) and *Spodoptera exigua* (Hubner) were evaluated (Cherry et al., 1997) successfully.

The first viral insecticide Elcar, containing the preparation of *Heliothis zea* NPV, was introduced by Sandoz Inc. in 1975 (Ignoffo and Couch, 1981). It has broad-spectrum action against species of *Helicoverpa* and *Heliothis* so controlling many important pests attacking a wide range of crops (Chakraborty et al., 1999). Although there are nearly 50 registered baculoviruses under different trade names available in different countries like NPV formulations of *HaNPV* and *Spodoptera litura* (*SINPV*) in India, NPVs of *Spodoptera littoralis* and *Spodoptera exempta* in Egypt and Kenya and *Anticarsia gemmatalis* NPV in Brazil, *Lymantria dispar* NPV and *Orgyia pseudotsugata* NPV in USA. Currently, at least two commercial products based on *Spodoptera* NPV are available in the USA and Europe; SPOD-Xk containing *Spodoptera exigua* NPV to control insects on vegetable crops and cut flowers in greenhouses, and Spodopterin containing *Spodoptera littoralis* NPV which is used to protect cotton, corn and tomatoes (Szewczyk et al., 2006). NPV registered products available in global market consist of Biotrol VHZ and Virion H of *Helicoverpa* spp., Gypcheck of *Lymantria dispar* in USA, Marestrin of *Mamestra brassicae* in Finland etc. The NPV based biopesticides marketed in India under different trades names includes

Spodo-Cide®, Spodopterin®, Heli-Cide®, Heliokill®, etc. (Erayya et al., 2013; Ramanujam et al., 2014). In India, two companies namely M/s Biocontrol Central Research Laboratory and M/s Multiplex at Bangalore are involved in mass production of baculoviruses belonging to *HaNPV* and *SINPV*. Only the *in vivo* production system of the baculoviruses has so far been economically viable due to high cost involved in the *in vitro* production systems (Kumar et al., 2005). The optimization of the production factors in *in-vivo* production methods is crucial to minimise the cost.

Environmental factors affecting in persistence of viruses in field

Solar radiation act as one of the most important factor affecting epizootics of *Spodoptera frugiperda* NPV in corn (Mitchell and Fuxa, 1990) but high pH of dew on cotton leaves inactivated NPV (Young, 2001). *Anticarsia gemmatalis* NPV sprayed on the upper surfaces of soybean leaves lost 60% or more activity within 2 days, whereas OB sprayed on the underside of the foliage lost 13% or less activity in the same period (Peng et al., 1999). NPVs persist on host plants only a short time, within which the virus must be ingested by a new host. NPVs generally persist only 1–32 days on surfaces of vegetation (Fuxa, 1989), although the viral population can persist for longer times in trees (Young, 2001). Exposure to UV irradiation resulted in substantial inactivation of the virus. High temperatures increase the rate of inactivation of baculoviruses which was supported by experiment where no larval mortality of *Diaphania pulverulentalis* of mulberry was observed when DpNPV was exposed to 50°C, indicating complete inactivation of virus (Pachiappan and Narayanaswamy, 2012). Increase in temperature from 15°C to 45°C had little effect on viral activity but a significant loss in viral activity was detected as temperature was increased to 45°C with exposure to UV irradiation (McLeod et al., 1977). It was found that the *SINPV* produced at 25°C was also found to be of superior quality in terms of low bacterial contaminants than at 35°C (Subramanian et al., 2006). Behaviour can affect NPV epizootics either on a localized basis or an area-wide effect. For example, gregarious feeding contributed to the spread of NPV in caterpillars and sawflies (Evans, 1987), and within-plant dispersal out of clumps was conducive to host–NPV contact and disease prevalence in *Spodoptera frugiperda* infesting corn (Mitchell and Fuxa, 1990). Migration apparently contributed to increased heterogeneity of *Spodoptera frugiperda* populations with respect to NPV susceptibility over the course of a growing season (Fuxa

et al., 1988). A well-known behavioural change in NPV-infected insects is their tendency to climb to elevated points on host plants shortly before death, thereby enhancing viral dissemination (Andreadis, 1987). Nutritional stress also changes susceptibility (Richter et al., 1987), for example, at the end of growing seasons when host plants become senescent and their food value changes. Stress severe enough to affect susceptibility to NPV also can result from other environmental factors such as high humidity and host crowding (Fuxa and Richter, 1999).

Advantages of NPV

The main advantages of these biocontrol agents are their specificity to target pests, safety to the non-target organisms, safe to environment and human health and can be used against pests which develop resistance to the conventional insecticides (Eranya et al., 2013). NPV is being one of the important biopesticide, as it is ecofriendly, having less residual toxicity, compatible with many chemical pesticides, self-perpetuating in nature. Therefore, their use is being encouraged in international concerns for a reduction in pesticides in the environment, and to restrict the development of resistance to chemical insecticides by target pests (Ahmad et al., 2003; Martin et al., 2005).

NPV as a component of IPM

Microbial pathogens of insects are intensively investigated to develop environmental friendly pest management strategies in agriculture. Due to their high degree of host specificity and insecticidal activity, baculoviruses have been exploited as environmentally sound microbial pesticides for pest management in agriculture and forestry (Moscardi, 1999; Szewczyk et al., 2006). Research efforts on the use of baculovirus pesticides for insect pest management in India started as early as in 1960s (Grazywacz, 2003). Nuclear polyhedrosis viruses recorded in India includes *Helicoverpa armigera*, *Spodoptera littura*, *Spodoptera exigua*, *Amsacta moorei*, *Agrotis ipsilon*, *Agrotis segetum*, *Anadividia peponis*, *Trichoplusia ni*, *Thysanoplusia orichalcea*, *Adisura atkinsoni*, *Plutella xylostella*, *Corcyra cephalonica*, *Mythimna separata* and *Phthorimaea operculella* (Eranya et al., 2013). Field evaluation of *Lymantria obfuscata* multiple nucleopolyhedrovirus for the management of Indian gypsy moth in Jammu and Kashmir, India were reported (Gupta et al., 2016). Optimization with respect to the host insect, insect diet, insect age, virus dosage,

incubation, environment, selection of harvesting time and preservation of virus infectivity consist some of the major factors (Gupta et al., 2007), in maximizing the yield of NPV production in individual larvae (Grazywacz et al., 1998). The combined use of microbial formulations has attained greater option among agricultural community as a successful tool in integrated pest management strategies (Purwar and Sachan, 2006). Integration of *Bacillus thuringiensis* and Nucleopolyhedrosis virus in order to determine their combine impact on survival of second and fourth instar larvae under laboratory conditions was observed as quite effective (Qayyum et al., 2015).

Disadvantages of NPV

One of the major problems in utilising baculoviruses as microbial pesticides has been their slow in action and lack of morphological changes in larvae in first stages of baculovirus development (Szewczyk et al., 2006). Rapid inactivation by the UV radiation in the fields comprises another limitation of the NPV formulation. The medium-wave UV portion of the sun's radiation (UV-B, 28-320 nm) acts as most important factor contributing to the photo inactivation of baculoviruses. They were rapidly inactivated after exposure to UV or natural sunlight, under natural field conditions (El Salamouny et al., 2000). Antioxidant or oxidative enzyme such as Dilodin, Inol, Vitamins, Folic acid, Riboflavin and Pyridoxine remain as promising compounds for protecting entomopathogenic viruses from UV rays (El-Helaly, 2013). Natural antioxidant such as green tea, black tea, eucalyptus and mango leaf extracts have been also reported as promising protective additives to baculoviruses (Baskaran, 2007). Therefore, formulations with UV protectants acting as adjuvants for increasing field persistence and efficacy have to be developed for large scale adaption of these microbial agents. The role of proteases to improve the insecticidal activity by eliminating the basement membranes of their hosts, in this manner facilitating the process of infection were reported (Harrison and Bonning, 2001). Koppenhofer and Kaya (2000) reported that neem seed kernel extract enhanced the activity of NPV by suppressing overall consumption, fecundity, survival, larval weight and growth rate of *Helicoverpa virescens*. The addition of moringa, rice bran filtrates (1%) to nucleopolyhedrovirus of cotton leaf worm *Spodoptera littoralis* (Bosid.) provided almost a complete protection to the inclusion bodies following exposure to artificial UV irradiation for 30 minutes (El-Helaly, 2013).

Biotechnological approaches for increasing the effectiveness of NPV

Genetic engineering offers a better scope for utilization of baculoviruses. A number of genetically modified baculoviruses with improved insecticidal activities have also been developed by the introduction of various foreign genes (Kamita et al., 2005; Inceoglu et al., 2006). These foreign genes include those that encode insect-specific neurotoxins from mite, scorpion, spider and sea anemone, metabolic enzymes and insect hormones that are capable of causing paralysis or death, or disturbing the physiology, development and behaviour of insects to make less crop damage (Ikeda et al., 2015). Chang et al. (2003) have explored another method for improvement of recombinant baculoviruses by cloning of insect toxic genes into the viral genome and expression during replication for quicker killing which may find application in future biopesticides construct. The most promising insect-specific toxin gene was probably the gene coding for AaIT toxin originating from scorpion *Androctonus australis* and reported that speed of killing by this recombinant baculovirus was increased by 40% and the reduced feeding damage by 60% (Inceoglu et al., 2001). The other toxic genes that have been inserted and expressed in baculovirus genome includes the *Buthus eupeus* insect toxin-1, the *Manduca sexta* diuretic hormone, the *Bacillus thuringiensis* ssp. *kurstaki* HD-73 deltaendotoxin, the *Heliothis virescens* juvenile hormone esterase, the *Pyemotes tritici* TxP-I toxin, *Androctonus australis* neurotoxin, Dol m V gene and T-urf 13 genes.

The most effective gene inserts have been the neurotoxins from *Androctonus australis* in *Bombyx mori*, from spider in *Spodoptera frugiperda*, Toxin 34 from *Pyemotes tritici* in *Heliothis zea*, Toxin 21 A from *Pyemotes tritici* in *Trichoplusia ni* and the T-urf 13 gene, which are responsible for cytoplasmic male sterility of maize (Eranya et al., 2013). Several major pesticide companies currently involved in the commercial development of these and other genetically enhanced viral pesticides. Development of recombinant clones of *Autographa californica nucleopolyhedrovirus* (AcMNPV) expressing proteases (Harrison and Bonning, 2001) was reported. Another approach to protect baculoviruses from UV radiation was proposed by Petrik et al. (2003) in which a recombinant AcMNPV was constructed which produces algal pyrimidine dimer specific glycosylase involved in the repairing of UV damaged DNA and observed that the recombinant virus was three times more resistant to UV inactivation. DNA photolyase genes were also found in baculovirus,

Chrysodeictis chalcites NPV (Van Oers et al., 2004) with potential to improve their UV resistance. Plant metabolites, mainly plant peroxidases also sometime cause inactivation of baculoviruses by generating free radicals (Sun et al., 2004). This inactivation can be reduced by addition of free radical scavengers such as mannitol or enzyme superoxide dismutase (Zhou et al., 2004).

Conclusion

Insect pathogenic viruses have long been recognised as potential environmentally safe alternatives to chemical insecticides. The development of widespread resistance to chemicals has also encouraged the development of biopesticides based on insect viruses as a means of overcoming this problem. NPV is known for high epizootic levels and is naturally occurring, self-perpetuating and safe to natural enemies due to host specificity. But, there is a scope to develop quality control guidelines and methodologies, systematic registration policies, to identify effective stains and to develop Ultraviolet resistant strains. In addition, the guidelines and training for implementation of biocontrol agents should be made available. There is a great probability of utilising genetically modified biopesticides that will gradually increase their share in pesticide market. Therefore, in this present scenario, formulation of an ecofriendly means of controlling pests to minimise the pesticides related problems and to ensure long term sustainable yield production through exploitation of microbial pesticides can added value in pest management in ensuring sustainable agriculture.

Conflict of interest statement

Authors declare that they have no conflict of interest.

References

- Ahmad, M., Arif, M. I., Ahmad, M., 2007. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Prot.* 26 (6), 809-817.
- Ahmad, M., Arif, M.I., Ahmad, Z., 2003. Susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to new chemistries in Pakistan. *Crop Prot.* 22,539-544.
- Andreadis, T.G., 1987. Transmission. In: Fuxa, J.R., Tanada, Y. (Eds.), *Epizootiology of Insect Diseases*. Wiley, New York. pp.159-176.
- Baskaran, R. K. M., 2007. *In vivo* screening of optical brighteners as UV protectants and their efficacy in enhancing the virulence of *Madurai* isolate of NPV of

- Amsacta albistriga* (Walker) (Lepidoptera: Arctiidae). J. Biol. Control. 21(1), 169-174.
- Blissard, G.W., Rohrmann, G.F., 1990. Baculovirus diversity and molecular biology. Annu. Rev. Entomol. 35, 127-155.
- Braunagel, S.C., Russell, W.K., Rosas-Acosta, G., Russell, D.H., Summers, M.D., 2003. Determination of protein composition of the occlusion-derived virus of *Autographa californica* nucleopolyhedrovirus. Proc. Natl. Acad. Sci. USA. 100, 797-802.
- Burand, J. P., 1991. Molecular biology of Hz-1 and *Oryctes* non occluded baculoviruses. In: Virology of Invertebrate (Ed.: Kurstan, E.). Marcel Dekker, New York. pp.111-126.
- Chakraborty, S., Monsour, C., Teakle, R., Reid, S., 1999. Yield, biological activity, and field performance of a wild-type *Helicoverpa* nucleopolyhedrovirus produced in *H. zea* cell cultures. J. Invertebr. Pathol. 73, 199-205.
- Chang, J.H., Choi, J.Y., Jin, B.R., Roh, J.Y., Olszewski, J.A., Seo, S.J., 2003. An improved baculovirus insecticide producing occlusion bodies that contain *Bacillus thuringiensis* insect toxin. J. Invertebr. Pathol. 84, 30-37.
- Cherry, A. J., Parnell, M. A., Grazywacz, D., Jones, K. A., 1997. The Optimization of *in vivo* nuclear polyhedrosis virus production in *Spodoptera exempta* (Walker) and *Spodoptera exigua* (Hubner). J. Invertebr. Pathol. 70, 50-58.
- Chih, Y. W., Wang, C.H., 2006. New cell line from *Lymantria xyliana* (Lepidoptera: Lymantridae) characterization and susceptibility to baculoviruses. J. Invertebr. Pathol. 93(3), 186-191.
- Desneux, N., Decourtye, A., Delpuech, J. M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81-106.
- El Salamouny, S., El-Sheikh, M.A.K., Elnagar, S., Huber, J., 2000. Prolongation of the UV persistence of nucleopolyhedroviruses by the lignin derived product. 33rd Annual Meeting Society for Invertebrate Pathology. Guanajuats Mexico University of Guanajuata, 13-18 Aug. 39p.
- El-Helaly, A. 2013. Additives for a baculovirus against ultraviolet effect. App. Sci. Report. 4 (1), 187-191.
- Erayya, J. J., Sajeesh, P.K., Upadhya, V., 2013. Nuclear polyhedrosis virus (NPV), a potential biopesticide: A review. Res. J. Agric. For. Sci. 1(8), 30-33.
- Erlanson, M., 2008. Insect pest control by viruses. Encyclopedia of Virology. 3rd Edn. pp.125-133.
- Evans, H.F., Entwistle, P.F., 1987. Viral diseases. In: Epizootiology of Insect Disease (Eds.: Fuxa, J.R., Tanada, Y.). Wiley & Sons, New York. pp. 257-322.
- Fernandes Neto, M.L., Sarcinelli, P.N., 2009. Agrotóxicos em água para consumo humano: uma abordagem de avaliação de risco e contribuição ao processo de atualização da legislação brasileira. Eng. Sanit. Ambient. 41, 69-78.
- Francki, R.I.B., Fauquet, C.M., Knudson, D., Brown, F. (Eds.), 1991. Classification and nomenclature of viruses. Fifth Report of International Committee on Taxonomy of Viruses. Archive of virology. Supplement 2.
- Fuxa, J.R., 1989. Importance of epizootiology to biological control of insects with viruses. Mem. Inst. Oswaldo Cruz. 84 (Supl. III), 81-88.
- Fuxa, J.R., Mitchell, F.L., Richter, A.R., 1988. Resistance of *Spodoptera frugiperda* (Lep.: Noctuidae) to nuclear polyhedrosis virus in the field and laboratory. Entomophaga .33, 55-63.
- Fuxa, J.R., Richter, A.R., 1999. Classical biological control in an ephemeral crop habitat with *Anticarsia gemmatalis* nucleopolyhedrovirus. Bio Control. 44, 403-419.
- Grazywacz, D., 2003. Development and registration of biopesticides in India and Thailand. In: Registration of Biocontrol Agents in Kenya (Eds.: Wabule, M.N., Ngaruiya, P.N., Kimmins, Silverside, P.J.). Proc. of the PCPB/ KARI/ DFID CPP Workshop, Nakuru, Kenya. pp. 101-111.
- Grazywacz, D., Jones, K.A., Moawad, G., Cherry, A., 1998. The *in vivo* production of *Spodoptera littoralis* nuclear polyhedrosis virus. J. Virol. Methods. 71, 115-122.
- Grazywacz, D., McKinley, D.J., Jones, K.A., Moawad, G., 1997. Microbial contamination in *Spodoptera littoralis* nuclear polyhedrosis virus produced in insects. J. Invertebr. Pathol. 69, 151-156.
- Gupta, R.K., Gani, M., Kaul, V., Bhagat, R.M., Bali, K., Samnotra, R.K., 2016. Field evaluation of *Lymantria obfuscata* multiple nucleopolyhedrovirus for the management of Indian gypsy moth in Jammu & Kashmir, India. Crop Prot. 80, 149-158.
- Gupta, R.K., Raina, J.C., Monobrullah, M.D., 2007. Optimization of *in vivo* production of nucleopolyhedrovirus in homologous host larvae of *Helicoverpa armigera*. J. Entomol. 4, 279-288.
- Harrison, R.L., Bonning, B.C., 2001. Use of proteases to improve the insecticidal activity of Baculoviruses. Biol. Control. 20, 199-209.
- Herniou, E.A., Arif, B.M., Becnel, J.J., 2012. Family *Baculoviridae*. In: Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses (Eds.: King, A. M. Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J.). Elsevier Academic Press, Amsterdam. pp.163-173.
- Huber, J., 1986. Use of baculoviruses in pest management programs. In: The Biology of Baculoviruses. Vol. II. Practical Application for Insect Control (Eds.: Granados, R.R., Federici, B.A.). CRC Press, Boca Raton, FL. pp. 181-202.
- Ignoffo, C.M., Anderson, R., 1979. Bio-insecticide. In: Microbial Technology (Eds.: Peppler, H. J., Parلمان, D.). Macmillan, New York. pp.1-28.
- Ignoffo, C.M., Couch, T.L., 1981. The nucleopolyhedrosis virus of *Heliothis* species as a microbial pesticide. In: Microbial Control of Pests and Plant Diseases (Burgess, H.), Academic Press, London. pp.329-362.
- IJkel, W.F.J., Westenberg, M., Goldbach, R.W., Blissard, G.W., Vlak, J.M., Zuidema, D., 2000. A novel baculovirus envelope fusion protein with a proprotein convertase cleavage site. Virology. 275, 30-41.
- Ikeda, M., Hamajima, R., Kobayashi, M., 2015. Baculoviruses:

- diversity, evolution and manipulation of insects. Entomol. Sci. 18, 1-20.
- Inceoglu, A.B., Kamita, S.G., Hammock, B.D., 2006. Genetically modified baculoviruses: A historical overview and future outlook. Adv. Virus Res. 68, 323-360.
- Inceoglu, A.B., Kamita, S.G., Hinton, A.C., Huang, Q., Severson, T.F., Kang, K. D., 2001. Recombinant baculoviruses for insect control. Pest Manag. Sci. 57, 981-987.
- Jayanth, K.P., 2002. Commercial production and market prospects of microbial pesticides in India. In: Microbial Biopesticide Formulations and Application (Eds.: Rabindra, R.J., Hussaini, S.S., Ramanujam, B.). Tech. Doc. No. 55, Project Directorate of Biological Control, Bangalore. pp.264-269.
- Jehle, J.A., Blissard, G.W., Bonning, B.C., Cory, J.S., Herniou, E.A., Rohrmann, G.F., Theilmann, D.A., Thiem, S.M., Vlak, J.M., 2006. On the classification and nomenclature of baculoviruses: A proposal for revision. Arch. Virol. 151, 1257-1266.
- Kamita, S.G., Kang, K.D., Inceoglu, A.B., Hammock, B.D., 2005. Genetically modified baculoviruses for pest insect control. In: Comprehensive Mol. Insect Sci. 6, 271-322.
- Katou, Y., Yamada, H., Ikeda, M., Kobayashi, M., 2010. A single amino acid substitution modulates low-pH-triggered membrane fusion of GP64 protein in *Autographa californica* and *Bombyx mori* nucleopolyhedroviruses. Virology. 404, 204-214.
- Kawasaki, Y., Matsumoto, S., Nagamine, T., 2004. Analysis of baculovirus IE1 in living cells: Dynamics and special relationships to viral structural proteins. J. Gen. Virol. 85, 3575-3583.
- Kopenhofer, A. M., Kaya, H. K. 2000. Interaction of a NPV with azadirachtin and imidacloprid. J. Invertebr. Pathol. 75, 84-86.
- Kumar, C.M., Sathiah, N., Rabindra, R.J., 2005. Optimizing the time of harvest of nucleopolyhedrovirus infected *Spodoptera litura* (Fabricius) larvae under in vivo production systems. Curr. Sci. 88,1682-1684.
- Kunimi, Y., Fuxa, J.R., Richter A.R. 1997. Survival times and lethal doses for wild and recombinant *Autographa californica* nuclear polyhedrosis viruses in different instars of *Pseudoplusia includes* Biol. Contr. 9,129-135.
- Long, G., Pan, X., Kormelink, R., Vlak, J. M. 2006. Functional entry of baculovirus into insect and mammalian cells is dependent on clathrin-mediated endocytosis. J. Virol. 80, 8830-8833.
- Lynn, E. D., Shapiro, M. 1998. New Cell Lines from *Heliothis virescens*: Characterization and Susceptibility to Baculoviruses. J. Invertebr. Pathol. 72, 276-280.
- Martin, T., Ochou, G.O., Djihinto, A., Traore, D., Togola, M., Vassal, J.M., Vaissayre, M., Fournier, D., 2005. Controlling an insecticide-resistant bollworm in West Africa. Agric. Ecosyst. Environ. 107, 409-411.
- McKinley, D.J., Moawad, G., Jones, K.A., Grazywacz, D., Turner, C.R., 1989. The development of nuclear polyhedrosis virus for the control of *Spodoptera littoralis* (Boisd.) in cotton. In: Pest Management in Cotton (Eds.: Green, M.B., Lyon, D.J. de B.). Ellis Horwood, Chichester, UK. pp.93-100.
- Mcleod, P.J., Yearian, W. C., Young, S.Y., 1977. Inactivation of Baculovirus *Heliothis* by ultraviolet irradiation, dew, and temperature. J. Invertebr. Pathol. 30, 237-241.
- Menn, J.J., Hall, F. R., 2001. Biopesticides- Present Status and Future Prospects. In: Methods in Biotechnology Vol. 5: Biopesticides Use and Delivery (Eds: Menn, J.J., Hall, F.R.). Humana Press, Totowa, NJ. pp.1-9.
- Mitchell, F.L., Fuxa, J.R., 1990. Multiple regression analysis of factors influencing a nuclear polyhedrosis virus in populations of fall armyworm (Lepidoptera: Noctuidae) in corn. Environ. Entomol. 19, 260-267.
- Moscardi, F., 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Annu. Rev. Entomol. 44, 257-289.
- Murphy, F.A., Fauquet, C.M., Bishop, D.H., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A., Summers, M.D. (Eds.), 1995. Virus Taxonomy-The Classification and Nomenclature of virus. VI Report of ICTV, Springer-Verlag, New York.
- Nagamine, T., Abe, A., Suzuki, T., Dohmae, N., Matsumoto, S., 2011. Co-expression of four baculovirus proteins, IE1, LEF3, P143, and PP31, elicits a cellular chromatin containing reticulate structure in the nuclei of uninfected cells. Virology. 417, 188-195.
- Nguyen, T.H.N., Borgemeister, C., Poehling, H.M., Zimmermann, G., 2007. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. Biocont. Sci. Technol. 17, 853-864.
- Oerke, E.C., Dehne, H.W., 2004. Safeguarding production - losses in major crops and the role of crop protection. Crop Prot. 23, 275-285.
- Ohkawa, T., Volkman, L.E., Welch, M.D., 2010. Actin-based motility drives baculovirus transit to the nucleus and cell surface. J. Cell Biol. 190, 187-195.
- Pachiappan, P., Narayanaswamy, K.C., 2012. Effect of sunlight and temperature on the Nuclear Polyhedrosis Virus of Mulberry Leaf-Roller, *Diaphania pulverulentalis* (Hampson). e-J. Biol. Sci. 5(1), 22-26.
- Passarelli, A.L., 2011. Barriers to success: how baculoviruses establish efficient systemic infections. Virology. 411, 383-392.
- Pawar, A.D., 2002. Quality control and registration requirements of microbial pesticides. In: Microbial Biopesticide Formulations and Application (Eds.: Rabindra, R.J., Hussaini, S.S., Ramanujam, B.). Tech. Doc. No. 55, Project Directorate of Biological Control, Bangalore. pp.228-263.
- Pedlowski, M.A., Canela, M.C., Terra, M.A.C., Faria, R.M.R., 2012. Modes of pesticides utilization by Brazilian smallholders and their implications for human health and the environment. Crop Prot. 31, 113-118.
- Peng, F., Fuxa, J.R., Richter, A.R., Johnson, S.J., 1999. Effects of heat-sensitive agents, soil type, moisture, and leaf

- surface on persistence of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus. Environ. Entomol. 28, 330-338.
- Petrik, D.T., Iseli, A., Montelone, B.A., Van-Etten, J.L., Clem, R.J., 2003. Improving baculovirus resistance to UV inactivation: increased virulence resulting from expression of a DNA repair enzyme. J. Invertebr. Pathol. 82, 50-56.
- Purwar, J.P., Sachan, G.C., 2006. Synergistic effect of entomogenous fungi on some insecticides against Bihar hairy caterpillar *Spilarctia oblique* (Lepidoptera: Arctiidae). Microbiol. Rev. 161, 38-42.
- Qayyum, M.A., Wakil, W., Arif, M.J., Sahi, S.T., 2015. *Bacillus thuringiensis* and nuclear polyhedrosis virus for the enhanced biocontrol of *Helicoverpa armigera*. Int. J. Agric. Biol. 17, 1043-1048.
- Rabindra, R.J., 2002. Current status of production and use of microbial pesticides in India and the way forward. In: Microbial Biopesticide Formulations and Application (Eds.: Rabindra, R.J., Hussaini, S.S., Ramanujam, B.). Tech. Doc. No. 55, Project Directorate of Biological Control, Bangalore. pp.1-12.
- Ramanujam, B., Rangeshwaran, R., Sivakmar, G., Mohan, M., Yandigeri, M. S., 2014. Management of insect pests by microorganisms. Proc. Indian Nat. Sci. Acad. 80(2), 455-471.
- Richter, A.R., Fuxa, J.R., Abdel-Fattah, M., 1987. Effect of host plant on the susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus. Environ. Entomol. 16, 1004-1006.
- Shapiro, M. 1986. *In vivo* production of baculoviruses. In: The Biology of Baculoviruses (Eds.: Granados, R. R., Federici, B. A.). Vol. 2. CRC Press, Boca Raton. pp.31-61.
- Sharma, N., Srivastava, R., 2013. *In vitro* production of nuclear polyhedrosis virus of *Helicoverpa armigera*. Persian Gulf Plant Prot. 2, 18-31.
- Skovmand, O., 2007. Microbial control in Southeast Asia. J. Invertebr. Pathol. 95, 168-174.
- Smaghe, G., Goodman, C.L., Stanley, D., 2009. Insect cell culture and applications to research and pest management. In Vitro Cell. Dev. Biol. Anim. 45, 93-105.
- Subramanian, S., Santharam, G., Sathiah, N., Kennedy, J.S., Rabindra, R. J., 2006. Influence of incubation temperature on productivity and quality of *Spodoptera litura* nucleopolyhedrovirus. Biol. Control. 37, 367-374.
- Sun, X., Sun, X., van der Werf, W., Vlak, J.M., Hu, Z., 2004. Field inactivation of wild-type and genetically modified *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus in cotton. Biocontrol Sci. Technol. 14, 185-192.
- Sundeeep, A.B., Mourya, D.T., Mishra, A.C., 2005. Insect cell line culture in research: Indian scenario. Indian J. Med. Res. 121, 725-738.
- Szewczyk, B., Hoyos-Carvajal, L., Paluszek, M., Skrzecz, I., Lobo de Souza, M., 2006. Baculoviruses-re-emerging biopesticides. Biotechnol. Adv. 24, 143-160.
- Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., 2001. Forecasting agriculturally driven global environmental change. Science. 292, 281-284.
- Van Oers, M.M., Herniou, E.A., Usmany, M., Messelink, G.J., Vlak, J.M., 2004. Identification and characterization of a DNA photolyase-containing baculovirus from *Chrysodeixis chalcites*. Virology. 330, 460-470.
- Volkman, L.E., 2007. Baculovirus infectivity and the actin cytoskeleton. Curr. Drug Targets. 8, 1075-1083.
- Wanger, T. C., Rauf, A., Schwarze, S., 2010. Pesticides and tropical biodiversity. Front. Ecol. Environ. 4, 178-179.
- Young, S., 2001. Persistence of viruses in the environment. In: Factors Affecting the Survival of Entomopathogens (Eds.: Baur, M.E., Fuxa, J.R.). South. Cooperative Ser. Bull. s-301/s-265, 35-69.
- Zhou, J., Blissard, G.W., 2008. Identification of a GP64 subdomain involved in receptor binding by budded virions of the baculovirus *Autographica californica* multicapsid nucleopolyhedrovirus. J. Virol. 82, 4449-4460.
- Zhou, M.Z., Sun, H.C., Hu, Z.H., Sun, X. L., 2004. SOD enhances infectivity of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrosis virus against *H. armigera* larvae. Virol. Sinica. 18, 506-507.

How to cite this article:

Bidyarani Devi, E., Premabati Devi, E., Deepshikha., 2016. An overview on Nuclear Polyhedrosis Virus (NPV) as a valuable biopesticide in enhancing ecofriendly management of insect pests. Int. J. Curr. Res. Biosci. Plant Biol. 3(8), 97-106. doi: <http://dx.doi.org/10.20546/ijcrbp.2016.308.016>