ABSTRACT

Pesticides driven from Plants, animals, and microbes such as (bacteria fungi, viruses, algae, nematodes and protozoa are replacing traditional chemical pesticides throughout the world. Bio pesticides are target specific and reduce the environmental risks. Bio pesticides are promoting sustainable agriculture development by reducing the environmental pollution. Various products have been registered and released, play important role in the agro-market. Regulations of insect populations are naturally occurs by Baculoviruses (entomopathogenic viruses). The pesticide based on baculovirus particles has been formed to control pest and their use is beneficial to reduce the risk of synthetic chemical insecticides. The present status and increase use of baculovirus based bio pesticides as replacement of chemical pesticides, its role in integrated pest management, have been discussed in this review.

Key word: Baculovirus, bio control agent, bio pesticides, life cycle, vector

INTRODUCTION

The baculoviruses belong to family Baculoviridae are circular large Double stranded (dsDNA) viruses contained within the enveloped nucleoprotein core of rod shape virion. It is believed that these virus have been evolved from Nudivirus family 310 million years ago (Thézé et al., 2011; Ikeda et al., 2015). Family Baculoviridae is divided into 4 genera, currently 66 species of baculovirus has been identified. Their natural hosts are many more including Lepidoptera, Hymenoptera, and Diptera (Jehele et al., 2006). These viruses have been used as bio pesticides, mammalian cells transduction and vectors for gene expression (Hofmann et al., 1995; Szewczyk et al., 2006; Chen et al., 2011). Baculoviruses are widely used as bio pesticides in crop field. Recombinant proteins used in research and as vaccines in both animals and humans has been prepared by utilizing these viruses (Lackner et al., 2008).

Habitat: Baculoviruses are used to control insects so they are present mostly at places where insects are present. Rain and wind serve as carrier of baculoviruses. Most of the agricultural crops product present on the shelves is "infested" by baculovirus particles (Heimpel et al., 1973). The spread of baculovirus particles, and their tests for registration can be used as evidence for their safety (Cory et al., 1997).

Host range: Generally host range of Baculoviruses is narrow, mostly they infect invertebrates (Szczeky et al., 2009). Approximately 600 host species of Baculoviruses have been described (Martignoni and Iwai, 1981). Baculoviruses have ability to infect more than one insect species. Autographa californica multiple nucleopolyhedrovirus (AcMNPV), extremely studied member of the family, can infect as many as thirty species from various genera of Lepidoptera. In culture baculoviruses are able to enter the mammalian cells, (Hofmann et al., 1995), but there is no information available regarding their replication in mammalian cells or other vertebrate cells. These viruses mostly infect the larva of moth species but they can also infect mosquito, sawflies and shrimps.

Morphology/genome organization: The baculoviruses has double stranded DNA genome that is large 80–180 kbp in length, covalently-closed. These viruses have rod shaped capsid and are surrounded by an envelope (Braunagel et al., 1998; Clem, 2007). NPVs virions are covered either single (SNPV)/ group of several virions (MNPV). They form occlusion body (OB) using polyhedron (a protein). Genome sizes of Baculovirus range from the smallest, 81,755 bp (Lauzon et al., 2004) to the largest 178,733 bp (Hayakawa et al., 1999). About 58 species of baculovirus have been sequenced; out of which 41 belongs to alpha baculoviruses, 13 beta baculoviruses, three gamma baculoviruses and one delta baculoviruses.

Baculoviruses encoded genes have effect on host physiology and behavior. Two types of enveloped virions are produced by Baculoviruses: embedded in 5–10 micron large protein crystals, and helps in horizontal transmission among insects, known as occlusion derived virions (ODV) and budded virions (BV) to move information from one cell to other (Clem and Passarelli, 2013). These viruses encode a DNA-directed RNA polymerase enzyme for the translation of viral genes and utilized baculovirus expression vector system to express the outer genes (Passarelli and Guarino, 2007; Rohrmann, 2008).

Baculovirus life cycle: Baculoviruses viruses are obligate parasite, need host for their reproduction and replication. Each virion produced more virus particles inside host cell and ultimately leads to the host cell death. Upon death of host insect, there is a chance that other insects can become infected when they come in contact with baculovirus. First step for the initiation of baculovirus life cycle is feeding of baculovirus. Two distinct forms of virus are involved in life cycle of Baculoviruses. Primary infection of the host occurs by Occlusion derived virus (ODV) while infected host cells release budded virus.
during secondary infection. There are three phases of baculovirus infection: Early infection takes 6 hours whereas late infection occurs within 6-24 hours post inoculation, and very late phase starts from 24-72 hours post inoculation. When insect feeds on contaminated plants having ODV initial infection begins. Host midgut releases protein matrix dissolved in the alkaline environment that fuses with epithelial membrane of the host intestinal cells and are transferred to the endosomes (Slack and Arif, 2006). Endosomes releases the nucleocapsid and transported to the nucleus. Actin filaments of host may be involved in this step. In the cell nucleus viral replication and transcription occur, new BV particles are formed. Infection spread systemically when basolateral side of host insect releases BV particles (Vollman, 2007). For BV particles budding host cell membrane with viral glycoproteins are required. During late phase of viral life cycle BV produced while ODV produced in very late phase to acquire and transfer the viral envelop from nucleus of host cell towards the occlusion body proteins and fixed it in matrix (Possee et al., 2010). When cells lyses it releases these occlusion bodies to spread infection to the other hosts. BV particles without envelope are more sensitive to environment as compared to ODV that are resistant to heat and light (Zimmer, 2014).

**Early phase:** When host cells are infected with baculovirus, transcription of host cells decease and virus increases due to the replication of virus particles (Guarino and Summers, 1987; Nobiron et al., 2003). RNA polymerase II enzyme is required for the replication of baculovirus (Huh and Weaver, 1990). Expression of early genes is usually regulated during transcription and viral DNA infects the surrounding sensitive cells and represents that transcription of early genes is not necessary for the infection. The lack of viral proteins requirements has been verified by the proteomic analysis of AcMNPV BV virion particles (lack regulatory proteins) (Wang et al., 2010). Larvae of Velvet bean caterpillar (Anticsarsia gemmatalis) are extremely resistant to AcMNPV. The infection almost stopped during replication (early stage), even the ODVs enter into the midgut and reached to tracheal cells and as a result of this early gene not transcribed (Chikhalya et al., 2009). So, the inhibition of the essential transactivator disturbed the viral gene expression and ultimately fails to produce infectious viral particles.

**Viral DNA replication:** Early gene products are precursor for the start of DNA replication in late phase of baculovirus. Inside cells AcMNPV infected with AcMNPV, genome replication happens between 6-8 hpi then after some time it begins to decline (Palomares et al., 2006). Six genes are essential for the replication of AcMNPV during transient DNA replication assay (Kool et al., 1994). These genes that are involve in DNA replication (Huang et al., 2011), encodes a homologous region (hr) binding protein, transcription activation factor (Leisy et al., 1995), ssDNA binding protein (lef-3) (Hang et al., 1995), helicase (DNA binding protein) (McDougal and Guarino, 2001), a putative primase (lef-1), a primase-associated protein (lef-2) (Mikhailov and Rohrmann, 2002) and a DNA polymerase (dnapol) (Hang and Guarino, 1999). Usually 4 genes (dnapol, p143, lef-1, and lef-2) are present in every baculovirus genome and other genes i.e-2, lef-7, pe38, and p35 are essential in cell lines of different lepidopteran genomes (Okano et al., 2007; Beas-Catea et al., 2014). Genomes of BmNPV and AcMNPV are highly similar but their host ranges are quite different (Gomi et al., 1997). As larvae and cell lines of Bombyx mori i.e., BmN are tolerant to BmNPV but in comparison AcMNPV is sensitive to infection (Morris and Miller, 1993). AcMNPV infected BmN cells showed cytotoxic effects which in turns led to reduced gene expression. In case of B. mori cells and larvae few amino acid alters AcMN were adequate to overcome the defects (Maeda et al., 1991). Block in AcMNPV infection of B. mori cells and cytotoxicity are suggested to stem from abnormal replication process (Rohrmann, 2008; Thiem and Cheng, 2009).

**Late phase /gene expression:** In the late replication cycle of baculovirus late gene expression encodes for structural proteins i.e. AcMNPV gene encoding proteins of nucleocapsids and BVs are transcribed in late phase, whereas, the genes responsible for the production of occlusion bodies are translated after post inoculation (18-76hpi) (Thiem and Miller, 1990). Early and late viral transcription opposes the decline in host (Nobiron et al., 2003). Sequences TAAG of promoter are conserved and essential components for the recognition of late promoters by viral RNA polymerase factors with Cis-acting sequences responsible for the late expression of gene in baculovirus (Ooi et al., 1989). As these nineteen genes are required for transcription of late genes and recognized as late expression factors (lefs). Although all events related to late transcription are dependent on DNA replication except four lefs (p47, lef-4, lef-8, and lef-9) that form viral RNA polymerase directly involved invitro transcription of baculovirus late promoters (Rapp et al., 1998). Baculovirus has unique feature which is the hypertension of late genes (Polyhydrin and P10). Rises in RNA polymerase transcription happens through the expression of factor (VLF-1) to late promoters of polyhedrin needed for occlusion body formation (Yang and Miller, 1999). Cells of gypsy moth Ld652Y are less tolerant to AcMNPV, but the genes replication and transcription of other class of viruses and their translation (host, viral proteins) is stopped by about 12 hpi (Thiem and Cheng, 2009).

**Gut interactions:** Baculoviruses primary target of infection is the larval midgut epithelium as they are transmitted orally. In the midgut lumen occlusion bodies are dissolved, and releases the embedded ODV, that result in infection of epithelial cells of midgut. Offspring’s of BV bud cross the basal lamina from the basal surface of the epithelium and infects the remaining larval tissues (figure 1). Attachment and entry of ODV depends mainly on different viral proteins that are found in envelop, known as PIFs (*per os* infectivity factors). It has been found that some PIFs form a complex under the ODV envelope, and interact with unidentified mid gut receptor to facilitate plasma membrane fusion (Peng et al., 2012). So, the novel fusion protein and virus attachment complex are formed by PIFs and contains unique genes that are conserved among related insect virus families as PIF homologs are found in the polydnaviruses and nudiavirus. However, they are also
involved in mutualism with parasitic wasps (Bézier et al., 2009; Burke et al., 2013).

Figure 1. Per os infection of baculoviruses. Anatomy of an insect larva is represented by the cross section (Clem and Passarelli, 2013).

After the completion of replication in midgut, most of the baculoviruses move towards basal liminal layer, cause other larval tissue infection. Homologs of fibroblast growth factor (vFGF) are encoded by midgut escaped baculoviruses. Basal laminal degradation occur due to the vFGF expression (induces a protease cascade). Virus cause infection of insect tracheal cells of basal lamina and then spread to the whole body (Means and Passarelli, 2010). In certain cases, few species of baculoviruses directly cross the midgut basal lamina, as shortly after oral infection these virions have been observed in the larval hemocoel (Clem and Passarelli, 2013).

Regulation of gene expression: Gene expression in members of family baculoviridae occurs in three stages as a cascade, each stage is dependent on earlier stages expression, then late, very late (Romanowsky and Ghiringhelli, 2001). Enzyme (RNA dependent RNA polymerase II) is responsible for the transcription of early genes while viral RNA polymerase transcribed genes in late and very late phase. Viral polymerase has four subunits, homologus to other identified polymerases. Promoters of both early and late stage are different and mainly consist of four nucleotide sequence TAAG. Baculovirus genome is circular molecule in which genes are randomly arranged and many overlapping genes have short intergenic sequences (Chen et al., 2013).

More than 200 transcription sites and genomic regions have been recognized where transcripts are directly involved in gene regulation by RNA interference mechanism. Baculovirus genome produced siRNAs (Jayachandran et al., 2012) after 12h post infection and viral transcripts contains 38% mRNA, whereas, after 48h polyadrin single virus transcript (24%) mRNA in the cell. Polyadrin promotor is usually used in baculoviruses gene expression system and also it contains 25% of the total proteins which is major constituent of occlusion bodies. Beside all these genes and transcripts 12 spliced mRNAs were identified. miRNAs regulation is one of the most important aspect for gene expression in baculoviruses (Singh et al., 2010). But some miRNAs stopped the transport of small RNAs from nucleus to other parts of cell and suppress the whole miRNA pathway (Singh et al., 2012). Baculovirus gene expression involved the mixture of full length and defective virus genomes in natural population of virus (Serrano et al., 2013). It has been assumed that this is might be optimized by expression of few genes required for infectivity.

Physiological and behavioral modification of host: Insect larvae infected with baculovirus shows several changes in behavior and its physiology (figure 2). Generally feeding is periodically paused in larva of Lepidoptera, to go through larval molts. However, due to the effect of viral enzyme becysteoyl UDP-glucosyltranferase (EGT) infected larvae with baculovirus do not undergo molt (O'Reilly and Miller, 1989). Ecdysone is the major insect molting hormone and EGT inactivate it, thus prevent larval molting. Dramatic death occurs due to abnormal growth of larva. Viral cathepsin like protease and chitinase enzymes expression caused liquefaction of infected corpses, and it helps in release of viral occlusion bodies. The amount of progeny virus ranges 10 million per mg of larval tissue (Clem and Passarelli, 2013). when caterpillar infects the virus infected plant and move to other hosts after getting virus and virus can spread to the leaves that will be consumed by new hosts (Clem, 2007). Larvae of gypsy moth were infected with L. dispar MNPV (wild type) revealed improved climbing behavior in comparison to mutant (Hoover et al., 2011). In some cases increased wandering of infected larvae results in increased virus transmission (Katsuma et al., 2012). According to phylogenetic analysis egt and ptp genes showed lepidopteran origin e.g., some addition genes, representing that baculoviruses have attained many beneficial genes during coevolution along with their hosts.

Figure 2: Climbing behavior of Baculovirus, known as “Wipfelkrankheit” (tree top disease), has been attributed to the viral egt gene (Hoover et al., 2011).

Current and future tactics for integrated pest management.

Human toxicity and infectivity: Viruses belong to family Baculoviridae are highly specific in terms of their host can only infect few species of insects. These characters make them best candidate for insect pest management of crop and forests with least impact on other hosts. Use of baculovirus products as biocontrol agents of insect pest is spread with large commercial scale around the world. Previously, since last fifty years these products are in use worldwide as microbial insecticides. Currently, safety issues related to
baculovirus products have been validated further by doing research on molecular events that helps to regulate the baculovirus life cycle, host range by increased applications of baculovirus for pharmaceutical uses. Basically, the safety of baculovirus products is intrinsically related to the host range and pathogenesis of viruses belongs to this family. For successive baculovirus infection replication process must happens relative to the prevailing environmental conditions and organism specific barriers. In each step of baculovirus life cycle there is need of long period availability of host and suitable environmental conditions.

Generally arthropods serves as hosts of Baculovirus (Doyle et al., 1990; Thiem and Cheng, 2009). Commercially available products of Baculovirus as biological pesticide have been tested on humans and other animals (Hauschild et al., 2011). Human infectivity and toxicity has been tested in labs on mammalian cells (e.g. rabbit, rat, mice). Active ingredients and end products of baculovirus pesticides have been injected (into the skin of animals), ingested and inhaled by, showing no harmful effects (Ashour et al., 2007). Many animal species have been tested with different species and different doses of baculovirus species.

In, in vitro studies, mammalian and other vertebrate cells shows non-permissive behavior to baculovirus pesticides (OECD, 2002). In some cases, uptake of BV particles have observed but lacks DNA replication, production of viral proteins or cyto-pathological effects have occurred. It has been studied that many animals take up baculovirus OBs but they do not produce baculovirus-specific antibodies (Groner, 1986). Chemically extracted AcMNPV ODVs from OBs were recently tested against A549, HepG2 and Human carcinoma cell lines (Makela and Oker-Blom, 2008). Inefficient binding and internalization in the cell shows no infection of the cells A549 and HepG2. There is no evidence of carcinogenic, mutagenic cytogenic or teratogenic effects of baculovirus pesticides (McWilliam, 2007).

**Relative effectiveness:** Baculovirus specific pesticides could be effective for the control of specific insect pests, as compared to chemical pesticides. The cost of specific pesticide spray is more in per hectare land while baculovirus in vitro production is less expensive (inside insect host). But, the expenses of insect rearing are add some cost to baculovirus products. Insect culture development system if maintained expenses of insect rearing are add some cost to baculovirus production is less expensive (inside insect host). Then cost of virus particles could be reduced.

**Pesticide compatibility:** There is controversy about the application of pesticides and baculovirus product at the same time in fields because chemical compound chloride hinder with the efficacy of virus particles and often damage them. Therefore research work is needed to explore the effectiveness of insecticides “cocktails” consisting of environmental friendly chemicals and baculovirus products.

**REFERENCES**


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