

Review: The effect and possible mitigation of UV radiation on baculoviruses as bioinsecticides

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Abstract. Anggraini E, Lau WH, Vadamalai G, Kong LL, Mat M. 2022. Review: The effect and possible mitigation of UV radiation on baculoviruses as bioinsecticides. *Biodiversitas* 23: 3721-3735. Indiscriminate use of pesticides has raised concerns over environmental and human health issues. A more environmentally-friendly biological approach to control insect pests is gaining importance. For decades, bioinsecticides based on viruses have been used to protect crops and forests. The viruses belonging to the *Baculoviridae* family are known to infect insect pests, and hence can be used as biopesticides. Baculoviruses are mostly specific to the host and are not lethal to the non-target organism. However, their usage is limited for field application due to a slow kill speed and sensitivity towards UV irradiation. This review paper discusses the life cycle of baculoviruses, their mode of infection and transmission as well as the influence of UV radiation on the effectiveness of baculoviral insecticides for field application.

Keywords: Baculovirus, insect pest, irradiated virus, UV radiation

INTRODUCTION

Chemical pesticides are commonly used to control insect pests in the field, but the indiscriminate use of chemical pesticides has raised concern over the pollution of natural resources and the development of resistant target pests, as well as detrimental effects on human health, useful insects and non-targeted organisms (Beas-Catena et al. 2014). There is substantial documentation of the adverse effects of synthetic chemical insecticides on beneficial insects of vegetable crops (Smagghe et al. 2013; Fernandes et al. 2016). Alternatively, biological control methods are becoming popular, particularly in controlling the insect pest population (Barratt et al. 2018). The use of biocontrol agents not only benefits the economy and the life of animals and users but also reduces the exposure of harmful chemicals to the environment (Chidawanyika et al. 2012). Microbial pesticides are less costly and environmentally friendly (Usta 2013; Kumari et al. 2014; Ruiu 2018). Entomopathogenic microbes such as viruses, bacteria and fungi are the common microbial biopesticides used in the field (Kvakkestad et al. 2020).

Entomopathogenic fungi (EPF) are claimed to be effective in controlling insect pests where they can infect disease-causing insects under the right conditions (Trizelia et al. 2015; Bamisile et al. 2021). EPF directly colonise the insect's body by penetrating the cuticle (Bamisile et al. 2021). To date, more than 700 species from about 90 different genera have been designated as insect pathogenic

fungi (Khachatourians and Qazi 2008), and 20 out of 31 orders of insects are infected by EPF (Araújo and Hughes 2019). However, EPFs require 2-3 weeks to kill their insect hosts (Singh et al. 2017). In addition, entomopathogenic bacteria, such as the commercial biocontrol agent *Bacillus thuringiensis* (Bt), are known as the most successful microbial insecticides against different orders of insect pests in agriculture (Jouzani et al. 2017). During sporulation, Bt releases insecticidal crystalline inclusions called Cry or Cyt toxins, which demonstrate a highly selective spectrum of insecticidal activity and kill insects within a confined area (Bravo et al. 2011). Nowadays, the crystal toxins of Bt can be expressed in plants to create crops resistant to certain insect pests (Abbas 2018). However, insects can develop resistance to Bt transgenic crops (Tabashnik et al. 2013; Tay et al. 2015) and bacteria toxins (Tetreau 2018).

Entomopathogenic viruses are well-known as very specific to target insects (Kalha et al. 2014), and they can cause epizootics in the natural population of insects (Sun 2015). For example, the *Lymantria xyliina* multiple nucleopolyhedrovirus (LyxyMNPV) is highly virulent to the casuarina moth *Lymantria xyliina* Swinehoe and has been investigated as a possible biopesticide for controlling outbreaks of *L. xyliina* Swinehoe (Nai et al. 2010). Among viruses that are known to infect insects, the *Baculoviridae* family is considered a potential pesticide for field application because they are host-specific and typically confined to a single or a small number of closely

related insect species (Haase et al. 2015). Moreover, they do not produce any toxins or metabolites that could harm human health (Landwehr 2021) and are not pathogenic to vertebrates (Amalfi et al. 2020). In addition, baculoviruses have polyhedral occlusive bodies (OBs) that confer resistance to adverse environmental conditions (Harrison and Hoover 2012a; Susurluk et al. 2013; Sajjan and Hinchigeri 2016). Furthermore, baculoviruses have been successfully controlled using the classical biological control methods (Ibarra and Del Rincón-Castro 2008). Introduction of the viruses into an insect population that has never been treated previously, and once inoculated and established, the virus will persist in the insect population without further application (Podgwaite 1985; Ibarra and Del Rincón-Castro 2008). Moreover, based on the efficacy, Nucleopolyhedrovirus (NPV), a member of baculoviruses, has been used to control pests biologically and has caused almost 90% larval mortality (Cuartas et al. 2014). NPVs have tremendous potential as bioinsecticides compared to other viral family members.

Baculovirus usage as a potential bioinsecticide may have potential limitations. For instance, UV radiation can affect the efficacy of baculoviruses (Zhang et al. 2016). Although they do not rely on the temperature and humidity to infect their host, their stability could be challenged by solar irradiation under field conditions in the ultraviolet spectrum at 290-400 nm (Bullock et al. 1970) particularly in the UV-B range (280-320 nm) (Lasa et al. 2007). Another study has revealed that extending the duration of UV exposure resulted in a decline in both virus concentration and insect mortality (Salama et al. 2017). UV radiation is also a challenge to other biological control agents such as EPFs and Bt. The mortality of *Spodoptera litura* decreased significantly when exposed to *Beauveria bassiana* irradiated under UV-C (Herlinda et al. 2020). *Beauveria bassiana* was unable to adjust to direct sunlight, and its conidia were swiftly destroyed by ultraviolet (UV) radiation (Jackson et al. 2010; Kaiser et al. 2019). Sunlight exposure could affect the efficacy of *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) against *Anopheles* larvae (Zogo et al. 2019). This review paper will give insights into the limitation of baculovirus as biocontrol agents, specifically the effects of UV radiation on the infection cycle of baculoviruses and the preventive measures against UV inactivation of baculoviruses.

Baculovirus

Baculoviruses are the major group of arthropod viruses that are well known as biocontrol agents against pests of agricultural crops. They are divided into four genera based on their target host (ICTV 2020). The genera *Alphabaculovirus* (baculoviruses are called nucleopolyhedrovirus or NPV) and *Betabaculovirus* (baculoviruses are called granulovirus or GV) infect lepidopteran insects while the genera *Gammabaculovirus* (NPV) and *Deltabaculovirus* infect hymenopteran and dipteran insects, respectively (Jehle et al. 2006; van Oers 2021). The name of baculovirus is derived from its rod-shaped nucleocapsids. Baculovirus contains double-stranded DNA (dsDNA), which is circular, supercoiled and

protected from the environmental hazards due to the presence of a polyhedral crystalline matrix (Rohrmann 2019). Its genome size ranges from 80-200 kb (Szewczyk et al. 2006), which encodes 70-180 genes. Of these, 31 core genes are well identified in all baculovirus genomes. These core genes are involved in various functions like infection, cell cycle arrest, replication, transduction, virus assembly, packaging and release (Miele et al. 2011).

The infection process of baculoviruses

During the life cycle of a baculovirus, two phenotypic virions, namely budded virions (BV) and ocular-derived virions (ODV), are produced at distinct chronological stages. BV is produced during the early stage of infection. It buds off from the cytoplasmic membrane as a single nucleocapsid per envelope and is involved in the secondary infection. ODV is produced during the latter phase of infection and embedded in a protein matrix, which is crystalline in nature and referred to as occlusion bodies (OB). Even though these two kinds of virions display similarities in their genome, they are different in their origins, envelope compositions and functions in the viral life cycle (Haase et al. 2015). The major proteins of the crystalline matrix in the OBs are called polyhedron (NPV) and granule (GV). This protein is very stable outside the host. Therefore, OBs can withstand different environmental conditions and prevail in the infectious state indefinitely.

The primary infection starts when OBs are ingested by susceptible lepidopteran larvae *per os*. The gastrointestinal tract of insects is the site of baculoviral infection. It can be divided into three categories: foregut, midgut and hindgut (Saxena et al. 2018). After ingestion, due to the extreme alkaline pH of the midgut, the OB is degraded to release ODVs. The ODVs are responsible for primary infection of the midgut epithelial cells. They pass through the peritrophic membrane (PM), which is made up of chitin and glycoprotein, lines the midgut and protects the cells from abrasion, cut, or insect pathogens (Terra 2001). Some baculoviruses encode an enzyme (metalloproteinase) containing divalent cations as an integral part of their structure, which helps them to cross PM. The enhancin is a metalloproteinase, which is believed to digest mucin, a component of PM, thereby allowing virus access to the epithelial cell surface (Wang and Hu 2019). The enhancin of GV is believed to increase the infectivity of some NPVs (Slavicek and Popham 2005). After crossing the PM, ODV will bind to the ODV-specific receptors and fuse to the brush border of the microvilli of the columnar epithelial cells, releasing nucleocapsids (Figure 1). Nucleocapsids travel through the cytoplasm to the nucleus which is the site for baculovirus replication (Harrison and Hoover 2012b). Transcription and translation of early genes then take place. Some proteins are translocated into the nucleus where nucleocapsids and virions are assembled. These nucleocapsids bud through the basal membrane of the cell as BV. It is presumed that during this stage, they obtain an envelope containing GP64 protein which can recognize a target cell membrane. The GP64 protein is encoded by the *GP64* gene and it plays a role in the attachment of BVs to the cell membrane (Hefferon et al. 1999). These BVs

disperse in the haemolymph to infect other cells during the systemic phase of infection (Becnel and White 2007; Rohrmann 2019). Most BVs escape the midgut basal lamina to infect larval tissues (Clem and Passarelli 2013). These secondary infections by the BVs attack all cells in the larval body such as tracheal cells, haemolymph, body fat and epidermis cells. Through the process of receptor mediated-endocytosis, they enter the cells and replicate in the nucleus (D'Amico and Slavicek 2012; Khorramnejad et al. 2021). During the late stage of infection, nucleocapsids are enveloped to form ODVs, which will be occluded in the polyhedral crystalline matrix as OBs. These OBs accumulate in the nucleus (Sajap et al. 2000). As a result of the accumulation of viral proteins and virions in the nucleus and the nucleus becomes swollen. Later, the host cells lyse, the tissues liquefy and the cuticle weakens and ruptures (Monteiro et al. 2012). This liquification of the insect host will contaminate the foliage, and consequently continue the viral transmission cycle to other insect hosts (Harrison and Hoover 2012; Elderd 2013), which could induce a self-propagating and positive-feedback loop (Elderd 2013). In some cases where infected insects only showed symptoms in their midgut and fat body while other tissues remain uninfected (Senthil-Nathan 2015). Some insects showed sub-lethal infection in which they do not die following the ingestion of viral OBs (Williams et al. 2017).

During the process of infection, the integument of larvae changes color, and the development, feeding and mobility of larvae decrease (Harrison and Hoover 2012b).

Spodoptera litura larvae infected with *Spodoptera litura* nucleopolyhedrovirus showed characteristics of polyhedrosis symptoms (Nazli-Huda et al. 2012). The integument becomes flaccid and pink, and the infected larvae stop feeding after three days of post-inoculation (Javier-Hila and Caoili 2020). Dorsal, lateral, and the ventral sides of larvae changed to a pinkish color compared to those of healthy larvae after five days post-inoculation. The larvae ruptured after seven days post-inoculation with single dose of 2.4×10^7 OBs, releasing white body fluids filled with OBs (Nazli-Huda et al. 2012). Shortly before death, the infected larvae migrate to the upper part of the plant. They died by hanging themselves on pseudopods, a behaviour of the tree top disease induced by baculovirus (Goulson 1997). *Spodoptera exigua* larvae infected with baculoviruses died in the top 10% of the leaves of chili plants and closer to the stems of healthy conspecific species of the same stage (Rebolledo et al. 2015). This was clear evidence that baculovirus had induced climbing behaviour in the susceptible hosts. Recent research has revealed that the *ecdysteroid UDP-glucosyl transferase (egt)* gene of *Lymantria dispar* multiple nucleopolyhedrovirus had caused this behaviour in the larvae of the gypsy moth *L. dispar* (Hoover et al. 2011). On the other hand, *Trichoplusia ni* and *S. exigua* infected with a mutant AcMNPV (with a deletion of the *egt* gene) still exhibited tree-top disease after infection (Ros et al. 2015). The tree-top disease may not be solely induced by *egt* gene which may not be applicable for all baculovirus-host interactions.

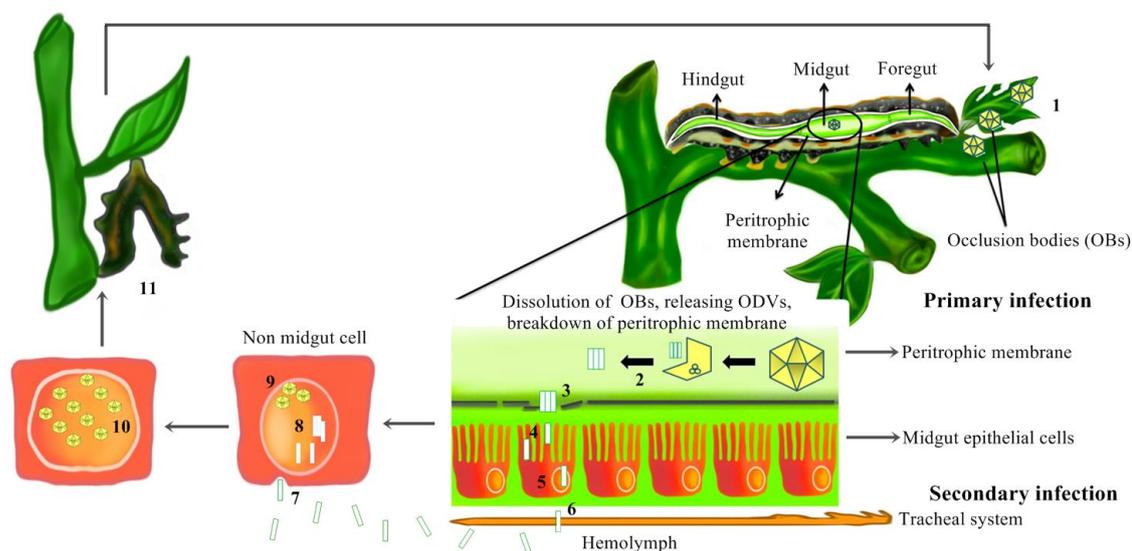


Figure 1. Replication and transmission of nucleopolyhedroviruses. (1) OBs are ingested by insects during the primary infection. (2) OBs are dissolved in the midgut of the insect, and (3) ODVs are released, passing through the peritrophic membrane and (4) fused with the microvilli of midgut epithelial cells to release the nucleocapsids. (5) The nucleocapsids enter into the nucleus and release the viral DNA, which allows replication to begin. Nucleocapsid progenies form in the nucleus, bud through the nuclear membrane and form budded viruses (BV). (6) During the secondary infection, these BVs disperse in the hemolymph and cause infection to other tissues. (7) Endocytosis allows BVs enter into the cells and replicate in the nucleus. (8) Newly formed nucleocapsids can either form BVs to infect other cells or (9) be enveloped to form ODVs that are occluded within OBs. (10) During late stage of infection, fully formed OBs are seen in the nucleus. (11) Upon death, the larvae hang on the uppermost leaves of the host plant and the integument ruptures, releasing the OBs into the environment. If other insects consume the contaminated leaves, the infection cycle will begin again

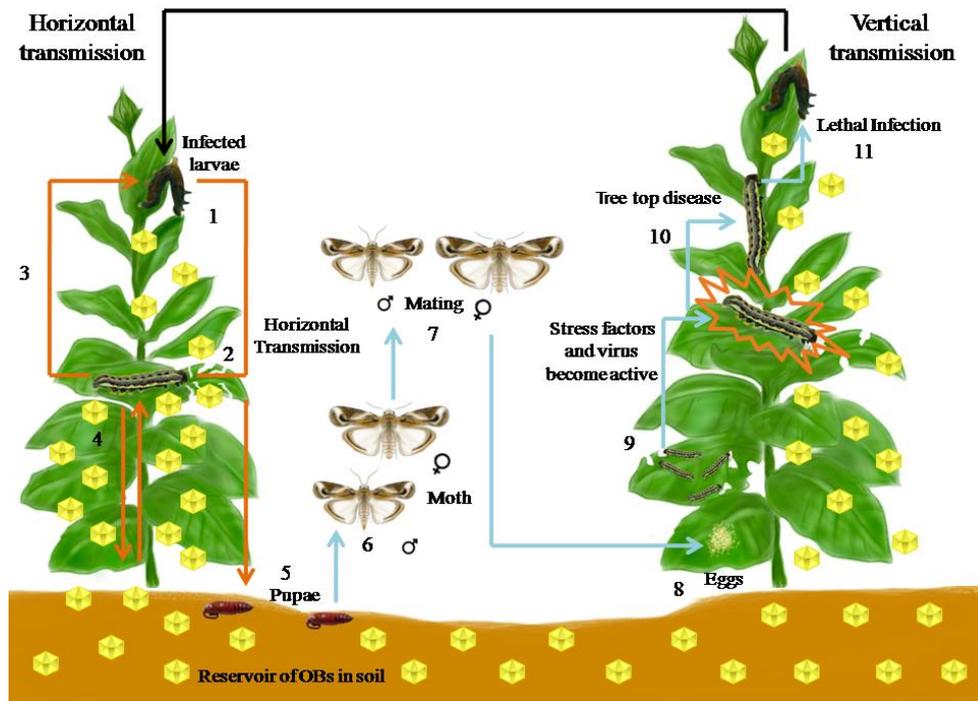


Figure 2. Horizontal and vertical transmission of baculoviruses (Adapted with permission from Williams et al. 2017). (1) After larvae ingest the OBs on the contaminated leaves, (2) some infected individuals develop a fatal condition and (3) release OBs on the host plant where they can be transmitted to other susceptible insects. (4) Rainfall will wash the OBs from the leaves into the soil, where they might be transferred back to plants via biotic and abiotic mechanisms. (5) The infected insects may be symptomless and continue to complete their lifecycle. (6) These adults might disperse, (7) mate and (8) lay eggs on other host plants. (9) The viruses are transmitted from the infected parents to their progenies. (10) Vertical transmission can be sustained for multiple generations until an elicitor or stressor transforms a latent infection into (11) a deadly disease, resuming the horizontal transmission cycle.

Transmission modes of baculoviruses

Insect baculoviruses have two types of transmission mode: horizontal and vertical (Figure 2). In horizontal transmission, viruses are transmitted from infected hosts to susceptible hosts, which are not in a parent-progeny relationship. During this stage of transmission, viruses sit and wait until they encounter a susceptible host and infect them. The viruses that retain virulence outside the host can infect a host of similar or next generation. In vertical transmission, the viruses are transmitted from the infected parents to their progenies. Vertical transmission is a mechanistic transmission of viruses across several generations (Williams et al. 2017). Infection of larvae with a viral infection does not always result in death. Some insects survive, reproduce and generate progenies. It has been suggested that the development of the disease can be overcome by metamorphosis (Burand and Park 1992; Kukan 1999).

UV radiation and its potential effect on baculovirus

Although the use of baculoviruses presents several advantages, they have their limitations in achieving their optimum goal of controlling pests in the field. Besides their limited host range and slow lethality, baculoviruses can be inactivated by UV radiation. The ultra-violet spectrum of solar radiation is the primary environmental parameter that

affects the field persistence of these viruses (Beas-Catena et al. 2014).

Solar radiation consists of ultraviolet or UV (200-390 nm), visible (390-780 nm) and infrared (more than 780 nm) radiation (El-Sharkawey et al. 2009). Around 56% of all electromagnetic radiation is infrared photons, 39% is visible light, and 5% is ultraviolet (UVA+UVB) radiation (290-400 nm) that reaches the Earth's surface (Lyons et al. 2020). The sun emits ultraviolet radiation in the form of UVA (320-390 nm), UVB (280-320 nm) and UVC (200-280 nm) bands (Hockberger 2002). UVC may be inhibited by the ozone layer however UVA and UVB reach the earth's surface after atmospheric filtration (Lubin and Jensen 1995). UVB rays inactivate insect pathogens and viruses under normal circumstances (Ignoffo et al. 1977; Asano 2005; Lasa et al. 2007). As obligate pathogens, baculoviruses have to remain viable outside their host to cause an effective infection inside their host (Redman et al. 2016). When directly exposing baculoviruses to sunlight in the field, the baculoviruses could lose their infectivity within 24 hours (Petrik et al. 2003). In general, direct exposure to solar radiation may impair the viability and infectivity of microorganisms owing to DNA denaturation and the synthesis of extremely reactive oxygen radicals (Tamez-Guerra et al. 2005). Solar radiation, particularly ultraviolet (UV) rays (300-400 nm), induces mutagenic and fatal alterations in nucleic acids (Bull et al. 1976). In

prokaryotic and eukaryotic microorganisms, ultraviolet radiation has become the primary genotoxic agent (Douki et al. 2017).

Single-stranded DNA viruses are more prone to UV radiation compared to dsDNA viruses (Gerba et al. 2002). Owing to the large size of nucleic acid and the structure of the capsid, dsDNA viruses are more resistant to UV inactivation. In addition, RNA viruses are more vulnerable to UV exposure because they have no genome repair systems (Wigginton et al. 2012; Elveborg et al. 2022). In contrast to DNA-based life forms, the majority of RNA viruses lack systems for detecting and repairing mismatches, therefore, the polymerase error was not corrected (Steinhauer et al. 1992; Elveborg et al. 2022). If a virus is inactivated by UV radiation, it will lose its biological activity. In many cases, pathogens will lose their efficacy in a few days after exposure to UVB (Martin and Domek 2002). Hence, the application of baculoviruses on foliage is hindered by UVB (Killick 1990).

UV radiation disrupts normal processes and leads to the formation of dimers. UV-induced dipyrimidine photoproducts such as cyclobutene pyrimidine dimers (CPDs), pyrimidine-pyrimidone 6-4 photoproducts (64PPs) (Mouret et al. 2006) and Dewar Valence isomers will be induced after exposure to UV radiation (Douki et al. 2017). CPDs occur from the [2+2] cycloaddition reaction between the C5-C6 double bonds of two adjacent pyrimidine bases, thymine-thymine (TT) or cytosine-cytosine (CC); however, the TT photoproduct that is a well-known thymine dimer is the hallmark of UV-induced DNA damage (Douki et al. 2017). Meanwhile, the 64PPs are generated from a single covalent bond formed between the 5' end of C6 and 3' end of C4 of adjacent pyrimidines (Rastogi et al. 2010). The dewar pyrimidinone is formed by a reversible isomerization of the 64PP upon further exposure

to UVA wavelengths around 325 nm (Matsunaga et al. 1991). Predominantly, DNA damage occurs in the bi-pyrimidine sequences upon excitation of thymine, the most photoreactive base, or, to a lesser extent, of cytosine (Cadet et al. 2014). CPDs, 64PPs and Dewar Valence isomers block the replicative DNA polymerases from passing through them when they reside on a template strand, and the transcription will be terminated (Ikehata and Ono 2011). Although DNA has a repair mechanism called nucleotide excision repair (NER), double-stranded DNA can be degraded due to failure in the repair (Ikehata and Ono 2011). This can result in death of the microorganisms.

DNA sensitivity to UV radiation depends on the purine and pyrimidine composition (Smith 1966). Due to the presence of a double-ring structure in purine, their resistance towards photoreaction increases ten folds in comparison with pyrimidine (Smith and Hanawalt 2013). Hence, more pyrimidine dimers are formed. The possibility of photodimerization is relative to the occurrence of pyrimidine sequences in the neighbourhood (Kleinermanns et al. 2013). Moreover, suitable orientation and positioning of the pyrimidine bases may result in dimerization. The precise order of adjoining base pairs and frequent occurrence of thymine determines the sensitivity of the virus to UV radiation (Figure 3). The precise mechanism of the inactivation of the virus due to UV radiation is still not completely understood. However, the hydrogen peroxides from one or more amino acids produced by UV radiation can reduce the vitality of baculovirus (Arora and Shera 2014). In viruses containing the dsRNA genome, UV radiation may result in the accumulation of uracil dimers (Williams 2017). UV radiation damages the viral genomes and capsid proteins (Eischeid and Linden 2011; Sigstam et al. 2013).

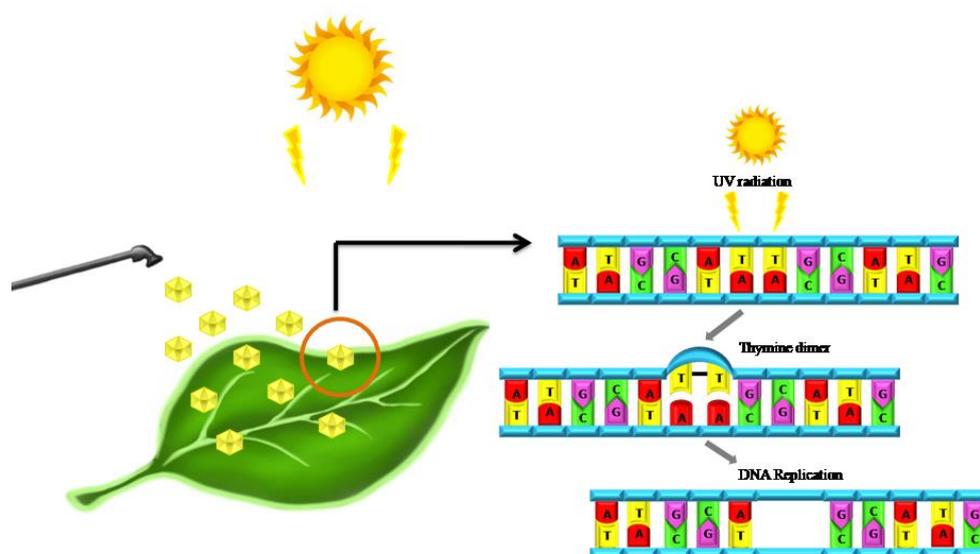


Figure 3. UV radiation damages dsDNA of baculovirus by forming thymine dimer which caused failure in DNA replication

Radiation at higher wavelengths and higher energy, such as UVA rays and visible light, can cause the inactivation of the virus if exposed for a longer period (Martin and Domek 2002). This exposure period is high in tropical regions and at higher altitudes, whereas it decreases with an increase in latitude. In temperate regions, there are variations in UV light due to cloud cover and precipitation (Williams 2017). The amount of shade provided by upper layers of plant parts or adjacent plants could reduce the exposure of OBs to solar UV rays (Maramorosch 2012). OBs present at the center part or bottom part of the plant will obtain a lesser amount of ultraviolet radiation and are likely to maintain integrity for longer than those present at the upper part. For example, *Heliothis* nuclear polyhedrosis virus was found to be rapidly inactivated on the upper leaf surface of cotton, soybean, and tomato when grown in field condition (Young and Yearian 1974). In addition, bioassay infection of *Panolis flammea* at 3 positions on the lodgepole pine tree: the upper, middle, and lower trees that were sprayed using purified *P. flammea* NPV; the results showed that the infected larvae were higher in the lower position of the tree with treatment (Killick and Warden 1991).

Methods to prevent UV inactivation of baculovirus

If baculoviruses are inactivated by UV radiation, their usage in the field will be hampered. Therefore, methods to enhance/prolong the persistence of the virus are required for effective utilization of this biocontrol agent against target pests.

Use of UV protectants

Several researchers have conducted studies to improve the effectiveness of entomopathogenic viruses and prevent them from UV inactivation. UV protectants used to protect the virus include dyes, optical brighteners, lignin derivatives or natural ingredients (Table 1). Oxidative enzymes such as dilodin, riboflavin, folic acids, inols, pyridoxine and vitamins were also claimed to be potential agents to protect entomopathogenic viruses from UV radiations (El-Helaly 2013). Several natural plant products have antioxidative potential to provide capacity to counteract UV-induced adverse effects on human skin (Dunaway et al. 2018). The composition of the customary sunscreens contain inorganic compounds, such as titanium dioxide (TiO₂), zinc oxide (ZnO) and minerals, which help to reflect and scatter UVA and UVB. Meanwhile, the mode of action of ZnO and TiO₂ is to absorb UV radiation (Cole et al. 2016). TiO₂ is more effective for UVB protection while ZnO is effective for UVA protection. The combination of these particles could enhance UV protection (Smijs and Pavel 2011). ZnO and TiO₂ are good UV protectants for *Cydia pomonella* GV isolate ZY (CpGV-ZY) (Wu et al. 2015). A recent study claimed that a novel formulation technology by encapsulating baculovirus in an ENTOSTAT-TiO₂ waxy coat has effectively safeguarded the efficacy of the sensitive viral DNA from damage by the UV radiation in the sunlight, and at the same time not phytotoxic (Wilson et al. 2020). However, the use of such metal-based particles for protecting human

skin has raised concern regarding their safety and potential toxicity.

Tinopal has been used as a UV protectant in the formulation of baculovirus-based biopesticide. It is an optical brightener derived from the stilbene compounds (Goulson et al. 2000). These compounds can absorb UV rays and reflect the light in the visible range (Martínez et al. 2009). The unformulated and formulated *Spodoptera frugiperda* NPV with Tinopal was examined. The research findings revealed that SfMNPV alone was more sensitive to UV than in mixture with 1.25 or 0.1% Tinopal C1101 (Mondragon et al. 2007). Tinopal could retain the efficacy of *S. frugiperda* NPV even after 4 hours of UV exposure (Mondragon et al. 2007). In the field studies conducted in Mexico and the United Kingdom on three distinct flower species, 0.1% or 1% concentrations of Tinopal CBS had significantly inhibited bees to flowers. A total of 64.6% rejection rate recorded on the flowers treated with Tinopal CBS compared to 5.1% on the control flowers (Goulson et al. 2000). Therefore, Tinopal is able to protect the baculovirus from UV radiation as well as to inhibit insects to the plants.

Some researchers have used natural compounds derived from leaves and fruits (referred to as herbal cosmetics) to protect entomopathogenic viruses from UV radiation (Table 1). Flavonoids are the most studied sunlight protectant which includes quercetin (3,4-dihydroxyflavonol) (Cefali et al. 2016). This compound is present in fruits (strawberries, grapefruits and apples), vegetables and beverages (tea and red wine) (Lakhanpal and Rai 2007). Quercetin has functioned as an anti-inflammatory, antioxidant, immunomodulator (Korać and Khambholja 2011), inhibits skin tumour formation (Nishino et al. 1984) and has a potential topical sunscreen (Saewan and Jimtaisong 2013). Due to the presence of aromatic rings in their molecular structure, flavonoids absorb UV rays between 200 and 400 nm, making them appropriate for use as a sunscreen agent (Cefali et al. 2016).

The use of inexpensive natural additives such as moringa, rice bran, cacao and green tea to sustain the effectiveness of *Spodoptera littoralis* multiple nucleopolyhedrovirus (*SpliMNPV*) against UV lights was analysed (El-Helaly 2013). Moringa exhibited the highest protection rate compared to rice bran, cacao and green tea. The moringa additive at 1% concentration could retain the activity of *SpliMNPV* (mortality of 93.24%) even after exposure to UV radiation. This could be due to the natural occurrence of high antioxidant content and anti-inflammatory compounds in moringa (Yong-Bing et al. 2019). Tea (polyphenol) has photo protective effect on human skin, reducing the number of sunburned cells and alleviating DNA damage (Li et al. 2014). Among the tea tested against the beet armyworm NPV (HaNPV), black tea was the best UV protectant with the highest larval mortality recorded at 90%. Black tea and lignin showed good result in protecting *Spodoptera exigua* MNPV (Salamouny et al. 2009). The combination of both black tea and lignin as UV protectant in biopesticides is safe and can be obtained easily. Plant phenolics such as caffeic acid, gallic acid and

chlorogenic acid were able to protect SeMNPV at concentration as low as 0.5 mM. The addition of a UV protectant in the baculovirus formulation has been proven effective in improving viral stability either in the laboratory or in field application. UV protectants derived from natural ingredients will be a great candidate in the biopesticide industry. However, in cases where chemicals are used as UV protectants in the viral formulation, its concentration must be kept as minimal as possible. It is important to study the harmful effect, if any, of chemicals used as UV protectant.

Selection of insect baculovirus resistant to UV irradiation

Insect baculoviruses with resistant capability to UV irradiation and maximal vertical transmission rate were selected. One strain of GV from a codling moth (CpGV) exhibited resistance to UV radiation under natural sunlight and in laboratory conditions (Brassel and Benz 1979). The resistance capacity of the altered strain was increased 5.6-fold compared to the original isolate, and its infectivity period was double that of the original isolate, i.e. it could stay for double the period in comparison with the original isolate (Brassel and Benz 1979). Witt and Stairs (1975) demonstrated that within a population of *Galleria mellonella* NPV (GmNPV) active against *Galleria mellonella*, one subpopulation was susceptible to low UV doses, while another subpopulation was susceptible only to high UV doses, equating to a nearly 1,000-fold difference in UV susceptibility. They hypothesised that this variety in UV reaction could be due to genetic variability and that it would be able to select UV-tolerant virus strains. Akhanaev et al. (2017) measured the relative rate of inactivation and the half-life of the virus to assess the UV tolerance of two LdMNPV strains from Western Siberia (LdMNPV-27/0) and North America (LdMNPV-45/0). The North American strain, previously demonstrated to be more virulent against *Lymantria dispar* larvae, was more sensitive to UV after 15 minutes of exposure to sunlight and lost its potency more quickly than the Siberian strain. Therefore, it is not recommended to utilise LdMNPV-45/0 in locations with high UV loads or at densities of forest stands. Another study reported the use of *Cryptophlebia leucotreta* granulovirus-SA (CrleGV-SA) as a commercial biopesticide against the false codling moth, *Thaumatotibia leucotreta*, in citrus and other crops (Mwanza et al. 2022). This virus strain was isolated from infected *T. leucotreta* larvae and subjected to UV irradiation in a climate chamber with simulating field settings under laboratory condition. This UV-resistant CrleGV-SA strain was thousand-fold more virulence than the wild-type isolate (Mwanza et al. 2022). Single Nucleotide Polymorphisms (SNPs) was

discovered in the 5th generation of UV-resistant CrleGV-SA which is believed to have improved the UV tolerance and pathogenicity of the virus.

Enhancing photostability of baculoviruses by genetic engineering

Isolation, insertion and expression of genes responsible for the DNA repair system in the genome of baculovirus will result in the production of UV-resistant baculovirus (Zhao et al. 2003; Ferrelli et al. 2018). Genetic modification research for improving the baculovirus resistance to UV inactivation is very much lacking. Petrik et al. (2003) had constructed a recombinant *Autographa californica* M nucleopolyhedrovirus (AcMNPV) that encoded cv-PDG, an algal virus-specific pyrimidine dimer glycosylase. The expression of cv-PDG resulted in a threefold reduction in the UV inactivation of budded virus compared to the wild type AcMNPV in plaque test using *Spodoptera frugiperda* Sf21 cells. However, the occluded viruses expressing cv-PDG did not become more resistant to UV inactivation than the wild-type AcMNPV when fed to *S. frugiperda* or *Trichoplusia ni* neonates. Several explanations for the lack of protection against UV inactivation in occluded virus were explained by Petrik et al. (2003). They assumed that the cv-PDG expression occurred in the midgut epithelial cells while the DNA repair pathways required to complete the repair process by cv-PDG were not operational in the midgut epithelial cells, therefore there was insufficient time for the viral DNA to be repaired. The insects periodically shed their midgut lining during moulting process into next instars; therefore the recombinant viruses were unable to initiate the replication in the midgut cells.

Baculoviruses as bioinsecticides in Asian countries

The effectiveness of baculoviruses as a bioinsecticide has been tested in several countries in Asia (Table 2). Research on the efficacy of wild baculoviruses is fundamental to finding out how effective the wild baculoviruses are and how they react to other active ingredients of bioinsecticides, such as bacteria or botanical pesticides, and synthetic pesticides when used in a combined application (Samada and Tambunan 2020; Landwehr 2021). The difference in the effectiveness of imported isolates and local isolates has been studied to ensure the efficacy and stability of both isolates for use in the local area (Akhanaev et al. 2017). It has been widely shown that the indigenous natural resources have provided a good control of insect pests (Grzywacz et al. 2014; Rathore et al. 2021).

Table 1. Research related to UV protectants used to prevent inactivation of baculoviruses during the past 10 years

Insect target	Virus	UV protectants (plant-based ingredients)	UV experiment	Result	Reference
<i>Spodoptera littoralis</i>	<i>SpliMNPV</i>	Orange oil 5% Tea tree oil 5% Marjoram 5%	UV lamp (Utalix; 23 sun UV, Germany)	Orange oil gave the best protection to the virus.	Sayed et al. (2020)
<i>Helicoverpa armigera</i>	HaNPV	Black tea Green tea Pepper Green cabbage Red cabbage Mango leaves Guava leaves Basil Dill Peppermint	Bacterial lamp 312 nm (Phillips TUV 30 W)	Black tea, dill, green cabbage, pepper provided the highest level of protection.	Ibrahim et al. (2019)
<i>Spodoptera littoralis</i>	<i>SpliNPV</i>	Moringa Cacao Green tea	Field experiment	Moringa could prolong the activity of the virus under field conditions.	El-Helaly (2020)
<i>Spodoptera exigua</i> First and second instar	<i>SpliMNPV</i>	Henna Green tea leave Clove flower	Field experiment	The addition of clove extract as a natural UV protectant had provided higher persistence of virus in the field.	Sukirno et al. (2018)
<i>Diaphania pulverulentalis</i> (Hampson)	DpNPV	DpNPV formulation (10% starch + 0.2% Tinopal + 1% Tween 80 + DpNPV)	UV lamp & sunlight	Tinopal showed significant protection to DpNPV when exposed to UV lamps and sunlight.	Prabhu and Mahalingam (2017)
<i>Spodoptera littoralis</i>	<i>SpliMNPV</i>	Henna whole Dates fruit (var. 'Sukari') without seed Black grape whole fruit Kiwi whole fruit, Olive fruit without seed Lemon whole fruit Pomegranate whole fruit without skin Red beetroot whole tuber Green tea whole leaves Clove whole flower	Under laboratory condition (UVB lamps 302 nm (FotodyneInc, New Berlin, WI, Germany) Field trials	Cloves, henna, green tea, pomegranate, and grape extracts exhibited a higher rate of virus protection compared to unprotected virus. In field conditions, clove and henna-based formulations had increased the persistence of the virus.	Sutanto et al. (2017)
<i>Spodoptera littoralis</i> neonate larvae	<i>SpliMNPV</i>	Cacao (5% and 10%) Green coffee (5% and 10%) Fresh green cabbage (5% and 10%) Fresh red cabbage (5% and 10%)	Natural sunlight in field	Cacao was the best protective additive.	El-Helaly et al. (2013)

<i>Spodoptera littoralis</i>	SpliNPV	Moringa Rice bran Cacao Green coffee	UV lamps with an absorbance under 300 nm	Moringa was the best candidate.	El-Helaly (2013)
<i>Spodoptera exigua</i>	SeNPV	Dust formulation: Husk charcoal Coconut shell charcoal Soot Powder Yam flour Liquid formula: Molasses Green tea filtrate Turmeric filtrate Yam filtrate	Sunlight (3000-4000 uW/cm ²)	The best dust formulations: coconut shell charcoal soot husk charcoal The best liquid formulations: molasses, turmeric filtrate yam filtrate	Samsudin et al. (2011)
<i>Chrysodeixis chalcites</i> (Esper)	ChchNPV-TF1	The photoprotective substances were divided into three groups: low protection (0-8%; kaolinite), intermediate protection (48-62%; green tea, moringa, bentonite and cacao) and high protection (87-100%; charcoal, iron oxide)	under laboratory conditions using a Crosslinker as UV light source at 200 J cm ⁻² field conditions	1% cacao and 1% charcoal were the best result for laboratory conditions. 1% charcoal treatment exhibited the highest protection.	Cakmak et al. (2021)
<i>Spodoptera littoralis</i> (neonate larvae on squash plants)	SpliMNPV	Nano aluminium oxide Nano zinc oxide Cacao (positive control)	Simulated UV light A set of four UV lamps	Nano zinc oxide was the best formulation.	El-Helaly and Sayed (2015)
<i>Helicoverpa armigera</i> third instar	HaNPV	Microencapsulations using Gelatin 3% and 5% Sodium alginate 3% and 5% Starch 3% and 5%	385 nm UVA 254 UVC With Entela lamp model UVGL 25 of 4 W	Gelatin was the best and the most stable formulation.	Gifani et al. (2015)
<i>Cydia pomonella</i> L	CpGV	ZnO (Hengxing [®] zinc oxide) 20 mg mL ⁻¹ TiO (Dengfeng [®] titanium dioxide) 20 mg mL ⁻¹	UVB light (20W) UV lamps model K5 B20H	ZnO and TiO ₂ were good UV protectants to CpGV-ZY isolate.	Wu et al. (2015)
<i>Pieris brassicae</i>	PbGV	Iron oxide (black) 1% and 2%) Robin blue (1% and 2%) Tinopal (1% and 2%) Congo (1% and 2%)	1. UV lamps (15 W Philips G15T8) 2. Field study	Congo red was more effective. In field conditions, Congo red and robin blue gave the best protection to virus.	Sood et al. (2013)
<i>Spodoptera litura</i>	SINPV	a) Sodium 2,5-bis(4-(2,4-disulfonate) phenyl)-1,3,4-oxadiazole with solvent: (1) DMF, (2) Acetone, (3) THF b) Sodium 2,5-bis(4-(2-sulfonate) phenyl)-1,3,4-oxadiazole with solvent: (1) DMF, (2) Acetone, (3) THF	a) 1. DMF 364 nm 2. Acetone 348 nm 3. THF 349 nm b) 1. DMF 348 nm 2. Acetone 344 nm 3. THF 346 nm	Sodium 2,5-bis(4-(2-sulfonate) phenyl)-1,3,4-oxadiazole compound showed greater protection to SINPV.	Zhu et al. (2013)
<i>Spodoptera frugiperda</i>	SfMNPV	Lignin encapsulated (sodium lignin, calcium chloride dehydrates)	1. Laboratory assay using a Suntest CPS (Hereus, Germany) 2. Field application	The spray-dried lignin encapsulated formulation protected virus.	Behle and Popham (2012)
<i>Spodoptera littoralis</i>	SINPV	Starch, clay, glycerine, extract of black tea	Natural light in field application	Clay and black tea were the best UV protectants.	El-Husseini et al. (2012)

Table 2. Efficacy test of baculoviruses as bioinsecticides in Asian countries during the past 10 years

Country	Host	Virus	Efficacy	References
Russia	<i>Lymantria dispar</i>	<i>LdMNPV</i> -27/0 isolated from Western Siberia, Russia <i>LdMNPV</i> -45/0 isolated from North America	The strain isolated in North America (<i>LdMNPV</i> -45/0) diminished its potency significantly and more rapidly than the strain isolated in Western Siberia (<i>LdMNPV</i> -27/0) when exposed to sunlight.	Akhanaev et al. (2017)
Turkey	<i>Malacosoma americanum</i> (F) <i>Malacosoma neustria</i>	<i>Malacosoma neustria</i> NPV Turkish isolate 3	The virulence of ManeNPV-T3 was determined under laboratory conditions using third instar <i>M. americanum</i> and <i>M. neustria</i> larvae, with 0.6×10^5 occlusion bodies per larva inducing 100% mortality after 10 days.	Demir et al. (2014)
Japan	<i>Adoxophyes honmai</i> (Lepidoptera: Tortricidae)	<i>A. orana</i> (AdorNPV) <i>A. honmai</i> (AdhoNPV)	AdhoNPV had a lower field effectiveness in reducing damage to tea plants caused by <i>A. honmai</i> larvae than the fast-killing AdorNPV. AdhoNPV transmitted horizontally at a substantially greater rate than AdorNPV.	Takahashi et al. (2015)
India	<i>Spilosoma obliqua</i>	<i>Spilosoma obliqua</i> MNPV	SpobMNPV recorded LC_{50} of 4.46×10^5 POBs mL^{-1} and ST_{50} at 72 h, respectively, against 3rd instar <i>S. obliqua</i> larvae.	Sujayanand et al. (2019)
Mongolia	<i>Lymantria dispar asiatica</i> Vnukovskij	LdMNPV-H from Heilongjiang Province, China LdMNPV-J from Japan LdMNPV-D from commercial product	LdMNPV-D, a commercial strain accessible from North America, was moderately pathogenic, while LdMNPV-J, a Japanese isolate, was the least harmful. The dose-response slope for the three virus isolates confirmed that the Chinese gypsy moth larvae were more homogeneous susceptible to LdMNPV-H and LdMNPV-D than to LdMNPV-J.	Duan et al. (2012)
Pakistan	<i>Spodoptera litura</i>	SpltNPV-Pak-BNG isolate Pakistan	The dose-to-death response and mortality rate of various instar larvae of <i>S. litura</i> were determined. Second and third instar larvae exhibited similar dose-response to mortality, whereas 4th instar larvae were less susceptible. The mean survival time followed a similar trend to the mean death time values and was identical for 2nd and 3rd instar larvae but was significantly prolonged for 4th instar larvae.	Ali et al. (2018)
Pakistan	<i>Spodoptera litura</i> F	<i>S. litura</i> NPV (V-SpltNPV) was isolated from infected larvae on a cotton crop	Spinosad combined with V-SpltNPV (1×10^8 OBs mL^{-1}) caused 100% mortality of 2nd instar larvae in a greenhouse experiment. A single application of V-SpltNPV (1×10^8 OBs mL^{-1}) had resulted in 52.63% of examined larvae.	Ayyub et al. (2019)
Pakistan	<i>Spodoptera litura</i> F	Nuclear polyhedrosis virus (NPV) and flubendiamide	For pupation and adult emergence of <i>S. litura</i> , combining NPV and flubendiamide showed to be more lethal at higher concentrations than applying them separately at lower concentrations.	Maqsood et al. (2017)
Pakistan	<i>Plutella xylostella</i>	<i>Plutella xylostella</i> granulovirus (PxGV) and <i>Azadirachta indica</i> (AZA)	Sublethal dose rate of PxGV and AZA mixture under laboratory condition caused higher larval mortality of <i>P. xylostella</i> .	Malik et al. (2020)
Pakistan	<i>Helicoverpa armigera</i> (Hubner)	Combination NPV and 0.1% spinosad	<i>H. armigera</i> was given various concentrations of NPV and spinosad individually, as well as a combination of NPV and 0.1% Spinosad. These results showed that effectiveness and tenacity of mixture caused significant mortality of larvae.	Nawaz et al. (2019)
Indonesia	<i>Spodoptera litura</i>	<i>Spodoptera litura</i> NPV (SINPV)	The investigation demonstrated that the interaction between the SINPV isolate and the virus concentration had a substantial effect on the feeding, mortality, LC_{50} and LT_{50} . Additionally, these potential characteristics varied across the <i>S. litura</i> larval instars. SINPV JTM 97c and SINPV JTM 02-5 was potential in controlling <i>S. litura</i> on soybean.	Bedjo (2017)
Indonesia	<i>Spodoptera exigua</i> Hubn	<i>Spodoptera exigua</i> NPV (SeNPV)	Within five days of infection, SeNPV killed 77.5% larvae in a laboratory trial. Additionally, SeNPV decreased the feeding capability of <i>S. exigua</i> by 0.43 g per day. Additionally, SeNPV reduced pupae weight by 0.063 g. In a greenhouse experiment, the application of SeNPV resulted in 100% larval mortality and a 10.43% reduction in the intensity of shallot damage caused by <i>S. exigua</i> .	Supyani et al. (2014)
Philippines	<i>Spodoptera litura</i> F	<i>Spodoptera picta</i> NPV (SppiNPV)	The SppiNPV produced signs and symptoms of baculoviral infection in <i>S. litura</i> larvae, indicating SppiNPV could cross-infect the <i>S. litura</i> larvae.	Javier-Hila and Caoili (2020)

Synchronisation of viral bioinsecticide application and bioecology of target insect

Knowledge of the insect pest behaviour and biology involved are prerequisites for successful bioinsecticide application. Insect behaviour encompasses a vast range of behaviours, such as movement, grooming, feeding, communication, reproduction, dispersal, flight, learning, migration, host or prey selection, diapause, and numerous reactions to environmental threats like temperature, humidity, parasites, and toxins (Hoy 2019). Understanding the behaviour of insect pest could improve pest management programs (Campbell 2006). Insect life cycles vary depending on the species. The complete and incomplete metamorphosis are the two most typical types. The life cycle is essential for controlling insects because, depending on the type of life cycle, an insect's habitat, behaviours, and appearance can change significantly over the course of its lifetime (Wilbur 1980).

For example, the newly hatched larvae of the tobacco armyworm, *Spodoptera litura* scrape the chlorophyll content and feed voraciously (Krisnawati et al. 2021). This will cause the leaf to appear yellowish-white. As the caterpillar grows, it consumes the leaves entirely, leaving behind only the stalks and midribs. Additionally, larval feeding becomes more aggressive as the larvae mature. The larvae eat in fragile pods initially, then burrow through the thick pod and consume the grains (Sunil et al. 2019). During the daytime, the larvae hide under the plants, cracks and crevices of soil debris, and during the night time, they feed on the plants (Natarikar and Balikai 2017). The intensity of the sun is low in the evening, and nocturnal insects become active at night. Since *S. litura* activity was observed in the dark, the application of the virus can be made late in the afternoon, thereby protecting the viral bioinsecticide from UV rays. According to Dezianian et al. (2010), younger larvae of *Plutella xylostella* L. were more susceptible to PxGV; however, the older larvae were less tolerant. Thus, the application of PxGV should be targeted at the early stages of larval development to obtain full control of the insect hosts.

Attention must be paid to the local climate as well when applying baculoviruses to the field. Indonesia has a tropical climate with two seasons throughout the year. In 2017, the average solar exposure was 4.1 to 8 hours per day. Less than 10% of days in the year 2017 achieved 12 hours of solar exposure per day (Hamdi 2019). Incoming solar radiation depends on many factors like altitude and latitude, aerosols, season, and cloud cover (Hillel et al. 2008). It is known that the average sunlight decreases sharply according to the height of the place. Moreover, solar UV radiation increased with altitude, which was mainly due to a decrease in the number of air molecules, ozone, aerosols and clouds in the atmosphere, in addition to surfaces being covered with snow (Gröbner 2013). The altitude effect plays an important role in the understanding of the UV radiation field in mountainous terrain (Schmucki and Philipona 2002).

Baculoviruses have polyhedral occlusive bodies (OBs) that shield the viruses from harsh environmental conditions

(Susurluk et al. 2013). Formulation of baculoviruses should focus on whether the carrier could affect the efficacy of OBs. Detergents such as sodium dodecyl sulphate (SDS), sodium deoxycholate and Triton X were often utilised in laboratory studies to extract OBs from the dead baculovirus-infected larvae or cells (Kato et al. 2011), and SDS could affect the efficacy of the baculoviruses (Nazli-Huda et al. 2012). As a result, the composition of bioinsecticides should be free of detergents. Knowledge of formulation is particularly essential to learn how to improve the stability of bioinsecticides before they are deployed in the field; also, the lifespan of microbial insecticides must be evaluated before commercialization (Ramanujam et al. 2014).

The knowledge regarding the synchronization between the target insect bioecology, virus characteristics and the weather or climate of the agroecosystem is essential for the effective use of baculoviruses to control the susceptible hosts in the field. In addition, the application time is crucial for successful insect control. The life cycle of an insect host varies in different agroecological zones. Therefore, knowledge of the life cycle of an insect host should be part of the training program for farmers using biopesticides.

In conclusion, baculoviruses have great potential to be used as bioinsecticides. The vulnerability of baculoviruses to UV radiation could be decreased through the use of various types of protective agents. Natural ingredients must be considered as the primary option for UV protectors since the purpose of using bioinsecticides is to produce green agricultural produce and products for the consumer, and the natural ingredients must be cheap and easy to get. If chemical UV protectants are used in the viral formulations, the quantity of the chemicals in the formulation must follow the safety standards and guidelines, so that the formulations are safe for the environment and non-target organisms. Research on the recombinant UV-resistant baculoviruses is still limited. The virulence and photostability of the wild-type viruses are the important criteria for selection. The target of insects, habitat, indigenous virus used, and time of applications of bioinsecticides are also crucial as these factors will affect the efficacy of the viral bioinsecticides in the field. Quality assurance is important in the production of baculoviruses for bioinsecticide industry as to ensure a sustainable production of functional virus product for insect pest control.

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