*Review Article*

Journal of Microbiology and Related Research
Volume 8 Number 1, January - June 2022
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.8122.3

# Micro Encapsulation for Ultraviolet Protection and Activity Enhancement of Baculoviruses

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**How to cite this article:**

Rakesh Kumar Gupta,Kamlesh Bali, Mudasir Gani/Micro Encapsulation for Ultraviolet Protection and Activity Enhancement of Baculoviruses/J Microbiol Relat Res. 2022;8(1): 19–22.

**Abstract**

Baculoviruses are dsDNA viruses and universally used biological control agents in the integrated pest management programmes. The foremost drawback of these viruses is their sensitivity towards ultraviolet radiation. Different materials have been tested by different workers to influence the sunlight protectant activity. Microencapsulation is currently an extensively studied approach in protecting baculoviruses from UV light as well as enhances the activity, efficiency and performance of baculoviruses formulations. Findings suggest that the microencapsulation technique could reduce the cost and enhance the efficacy of baculoviruses. On the other hand, it can also reduce the use of agrochemicals and their residues in the environment and food materials.

KEYWORDS: Microencapsulation; baculovirus; biological control.

## INTRODUCTION

Biological control of agricultural pests has gained importance in recent years due to increased pressure to reduce the use of agrochemicals and their residues in the environment and food. Over the past two decades, baculoviruses have been commercialized for the control of codling moth (*Cydia pomonella*), gypsy moth (*Lymantria dispar*), corn earworm (*Helicoverpa zea*), tobacco budworm (*Heliothis virescens*), beet armyworm (*Spodoptera exigua*), and the cabbage looper (*Trichoplusia ni*) in the United States; the rhinoceros beetle (*Oryctes rhinoceras*) in the Pacific; the velvetbean caterpillar (*Antacarsia gemmatalis*) in Brazil. In addition, large scale control programs for periodic forest pests, such as the Douglas-Fir tussock moth (*Orgyia pseudotsugata*), Eastern Spruce Budworm (*Choristoneura fumiferana*), and European pine sawfly (*Neodiprion sertzfer*), have been conducted in countries worldwide, including Canada, the United States, the United Kingdom, Finland, Norway, Sweden, Austria, Italy, Poland, and the Soviet Republic (former USSR). Although insect viruses are highly effective against their target insects, until today, baculovirus insecticides and their application as bio-insecticides was limited to control pest insects worldwide as the performance of these viruses are susceptibility to ultraviolet (UV) radiation. For commercialization, formulations that provide protection from sunlight and rainfall can significantly improve the residual activity of baculoviruses. Following application to plant surfaces, baculovirus occlusion bodies (OBs) are rapidly inactivated by solar ultraviolet (UV) radiation, particularly in the UV-B range of 280–320 nm. This radiation directly affects the nucleic

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**Received on:** 22.04.2022

**Accepted on:** 25.05.2022

acids, modifying or denaturing them, preventing virus infection and replication. Formulation of insect pathogens is recognized as one of the most important priorities in biopesticide research, especially for developing countries (Harris and Dent, 2000). It is well established that the efficacy of entomopathogens that act by ingestion can be improved by the use of formulations that include feeding stimulants which increase the consumption of the pathogen resulting in enhanced prevalence of disease and improved pest control. In this respect, the use of flour and starch-based granular formulations has been the subject of recent interest for the delivery of *Bacillus thuringiensis* and nucleopolyhedro viruses to noctuid pests.

## CASE REPORT

Certain formulations can improve the degree of pest control through the use of baits or feeding stimulants that enhance the pest's consumption of viral inoculum while simultaneously protecting. Commonly used unpurifed aqueous preparations typically have high levels of bacterial contaminants, often making them unpleasant to handle, as well as potentially hazardous to human health (Grzywacz *et al*., 1997), yet purification of baculoviruses prior to formulation is expensive, and leads to large losses of polyhedra (Guillon, 1997). Recent studies have indicated that phagostimulant maize flour-based formulations of *Bacillus thuringiensis* Berliner and nucelopolyhedrovirus resulted in improved control of various species of noctuid pests (Morales Ramos *et al*., 1998, Tamez-Guerra *et al*., 1998). Moreover, the maize flour formulation o-ered increased protection from UV radiation and improved the rainfastness of the inoculum during simulated rainfall (Tamez-Guerra *et al*., 2000 a, b). The use of granular feeding stimulants has a number of advantages that may compensate for the increased formulation cost. These include the ability to use a lower quantity of pathogen inoculum to achieve satisfactory pest control (Bartelt *et al*., 1990) and the ability to target applications precisely to the feeding site of the pest (e.g., application of granules directly to the maize leaf whorl) (McGuire *et al*., 1994). Moreover, the opacity of granules may protect the pathogen from UV degradation (Bartelt et al., 1990) and may improve the rainfastness of the application (McGuire *et al*., 1996; Tamez-Guerra *et al*., 2000 a, b). Past attempts have been made to provide formulations wherein an insecticidal virus and a sunscreening agent are maintained in close contact after dispersion of the formulation onto vegetation. However, when formulations containing both virus and sunscreening agent were dispersed in the field, the sunscreening agent was no longer in close enough contact to the virus to be effective at reducing the exposure of the viruses to the damaging effects of ultraviolet light. Therefore, microencapsulation - a process in which tiny particles or droplets are surrounded by a coating to give small capsules of many useful properties has been employed. Spray drying serves as a microencapsulation technique when an active material is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. Microcapsules containing virus and sunlight protectant were found to be more stable than virus alone. Very promising results have been obtained by the Agricultural Research Service of the USDA regarding the encapsulation of biopesticides made of species-specific nucleopolyhedroviruses (NPV) isolated from several insects, including celery looper *Anagrapha falcifera* alfalfa looper *Autographa californi*a, codling moth *Cydia pomonella* and fall armyworm *Spodoptera frugiperda*. Microencapsulation of the UV sensitive *Cydia pomonella* granulovirus in powder form with the encapsulated virus showed increased photostability and strongly enhanced half-life of the encapsulated virus compared to the untreated virus in Germany. Similarly a desirable formulation through microencapsulation of CuniNPV (mosquito) occlusion bodies together with magnesium into a particle that is being developed for delivery to the larval mosquito. Since, inactivation of baculoviruses may be also caused by plant metabolites such as peroxidases which generate free radicals (Hoover *et al*., 1998), it can be reduced by addition of free radical scavengers such as mannitol or enzyme superoxide dismutase to baculovirus preparations (Zhou *et al*., 2004). Additives such as Lysine KKL, polyglucine, a by-product of citric acid production and molasses of peat increased the retaining of viral polyhedrae on apple leaves after artificial rainfall and gave 0.5 to 3 times higher larval mortality than that in the control. Luo *et al.* (2021) prepared *S. litura* nucleopolyhedrovirus microcapsules by the complex coacervation method using *S. litura* nucleopolyhedrovirus as the core material, gelatin, and CMC as the wall materials, and tea polyphenols as the curing agent. The particle size of the prepared virus microcapsules was 13 μm, the drug loading was 43.87%, the embedding rate was 62.53%, and LC50 of the microencapsulated virus was 8.36 × 104 (PIB/ml) after 192 h of treatment. The survival ability of microencapsulated *S. litura* nuclear polyhedrosis virus in the field environment was significantly higher than that of unembedded

virus. Similarly, Wilson *et al.* (2020) prepared micro-encapsulated baculovirus in an ENTOSTAT wax combined with a UV absorbant (titanium dioxide, TiO2). Importantly, this capsule protects the sensitive viral DNA from degrading in sunlight, but dissolves in the alkaline insect gut to release the virus. The new formulation has a shelf-life at 30°C of at least 6 months, which is comparable to standard commercial biopesticides and has no phytotoxic effect on the host plants. Taken together, these findings suggest that the new formulation technology could reduce the costs and increase the efficacy of baculovirus biopesticides, with the potential to make them commercially competitive alternatives to synthetic chemicals.

## GOALS AND NOVELTY OF APPROACH

- To develop standard formulation for precise delivery of the baculovirus to the feeding site of the target.
- To develop improved formulations for ultraviolet protection and activity enhancement using microencapsulation technique.
- To develop phagostimulant nucleopolyhedrovirus formulations for control semi-cryptic pests such as baits or granules.
- To compare improved formulations of viruses for storage and efficacy.

Typically, baculoviruses have a narrow host range that limits the potential market size of the product when compared with broad spectrum products such as *Bacillus thuringiensis* and chemical pesticides. Therefore, improved formulation technology is required for taking advantage of the unique properties of baculoviruses. It is well established that the efficacy of entomopathogens that act by ingestion can be improved by the use of formulations that include feeding stimulants which increase the consumption of the pathogen resulting in enhanced prevalence of disease and improved pest control. Formulation research proposal presented here addressed to incorporate potent additives of virus preparations retain virus persistence on plants after the rain such as Lysine KKL, polyglucine, a by-product of citric acid production and molasses of peat. Besides, novel adjuvants that improve the pathogenicity of the virus to late instar larvae and improve the persistence of the virus on leaf surfaces. One group of adjuvants of particular interest has been the optical brighteners, derived from stilbene compounds, such as Tinopal LPW or Blankophor BBH. These compounds appear to degrade the peritrophic membrane in the insect midgut, thus increasing the probability of infection of midgut epithelial cells. They can also reduce the rate of sloughing of infected cells and inhibit the suicide response of infected cells, known as apoptosis, thereby increasing the number of foci of infection in each insect that consumes viral OBs. The ingredients used to produce these formulations will be selected based on previous research for extended residual activity (Tamez-Guerra *et al.*, 2000, McGuire *et al*., 2001) virus encapsulation, and storage stability (Tamez-Guerra *et al.*, 2002). These shall include Baculovirus: for selective insects adjuvants as stickers, UV protectants and phagostimulants antioxidants or radical scavengers besides the addition of folic acid, pyridoxine, riboflavin and charcoal.

## DISCUSSION

The methodology will be focused on the preparation of granular/bait formulation by mixing optimized quantity of ingradients like pregelatinized cornstarch, oil, and distilled water to form a paste and its subsequent transformation into bait/granules followed by air drying through fan ventilator. Feeding stimulants that use a lower quantity of pathogen inoculum to achieve satisfactory pest control and the ability to target applications precisely to the feeding site of the pest (e.g., near the base of plant for *Agrotis ypsilon* and application of granules directly to the maize leaf whorl for *Chilo partellus* besides coddling moth *Cydia pomonella.* Another important approach envisaged in this is encapsulation which is the process of mixing microbes with a matrix-forming material, such as cornstarch, that has been partially gelatinized that is, heated to enable water absorption. When the cornstarch-microbe mixture is added to water and then dried, the microbes become entrapped in protective particles so small they can barely be seen without a microscope. Natural (lac or lignin) or synthetic acrylic based polymeric encapsulating agent which is generally soft and thus easily ingested by a target insect.The method will involve (i) mixing (A) an encapsulating polymer comprising (B) a sunscreening agent and (C) a solvent comprising at least one of polyethylene glycol, propylene glycol, a methylene chloride and appropertiate solvents like propylene glycol mixture, tetrahydrofuran, tetrahydropyran, furan, and pyran; A number of schemes for preparing microencapsulated viruses are now described. Among them spray drying is most common.

Finally the formulations of biopesticides can be tested on farmer scale and shall be made available to them by blending the microbial component with carriers and adjuvants for better protection from unfavourable environments, enhanced survival of the bio-agent, controlled rates of release, as well as improved bioactivity, shelf life, and stability.

## CONCLUSION

Baculoviruses have been commercialized and gained prime importance worldwide in recent years for the control of many pests. Although baculoviruses are highly effective against their target insects, until today, their application as bio-insecticides was limited as the performance of these viruses is susceptible to UV radiation. Therefore, microencapsulation a process in which tiny particles or droplets are surrounded by a coating to give small capsules of many useful properties has been employed. Microcapsules containing virus and sunlight protectant were found to be more stable than virus alone. Very promising results have been obtained by the Agricultural Research Service of the USDA regarding the encapsulation of biopesticides made of species-specific nucleopolyhedroviruses (NPV) isolated from several insects. The findings discussed above suggest that the new formulation technology could reduce the costs and increase the efficacy of baculovirus biopesticides, with the potential to make them commercially competitive alternatives to synthetic chemicals.

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