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REVIEW ARTICLE **OPEN ACCESS**

Nuclear Polyhedrosis Virus (NPV): An overview

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ABSTRACT
Nuclear polyhedrosis viruses (NPV) are baculoviruses, obligate pathogens with rod- shaped nucleocapsid containing double stranded circular DNA.The normal way of infection is by ingestion of polyhedra or in some cases virions can infect host insects. These days the use of chemical pesticides affects the environment and cause health hazards to humans, animals and beneficial insects also. Nuclear Polyhedrosis virus is a good alternative to the synthetic pesticides. This virus can control lepidopteran insects of cultivated crops like potato, cotton, cabbage etc. This narrow spectrum biocide only targets the specific host i.e., insect pests not the non-target hosts which includes beneficial insects. Mass culture of NPVs on Spodopteralitura is easily possible by using natural diet, castor leaves under laboratory condition in plastic buckets. Spraying of NPV formulation should be late within the day when peak sunshine or evening escapes sun's rays to safeguard the microorganism particles to enhance the effectiveness of NPV.
Keywords: Biopesticide, Entomopathogens,Nuclear Polyhedrosis Virus (NPV), Baculoviruses, Lepidoptera, Helicoverpa spp., Spodopteraspp.

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INTRODUCTION

Biopesticides or 'biological pesticides' is a part of biological control where pest management is through organisms of biological origin or their products like virus, bacterium, fungus and protozoan [37]. In Pest management mechanisms involve parasitic,predatory, or chemical relationships. Pest refers to any organism (insect, non-insects, microbial pathogens, weeds etc. detrimental to man and his properties. European Union told biopesticides is a type of pesticide which is based on microorganisms or natural products.

HISTORY OF NPV

In history During 1892 Germany first introduced NPV into the population of *Lymantria monacha* in pine forest. During 1913 first field application of NPV was against gypsy moths in the U.S.A; a Californian farmer collected NPV killed larvae of *C.eurytheme* as an inoculum for application on alfalfa crop. In 1975, U.S.A registered first viral insecticide(Elcar) used against cotton bollworm (*Helicoverpa Zea*) [1, 39]. Baculoviruses give an alternate of chemical insecticide for controlling different insect pest because it don't have any residue effect and don't have any harmful effect on beneficial predators and parasites.The *Helicoverpa* and *Heliothis* attack more than 65 crops like maize,wheat, sorghum various legumes, solanaceous crop, malvaceous crop, etc.[8]. Many important spp. of *Helicoverpa* and *Heliothis* found sensitive to NPV i.e., *Helicoverpa armigera, Helicoverpa assulta, Heliothis virescens* and *Helicoverpa zea.*

STRUCTURE

Nuclear polyhedrosis virus is an obligate pathogen.The virus is rod- shaped nucleocapsid(250-400*40-70nm),containing double stranded circular DNA [36, 58]. Nucleocapsids are enclosed within an envelope called virions consisting of a proteinaceous polyhedral occlusion body inside which the virions or virus rods are embedded [4, 15]. Due to alkaline gut juice the viruses are liberated, which attack the nuclei of cell tissue,fat bodies, hemocytes, tracheal matrix, ganglia and brain [18].

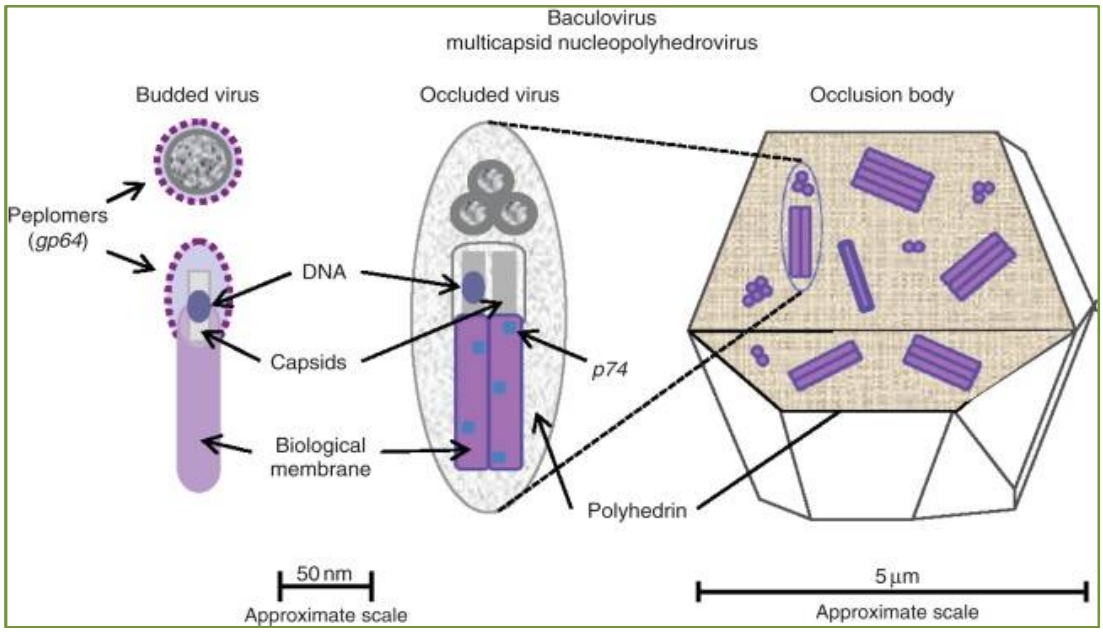

Fig. Structure of Baculovirus; a multicapsidnucleo-poly-hedro-virus[20]

INFECTION PROCESS

The normal way of infection is by ingestion of polyhedra or in some cases virions. Larvae should be susceptible; when polyhedra are ingested with the food the occlusion protinacious bodies start dissolve under the alkaline (pH>9)condition. The virions infect the epithelial cell of midgut; in the nucleus of infected cells newvirions are produce and infect cells of hemocoel and other tissue such as fat body [47]. In that tissue occlusion of virions in polyhedra take place & that possess keep going until cell lysis. After 24 to 72hr infection polyhedra can be detected in the nuclei of infected cells. After a few days larvae die and spilled on the foliage and soil. After that predators and parasite eat or distribute to other insect or place [13, 43].

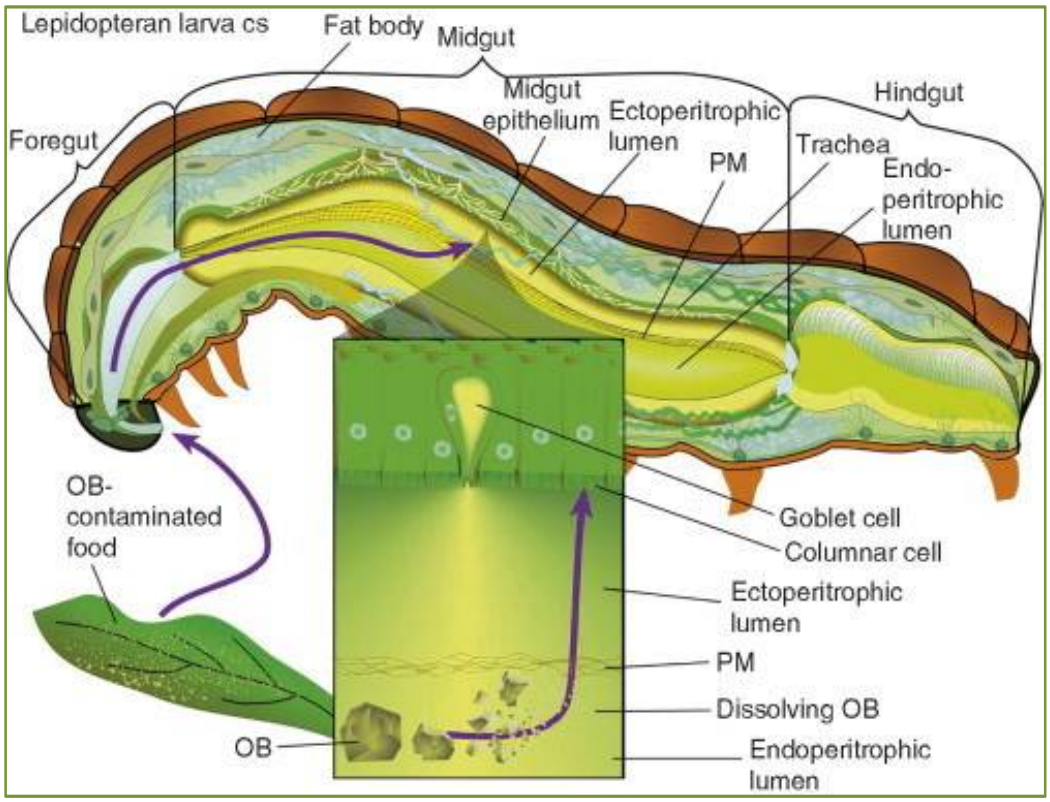

Fig. Mechanism of infection of lepidopteran larvae by NPV**[57]**

Infected insects are dull in color, less active and the larvae turn reddish pink on the ventral side. With advanced stages, the worms become flaccid, the skin becomes very fragile and eventually breaks down [11]. Infected larvae hang upside down on plants. This is called "tree top disease" or "wipfelkrankheit" Diseases caused by NPV in silkworms are called Grasserie [2].

THE EFFECT OF ENVIRONMENT ON NPV

The virus came out from the dead larvae which are resistant to abiotic factors such as humidity, drought, pressure etc.become inactive by sunlight and UV radiation range 280 to 320 nm is harmful for the NPV[32].

Effectiveness of NPV depends on environment

As foliar application virus can be inactive after a few days but in soil polyhedra can stay active for more than 10 years [26].During rain splash polyhedra can contaminate foliage.

MASS PRODUCTION OF NPV

The mass production of the NPV is carried out in the early fifth instar stage of the *Spodoptera litura* which yields the maximum amount of the NPV. Therefore in the host culture laboratory a continuous culture of the insects is maintained with proper handling procedures[5].

The larvae are grown in a diet held in 5ml glass vials when the larvae reach the appropriate stage they are transferred to virus production facility. The NPV is multiplied by feeding the semi synthetic diet coated with a clean inoculum of the NPV that has previously been standardized. This is accomplished by placing aliquots of 10ml of viral suspension of concentration $1x10^8$ polyhedral occlusion bodies (POB)in the center over the diet surface either in glass vials and spreading the suspension uniformly all over the surface with a polished glass rod. Larvae are released singly after 15 min, into each glass vial/cell and incubated at 25°C for 10 days. The larvae begin to die from 5th day onward. The cadavers are collected individually and transferred to 500ml plastic containers and frozen immediately until processing. The processing method is similar to that of *Helicoverpaarmigera*(TNAU) [56].

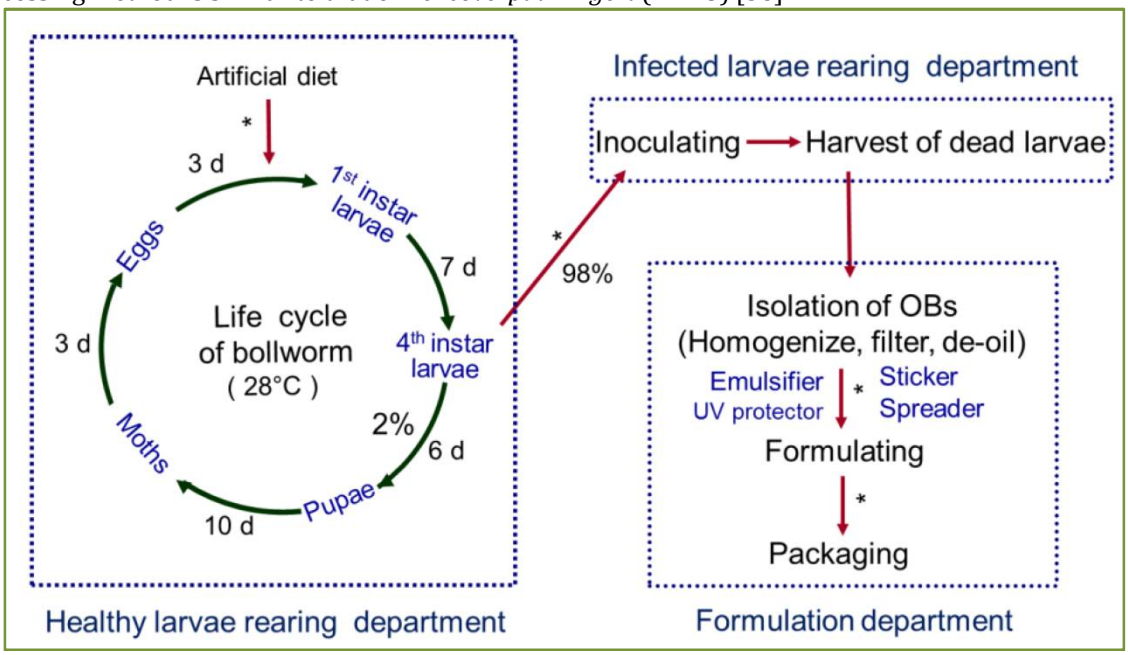

Fig. Infection cycle by NPV**[54]**

The cadavers are unit homogenized in sterile ice cold water at the magnitude relation 1:2.5 (w/v) during a liquidizer or cool all glass pestle and mortar. The material is filtered through double stratified cloth and repeatedly washed with water. The magnitude relation of water to be used for this purpose is 1:7.5-12.5 (w/v) for the first weight of the corpse processed [30].The leftover mat on the cloth is discarded and also the filtrate may be semi-purified by differential activity. The filtrate is centrifuged for 30-60 sec at 500rpm to get rid of junk. The supernatant is next centrifuged for 20min at 5,000 rpm. Then the pellet containing the solid occlusion bodies (POB) is suspended in sterile water and washed 3 times by centrifuging the pellet in water at low rate followed by activity at high rate. The pellet finally collected is suspended in water and created upto a famed volume that is important to calculate the strength of the letter box within the refined suspension[60].

IN VIVO PRODUCTION TECHNIQUE

All industrial production of NPV for use as biopesticide is therefore currently done in vivo by infecting, rearing and harvesting the whole insect. Systems ranging from simple home pesticide involving farmers using the field collected larvae to the computer controlled robot operated mass production facilities with capacity of 0.25-1 million ha per annum. Examples of such modern mass production facilities have been built in France, Canada and USA [35, 58]

In vivo production technique completes into three steps: 1. Infect larvae susceptible species, 2. Grow infected larvae for a time to allow the infection to develop and the virus to replicate and 3. Harvest the larvae and extract the virus [35].

However in practice maintaining a sustained production of virus has not been found to be easy. It is only with well- trained staff, adequately developed production procedures, the appropriate equipment and a high standard of process quality control that effective production can be both attained and maintained [16].

TYPICAL PROBLEMS FOUND WITH PRODUCTION

1. Reduced production rate per insect over time.
2. Contamination of the system by other competing pathogens reduces the NPV yield as well as lowering the quality of the product.
3. Failure to maintain a supply of healthy insects [16].

The causes of problems are various but important factors are

1. Poor quality insects; impure inoculum.
2. Inappropriate dosing.
3. Poor rearing.
4. Unsuitable harvesting.
5. Poor sanitation.

MEASURING THE STANDARD OF VIRUS

Measure the standard of virus by Haemocytometer.Either dark field or section distinction magnifier is required to count the solid inclusion bodies (PIB) employing a Haemocytometer.The dose of virus is expressed as the larval equivalent (LE) and one LE is $6x10^9$ POB [31, 32].

APPLICATION OF NPV

Spraying the NPV late within the day when peak sunshine or evening escape sun's rays to safeguard the microorganism particles to enhance the effectiveness of NPV. Adding ultraviolet light absorbent use of 1ml capacity unit of robin blue to a liter of spray solution has been reported as improving the effectiveness of the NPV [16]. Also, addition of an adhesive like teepoland and a phagostimulant like jiggery is suggested to improve the efficacy. The dosage lies between 250-500 LE/ ha relying upon the foliage density [1].

Application Systems

1. **High-volume**

Many Spraying systems we can use for applying nuclear polyhedrosis viruses in crops. High-volume applications were made with an azo-propen hand sprayer using a birchmeier helicon sapphire nozzle100, pressure 4 bar.This sprayer nozzle should be 50 cm over the crop during spray time [3].

2. **Low-volume**

Low-volume spinning disc applications were made by micron-ulva eight spinning discs. This sprayer nozzle should be 75 cm over the crop during spray and droplets angel should be 50 degree. Volume should be apply3 ml virus suspension in water with 20% mineral oil per meter square [3].

3. **Ultra-low volume electrodynamic sprayer**

In ultra-low volume electrodynamic sprayer use of electrod provided by ICI [7], in this sprayer nozzle position should be 50 cm above the crop [3].

MAJOR INSECT PESTS AND THEIR MANAGEMENT BY NPV

Spodoptera litura

Spodoptera litura (Lepidoptera: Noctuidae) is a polyphagous pest of different crops and it has attended global importance due to its damaging range and insecticide resistance problems.SlNPV can be good alternate for the control of *Spodoptera litura* on cabbage [33, 56].

MODE OF APPLICATION OF SlNPV

SlNPV is applied in the field@ rates of 500 lit/ha. Two sprays are required; first spray 45th days after transplanting of cabbage in the field and second spray at the time of the 60th day after transplanting.

Helicoverpa armigera

Helicoverpa armigera Hubner (Noctuidae: Lepidoptera) ranks as the most important

lepidopteran pest in South Africa [1, 17] Included in itswide range of economically important agricultural crops is citrus, on which it can be aserious pest The moths lay their eggs on or near the blossoms during spring.

MODE OF APPLICATION OF HaNPV

A concentration of $1.15x10^7$ OBs/ml of HaNPV is sprayed using a knapsack resulted in a100% reduction in *H. armigera* larval infestation within 7 days on tomato plants in ahot house environment. A 10-fold lower concentration, $1.15x10^6$ OBs/mL, resulted in a 100%reduction within 16 days.**Table 1 and Table 2.**

Table 1 Worldwide infestation by larvaeof *Spodoptera*spp. on various plant species

strawberry	Lettuce	sage	Citrus	coffee
sugar beat	Maize	Potato	Chilli	cotton
Sweet pepper	Marigold	Safflower	carrot	Eggplant
wheat	Tomato	sesame	Cabbage	Eucalyptus
Turnip	millet	Purslane	Bean	Grape
sunflower	Mint	Pear	Barlley	Spinach
Rice	Onion	Tobacco	Apple	Jute
Pea	Lentil	Redish	Alfalfa	Indigo

Source: [16, 30, 60]

Table 2: Insect pests of plants and their management by NPV

Scientific name of Host insect	Common name in India	Associated crops	References
Helicoverpa armigera	cotton bollworm	Cotton, Tomato, Wheat	[38, 43]
Helicoverpa zea	Corn earworm	Maize	[17, 48]
Heliothis virescens	Tobacco Budworm	cotton	[24]
Hyphantriacunea	Fall webworm	Mulberry	[22, 61]
Buzurasup pressaria	Tea looper	Tea & Tung oil tree	[36, 61]
Autographa californica	Alfalfa looper	Cabbage, cotton & Ornamentals	[55]
Anticarsiagemmatalis	Bean caterpillar	Soybean	[41, 60]
Spodopteralitura	Tobacco cutworm	Vegetables, rice and peanuts	[33, 56,58]
Spodopteraexigua	Army worm	Cabbage, Cotton, Amaranthus and Sunflower	[23, 42]
Spodopterafrugiperda	Fall armyworm	Maize	[44]
Spodopteralittoralis	cotton leaf worm	Cotton	[30]
Lymentiradispar	Gypsy moth	Forest	[31]
Mamestrabrassicae	Cabbage moth	Cabbage	[66]

CONCLUSION

By this review paper we can conclude that Organic foods are the safe food for the human body. NPV is a very good option of bio pesticide available for farmers which will not cause any harm to nature.The initial cost may be little high but within *invivo* technique we can replace the chemical pesticides in long term. For production of NPV skilled farmers are required which may be fulfilled by giving training to the farmers.

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