

UNIT-IV

PESTICIDES

Pesticides are substances that are meant to control pests.^[1] The term pesticide includes all of the following: herbicide, insecticides (which may include insect growth regulators, termiticides, etc.) nematicide, molluscicide, piscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial, and fungicide.^[2] The most common of these are herbicides which account for approximately 80% of all pesticide use.^[3] Most pesticides are intended to serve as plant protection products (also known as crop protection products), which in general, protect plants from weeds, fungi, or insects. As an example - The fungus Alternaria is used to combat the Aquatic weed, Salvinia.

In general, a pesticide is a chemical (such as carbamate) or biological agent (such as a virus, bacterium, or fungus) that deters, incapacitates, kills, or otherwise discourages pests. Target pests can include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, or spread disease, or are disease vectors. Along with these benefits, pesticides also have drawbacks, such as potential toxicity to humans and other species.

The Food and Agriculture Organization (FAO) has defined *pesticide* as:

any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.^[4]

Pesticides can be classified by target organism (e.g., herbicides, insecticides, fungicides, rodenticides, and pediculicides^[5] – see table), chemical structure (e.g., organic, inorganic, synthetic, or biological (biopesticide),^[6] although the distinction can sometimes blur), and physical state (e.g. gaseous (fumigant)).^[6] Biopesticides include microbial pesticides and biochemical pesticides.^[7] Plant-derived pesticides, or "botanicals", have been developing quickly. These include the pyrethroids, rotenoids, nicotinoids, and a fourth group that includes strychnine and scilliroside.^{[8]:15}

BIO-PESTICIDES

Biopesticides can be classified into these classes-

- Microbial pesticides which consist of bacteria, entomopathogenic fungi or viruses (and sometimes includes the metabolites that bacteria or fungi produce). Entomopathogenic nematodes are also often classed as microbial pesticides, even though they are multi-cellular.^{[4][5][6]}
- Bio-derived chemicals. Four groups are in commercial use: pyrethrum, rotenone, neem oil, and various essential oils^{[7][8]} are naturally occurring substances that control (or monitor in the case of pheromones) pests and microbial diseases.
- Plant-incorporated protectants (PIPs) have genetic material from other species incorporated into their genetic material (*i.e.* GM crops). Their use is controversial, especially in many European countries.^[9]
- RNAi pesticides, some of which are topical and some of which are absorbed by the crop.

Biopesticides have usually no known function in photosynthesis, growth or other basic aspects of plant physiology. Instead, they are active against biological pests. Many chemical compounds have been identified that are produced by plants to protect them from pests so they are called antifeedants. These materials are biodegradable and renewable alternatives, which can be economical for practical use. Organic farming systems embraces this approach to pest control.

BIO-PESTICIDES:

VIRAL ORIGIN

Baculoviruses are specific to individual insect host species and have been shown to be useful in biological pest control. For example, the *Lymantria dispar* multicapsid nuclear polyhedrosis virus has been used to spray large areas of forest in North America where larvae of the gypsy moth are causing serious defoliation. The moth larvae are killed by the virus they have eaten and die, the disintegrating cadavers leaving virus particles on the foliage to infect other larvae.

Baculoviridae family is a classic example of this kind of insecticides which are arthropods. However, baculoviruses infect only a few arthropod species. Apart from this, there are some viruses that can control insects such as sawflies and Lepidoptera such as NPV, granulosis virus, entomopox virus, etc.

NPV

What is NPV?

NPV stands for nucleopolyhedrovirus. NPV is a viral disease of *Helicoverpa* caterpillars that occurs naturally in the Australian environment. Australian farmers have access to commercially produced formulations of NPV for the control of *Helicoverpa* infestations in crops. NPV is safe and environmentally friendly. It is ideally suited for

inclusion in an integrated pest management (IPM) approach to controlling *Helicoverpa armigera* and *H. punctigera*, the major insect pests in grain systems.

Where can NPV be used?

NPV can be used in a variety of field crops, including sorghum, cereals, chickpea, cotton and maize. In sorghum, NPV is the preferred product for *Helicoverpa* management, not only because it is very effective (frequently giving more than 90 per cent control) but because it preserves the full range of beneficial insects in the crop (e.g. *Microplitis* and *Trichogramma* wasps).

In crops other than sorghum, it is important to have realistic expectations of what NPV can achieve. In these crops, control varies and depends on a range of factors.

NPV can persist for years in a protected environment such as the soil, but is rapidly killed by exposure to ultraviolet light (sunlight) and high temperatures.

Why is NPV different to conventional insecticide?

One of the key differences between NPV and a conventional insecticide is that NPV is applied as a live disease. Therefore it is important to understand the NPV lifecycle to understand how it works.

Helicoverpa larvae have to eat NPV particles to become infected. These particles are called polyhedral inclusion bodies (PIBs). One PIB can be enough for NPV to successfully infect and kill the caterpillar. Once the PIB is ingested, the virus infects the gut cells, spreading to the blood within 24 hours, and then to almost all the tissues in the body.

Impact of NPV on pests

NPV can kill young larvae within 4 days of ingestion, older larvae within 5 to 7 days, depending on dose and temperature. The higher the dose and/or temperature, the faster the rate of infection and death. However, cool to cold temperatures can prolong the time NPV takes to kill its host to more than 10 days.

Diseased larvae typically climb to the top of the plant to die. Shortly after death, an infected larva's body becomes flaccid and its skin ruptures, releasing millions of infectious virus particles back into the environment. When larval numbers are high, waves of natural infection can develop as more larvae become infected, die, and spread the infection, resulting in an outbreak.

GRANULOSIS VIRUS:

Granuloviruses are in the family of insect viruses called the baculoviridae. These viruses target insects and are popular as insecticides for farmers targeting lepidopteran larva. The viruses are present in many historical records for causing silkworm 'jaundice' and have been used as biopesticides since WWII. There are currently 17 species in the genus *betabaculovirus*. These viruses are popular due to their selectivity of only attacking

insects from the order lepidoptera. Granuloviruses are well known for their unique ability to completely liquify their hosts in order to spread to more hosts, a trait they share with the closely related nuclear polyhedrosis viruses. The type species of granuloviruses is the species *Cydia pomonella granulosis virus* (CpGV), which only affects *Cydia pomonella* larva. *C. pomonella* larva are pests of various fruits in agriculture, causing great loss of ripened fruits. Granulovirus has been used as a pesticide since World War II (Federici, 1997); however, *C. pomonella* has been developing resistance to granulovirus since 2005 (Sauer, 2017). Granuloviruses are useful in the pesticide industry due to their ability to efficiently kill lepidopteran pests, such as *C. pomonella*, to protect crops without damaging them or harming the consumer of the crops. Extensive research is being done on more uses and applications of baculoviridae to operate as pesticides against more specialized or resistant lepidopteran pests.

Granulosis was discovered in the early 1960's, and was found to only infect the codling moth (*C. pomonella*) and species closely related to it. The codling moth bores into apples, which makes them unfit for human consumption. Spraying apples with granulosis virus significantly decreased the amount of damaged apples, and killed most codling moth larvae before they could enter the fruit; most died after feeding on the epidermis of the treated fruits, which are still suitable for human consumption (Falcon et al, 1968). Granuloviruses are produced for commercial use on crops by infecting large numbers of lepidopteran larva in a lab setting (D'Amico, Podgwaite, 2015). Upon liquification, the larva and virus products are brought to a processing facility where the liquid is converted into a powder mix (D'Amico, Podgwaite, 2015). This powder is then mixed with water and sprayed on the crops to protect the crops from lepidopteran pests (D'Amico, Podgwaite, 2015). Larva that are killed by the granuloviruses become shiny in appearance and latch on to the leaves of the plant they are on (D'Amico, 2015). The bodies of the larva are generally very flimsy and rupture after proteases break through epithelial layer of the host, causing a release of viral particles into the environment (D'Amico, 2015).

Granuloviruses occlusion bodies contain one or rarely two virions and are about 0.16-0.30 μm by 0.30-0.50 μm in size. The nucleocapsid of the virus contains a double-stranded circular-shaped strand of DNA (Fields Virology, 2013). The outside of the nucleocapsid contains proteins that form ring-shaped subunits through their interactions. The end of the virion contains many unique proteins that are not found anywhere else in the virion, including the protein pp78/83 which aids in assembly of actin and production of offspring viruses (Shuler, Michael L. et al, 1995). Occlusion bodies (Occluded virus, or OV) of granuloviruses contain one or two virions that are wrapped in a protein called granulin (a protein that distinguishes granuloviruses from nuclear polyhedrosis viruses) (Fields Virology, 2013). Occlusion bodies are crystalline gene products that are responsible for the primary viral infection in the gut basal cells. Their function is to release nucleocapsids into gut epithelial cells during infection (Rohrmann, 2013). These occlusion bodies are oval shaped and referred to as 'granules' due to their appearance under a microscope. (Fields Virology, 2013). Granuloviruses also encode homologs of LD130, an envelope fusion protein (Fields Virology, 2013). Granulovirus replication is biphasic cycle, where the budded viruses are formed prior to the occlusion viruses. Budded viruses are produced after

primary infection and are simply nucleocapsids surrounded by a membrane (Rohrmann, 2013). Their function is for cell-to-cell transmission of granulovirus.

The granulovirus replication cycle begins when the occluded form of the virus is consumed by hosts off leaves, where it is dissolved in the alkali environment of the *C. pomonella* larva midgut (Summers, 1971). The cells endocytose the virus released from the occlusion body, which is carried by actin filaments to the nucleus. At this point, the virus uncoats and releases its genome into the nuclear pores. The genome is then incorporated into the host genome and is transcribed by host machinery. Budded viruses are formed using host machinery to infect more cells in the organism, as occluded viruses cannot be transmitted between cells due to presence of granulin, which can not enter cells (Fields Virology, 2013). The assembled budded virus buds off the basal side of gut lumen cells, where it moves to infect new cells. Each budded virus particle buds out from the cell with the LD130 envelope fusion protein, which allows the budded virus to anchor onto the next gut cell. The cell then endocytoses the nucleocapsid of the budded virus and the replication cycle repeats. Granuloviruses encode proteases, such as chitinase, that degrade host proteins and liquify the host, which is the cause of its death (Luque et al, 2001). During these late stages of replication before the larva die, the granulosis viruses begin to express a gene called 'egt' in gut cells (Zimmer, 2014). This gene causes the larva to become agitated and move up the fruit/leaves they are on (Zimmer, 2014). This forced behavior ensures that the viral particles spread further upon liquification of the host (Zimmer, 2014). Scientists are interested in this mechanism of behavior because it is uniquely controlled by a singular gene (Zimmer, 2014). During very late stages of replication, as the larva begin to move to the top of the plant, nucleocapsids remaining in the host cell nuclei are used to form new occluded viruses to be spread upon host liquification (Fields Virology, 2013). The occlusion viruses are produced due to their enhanced survivability in the environment (Fields Virology, 2013). This is because the occlusion body is efficient at protecting viral particles from heat and ultraviolet light protein denaturation while the budded virus membrane does not protect the virus from environmental damage (Fields Virology, 2013).

Fungus Origin:

Entomopathogenic Fungi

I. *Metarhiziumanisopliae*

This fungus was reported for the production of toxins such as destruxin B and desmethyldestruxin B in silkworm larvae. Apart from the toxin, *M. anisopliae* culture filtrate is toxic to coleopteran hemocytes *in vitro* producing changes in organelles, the extract of mycelium is toxic to adult house fly.

II. *Beauveria bassiana* and *B. brongniartii*

These fungi were reported for the production of beauvercin which is a depsipeptide, comprises of a cyclic repeating sequence of 3 molecules of N- methyl phenylalanine alternating with three molecules of 2 hydroxyisovaleric acid. Apart from these beauverolides and bassianolide are other cyclopeptides produced by these fungi. These are also reported to attack insects.

III. *Verticillium lecanii*

The target hosts for this kind of fungus are scale insects and aphids. The control of aphids and scales is done by applying *V. lecanii* conidia suspended in phosphate buffer containing 0.2% Triton X-100 as a wetting agent by using sprays.

IV. *Hirsutiellathompsonii*

It was reported as a potential killer of *Phyllocoptrulaoleivora* also known as citrus rust mite, *Eriophyes sheldoni* (citrus bud mite), and *E. guerreronis* (coconut flower mite). The formulation of conidia of *H. thompsonii* is commercially used in the USA.