Biopesticides

By - Dr. Ekta Khare

Introduction

- **Pest** is competitor of humanity – broadest sense
- Major pest control method is chemical, but the xenobiotics adverse effect on non-target organisms, and also it leas to new bio type pest resistant against pesticide.

- **Biological control** is the beneficial action of parasites, pathogens, and predators in managing **pests** and their damage.
- Microbial pathogens of insects are intensively investigated to develop environmental friendly pest management strategies in agriculture and forestry.

- Microbial insecticides:

Single cell organisms, such as bacteria, fungi, Protozoa and viruses have been mass produced & formulated for use in a manner similar to insecticides.

Product containing these organisms are regulated by the environmental protection agency (EPA) & use is governed by **the Federal insecticide, fungicide & Rodenticide Act (FIFRA).**

- Microbial insecticides can be

1. **Microbially produced toxic substance**
2. **Organism**

Microbial pesticides

- Should be virulent & cause disease to pest at recommended concentration
- Should not be sensitive to moderate environmental variations
- Should rapidly establish disease in pest
- Specific to pest population so as not to cause harm in non –target population.

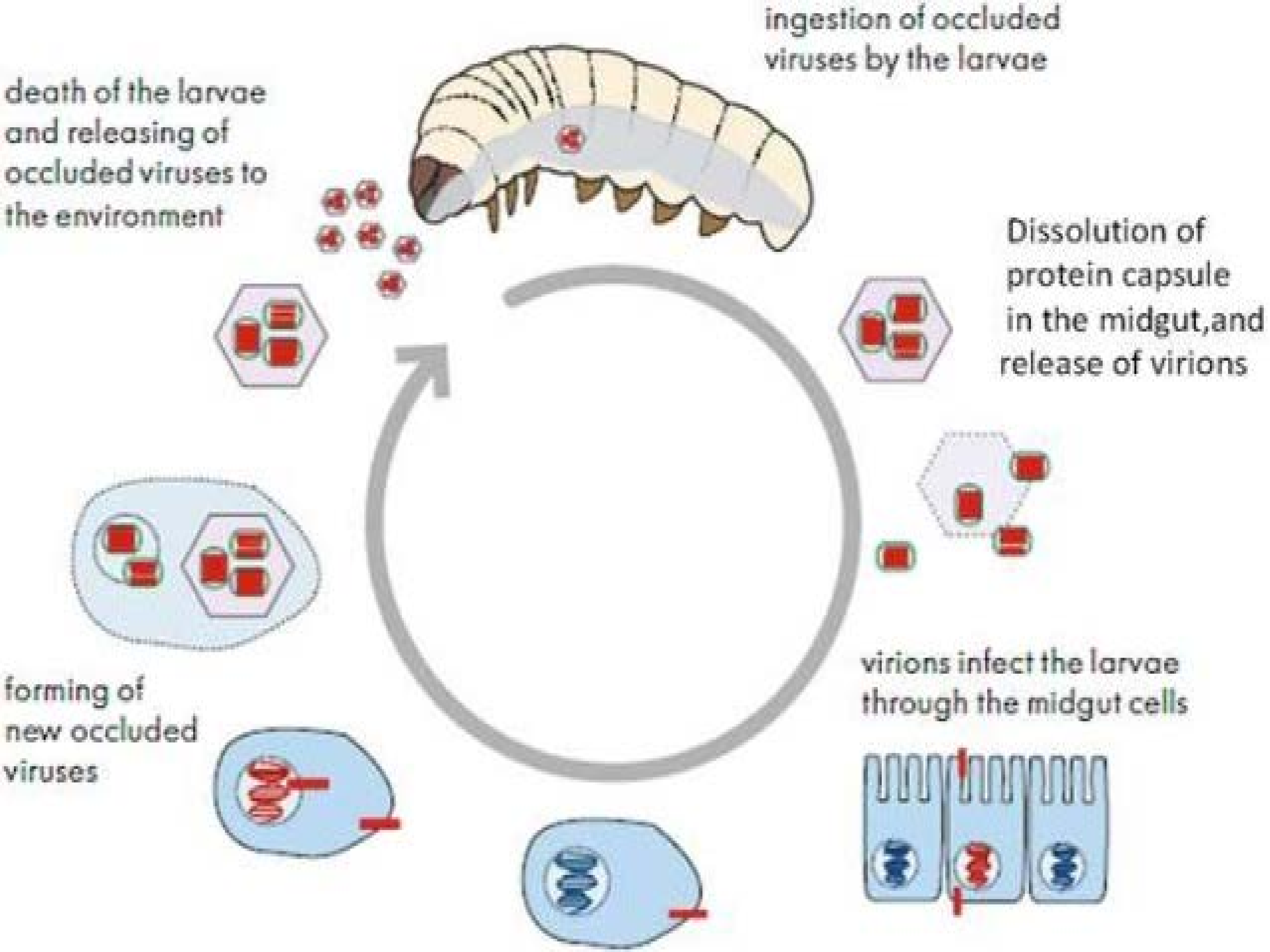
- Types of microbial pesticides:
  - Viral:
    - Among the insect viruses baculoviruses (Nuclear polyhedrosis virus, NPV; cytoplasmic polyhedrosis viruses, CPV; and Granulosis virus, GV) are the most promising for insect control particularly of Lepidoptera and Diptera because of their specificity.
    - NPV and GV are mainly used.
  - Bacterial:
    - The most widely used bacterial pathogens include subspecies or strains of *Bacillus thuringiensis*. Other than this *Serritia marcescens, Bacillus sphaericus, B. popilliae, B. lentimorbus* and *Pseudomonas aeruginosa* also have importance.
    - Each one of the strains produces different mix of toxins and specifically kills one or a few related species of insects.
  - Fungal:
    - Entomopathogenic fungi like *Beauveria* spp., *Metarhizium* spp., *Lecanicillium* spp. and *Isaria* spp. have been developed as successful mycoinsecticides for various groups of insect pests.
  - Protozoan:
    - They are generally host specific and slow acting, producing chronic infections. The infection results in reduced feeding, vigour, fecundity and longevity of the insect host.
    - Although they are undoubtedly important in natural biological regulation of insect populations, they do not possess the required attributes for a successful microbial insecticide.

Baculoviruses as Insect Pathogens

- Baculoviruses are a very diverse group of viruses with DNA double-stranded, circular, supercoiled genomes, with sizes varying from about 80 to over 180 kb, that encode between 90 and 180 genes.
- Members of the Baculoviridae are characterized by their presence in occlusion bodies called polyhedra for NPVs and CPVs, and granules or capsules for GVs.
- The occlusion body consists of a crystalline matrix composed of a protein called polyhedrin in NPVs and CPV, and granulin in GVs.
- Baculoviruses have been described in over 600 insect species.
- The NPV from *Autographica californica* is one of the most intensively studied species.

Infection

- Infection with baculoviruses occurs when a susceptible host eats the polyhedra or granules, which are dissolved in the basic digestive gut juices.
- The virions are released when the protein matrices dissolve.
- The lipoprotein membrane surrounding the virus fuses with plasma membrane of the gut wall cells and liberates nucleocapsids into the cytoplasm.
- The nucleotide transports virus DNA into the nucleus of the cell and virus gene expression begins.
- The virus multiplies rapidly and eventually fills the body of the host with virus particles, primarily the fat body, epidermis, and blood cells.
- Insect larvae infected with baculoviruses usually die from 3 to 12 days after infection depending on viral dose, temperature and the larval instar at the time of infection.
- The infected larvae are extremely fragile to the touch, rupturing to release fluid filled with infective virus particles.
- **This** tendency to remain attached to foliage and then rupture is an important aspect of the virus life-cycle. Infection of other insects will only occur if they eat foliage that has been contaminated by virus-killed larvae.


ingestion of occluded viruses by the larvae
death of the larvae and releasing of occluded viruses to the environment
Dissolution of protein capsule in the midgut,and release of virions
virions infect the larvae through the midgut cells
forming of new occluded viruses

Virus formulation

- NPVs are most commonly considered for development of microbial insecticides.
- Since no fermentation technology is available for the NPV and GV production, the products are required to be produced on the respective insect hosts and formulated in small scale cottage industries or medium sized commercial units.
- This has been one of the impediments for the large-scale commercial production of baculoviruses.
- Another limitation of the NPV or GV formulation is its rapid inactivation by the UV radiation in the fields.
- Therefore, formulations with suitable adjuvants (UV protectants) for field persistence and efficacy have to be developed for large scale adaption of these biocontrol agents.
- Research has to be focused to develop NPV and GV production in insect tissue cultures so that large-scale production and commercialization can take place for increased uptake of these biocontrol agents.

Commodity	Insect pest	Virus Used
Apple, pear, walnut and plum	Coding moth	Coding moth granulosis virus
Cabbage, tomatoes, cotton, (and see pests in next column)	Cabbage moth, American bollworm, diamondback moth, patato tuber moth, and	Cabbage army worm nuclear polyhedrosis virus
Cotton, corn, tomatoes	Spodoptera littoralis	Spodoptera littoralis nuclear polyhedrosis virus
Cotton and vegetables	Tobacco budworm Helicoverpa zea, and Cotton bollworm Heliothis virescens	Helicoverpa zea nuclear polyhedrosis virus
Vegetable crops, greenhouse flowers	Beet armyworm (Spodoptera exigua)	Spodoptera exigua nuclear polyhedrosis virus
Vegetables	Celery looper (Anagrapha falcifera)	Anagrapha falcifera nuclear polyhedrosis virus
Alfalfa and other crops	Alfala looper (Autographa californica)	Autographa californica nuclear polyhedrosis virus
Forest Habitat, Lumber	Douglas fir tussock moth (Orgyia psuedotsugata)	Orgyia psuedotsugata nulcear polydedrosis virus
Forest Habitat, Lumber	Gypsy moth (Lymantria dispar)	Lymantria dispar nuclear polyhedrosis virus

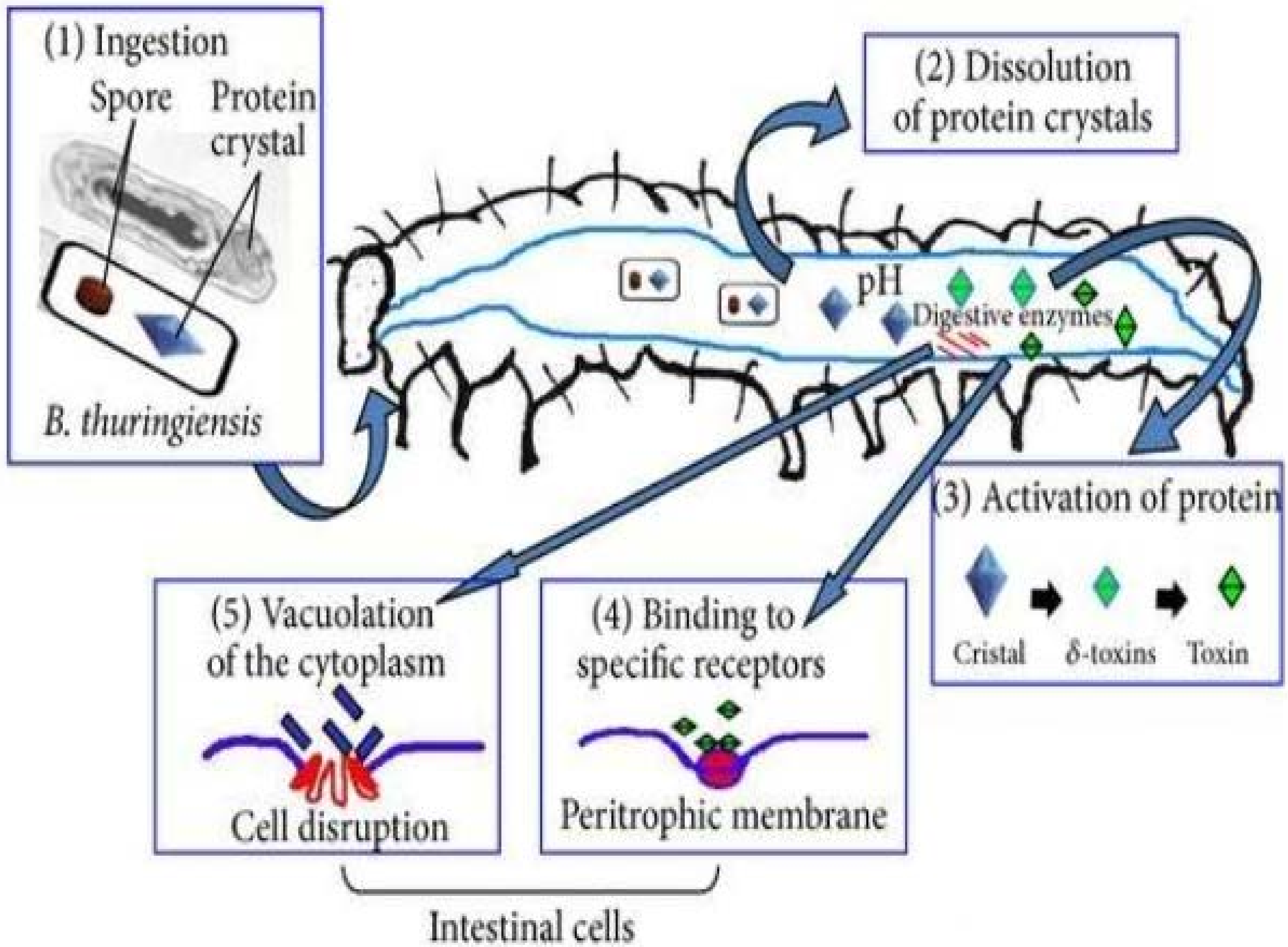
Bacterial insecticides

HISTORY OF BT

- Discovered in japan in 1901 by Ishiwata
- Officially described by berliner in 1915, isolated from mediterranean flour moth in province of thuringia in 1911
- US production of a subspecies thuringiensis in late 1950's
- Discovery of highly active subspecies kurstaki HD-1 by dulmage, 1960's, commercial production 1970s
- Discovery of mosquito, black fly active subspecies israelensis by goldberg and margalit, 1980's
- Discovery of beetle-active subspecies morrisoni by krieg, 1980's

Bacillus thuringiensis

- *Bacillus thuringiensis* (Bt) is a Gram-positive, rod-shaped, spore-forming entomopathogenic bacterium characterized at the species level by the production upon sporulation of a parasporal inclusion body, the crystal which is toxic to insects and other invertebrates and is the most commonly used commercial biopesticide worldwide.
- The cry genes code for proteins with a range of molecular masses from 50 to 140 kDa.
- Upon ingestion by the susceptible insect larvae, protoxins are solubilized in the high alkaline pH of midgut and proteolytically digested by midgut proteinases to release the toxic fragments of approximately 55-68 kDa.
- A generally accepted model for Cry toxin action is that it is a multistage process.
  - The activated toxin binds to receptors located on the apical microvillus membrane of epithelial midgut cells.
  - Important insect proteins have been identified as receptors for Cry toxins viz., aminopeptidase N (APN) and Cadherins. Binding of the toxin to the receptor leads to change in the toxin's conformation, allowing toxin insertion into the membrane.
  - Oligomerization of the toxin follows and this oligomer then forms a pore that leads to osmotic cell lysis and larval death



(1) Ingestion
Spore
Protein crystal
B. thuringiensis
(2) Dissolution of protein crystals
pH
Digestive enzymes
(3) Activation of protein
Cristal
δ-toxins
Toxin
(4) Binding to specific receptors
Peritrophic membrane
(5) Vacuolation of the cytoplasm
Cell disruption
Intestinal cells

Bacillus thuringiensis formulation

- The final fermented Bt broth usually comprises of spores, cell debris, inclusion bodies, enzymes and other residual solids, these have to be recovered efficiently to be utilized in subsequent formulation step.
- Harvesting microorganisms from submerged fermentation is often difficult due to low concentration of products, their thermolabile nature and in some cases, poor stability.
- Stabilizing adjuvants may have to be incorporated in postharvest operation to prevent spore mortality and/ or germination.
- Rapid drying or addition of specific biocidal chemicals may be required to prevent growth of microbial contamination.
- Most commercial Bt products contain insecticidal crystal proteins (ICP), viable spores, enzyme systems (proteases, chitinases, phospholipases), vegetative insecticidal proteins and many unknown virulent factors along with inerts/ adjuvants
- However, at the final stage after fermentation, lactose (5%) was sometimes added as a cryoprotectant to prevent clumping during storage and lactose-acetone co-precipitation could be used as a sequential step to centrifugation to achieve higher -endotoxin recovery efficiency.
- The final products (powder/suspension) are suitably formulated as aqueous (flowable) or oil concentrate, spraying powder, or granulates.

Commercial bacterial insecticides

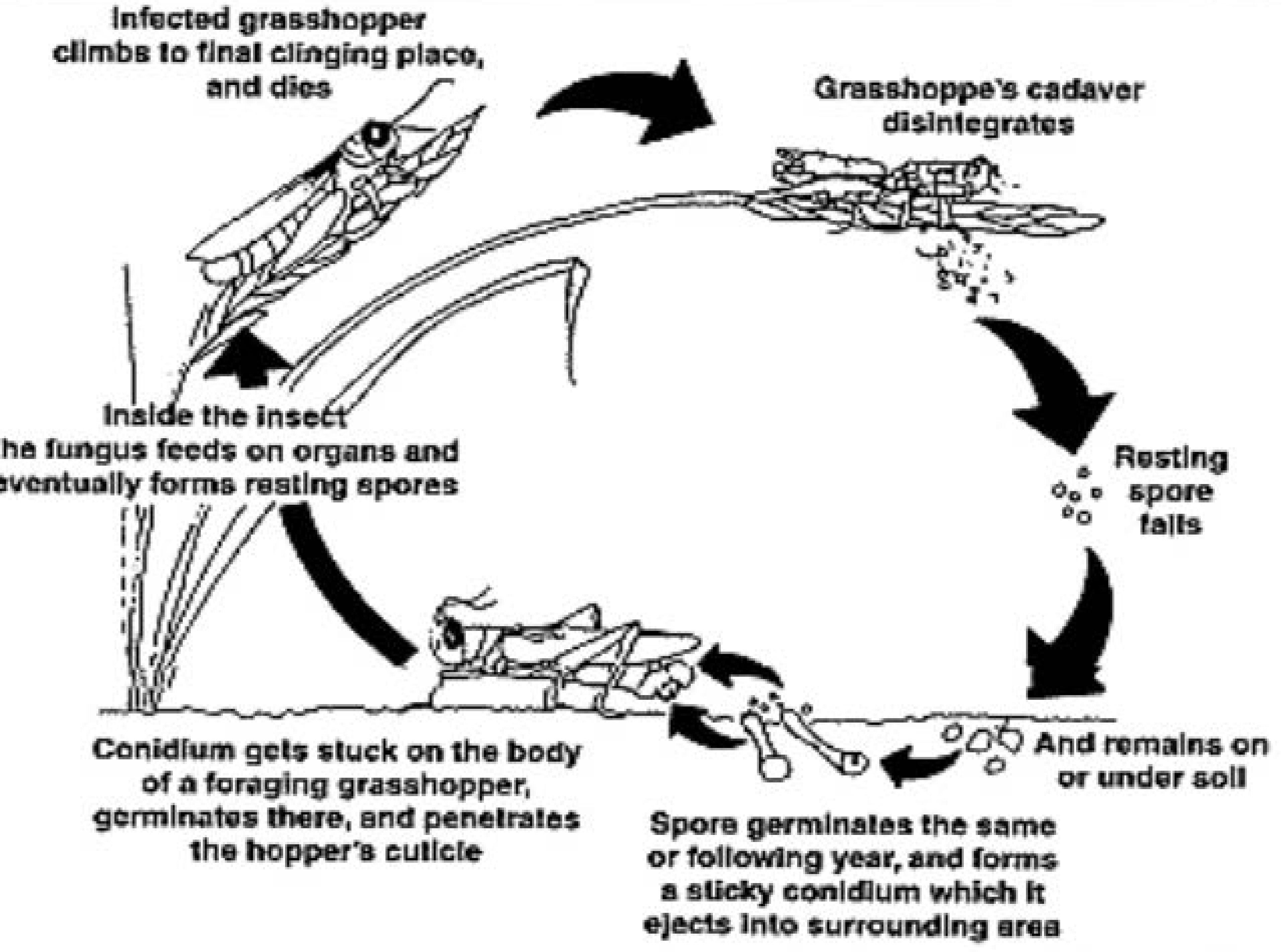
organism	Product name
1. *Bacillus thuringiensis var. kurstaki*	Dipel®, Javelin®, Thuricide®, Worm Attack®, halt Caterpillar Killer®, Bactospeine®, and SOK-Bt®
2. *B.thuringiensis var. aizawai*	Certan®
3. *B. thuringiensis var. israelensis (Bti)*	Vectobac®, Teknar®, Bactimos®, Skeetal®, and Mosquito Attack®.
4. *Bacillus popillae and Bacillus lentimorbus.*	Doom®, Japidemic®, Grub Attack®,
5. *Bacillus thurigiensis var. san diego,*	M-One®,

Fungi as Insect Pathogens

- Fungal pathogens have certain advantages in pest control programmes over other insect pathogens like bacteria and viruses.
- Mass production techniques of fungi are much simpler, easier and cheaper than those used for Bt and NPVs.
- Fungi unlike bacteria or viruses directly infect through insect cuticle and do not require ingestion for infection and so sucking insects are also infected by entomopathogenic fungi.
- Entomopathogenic fungi play an important role in the natural pest control in various crops through epizootics.
- More than 750 sp. of fungi, mostly from hyphomycetes and entomophthorales are pathogenic on insects, many of them offer great potential for pest management e.g. *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *M. anisopliae* var. *acridium*, *Lecanicillium* spp., (previously *Verticillium lecanii), Hirsutella thompsonii, Nomuraea rileyi* and *Isaria fumosorosea* (previously *Paecilomyces fumosoroseus*).
- Fungi infect insects of almost all orders, most common on Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera.

Infection

- Entomopathogenic fungi from hypomycetes group are opportunistic pathogens and usually cause insect mortality by nutritional deficiency, destruction of tissues and by release of toxins.
- Cuticular degrading enzymes of entomofungal pathogens like chitinase, protease and lipase play an important role in the pathogenicity of these organisms on insects in the breakdown of insect cuticle for penetration of fungal germ tube into the insect body.
- The entry of entomopathogenic fungi through the insect cuticle is considered to occur by a combination of mechanical pressure and enzymatic degradation.
- Several mycotoxins like, Beauvericin, Beauverolides Bassianolide (by B. bassiana, V. lecanii, Paecilomyces spp.) and Destruxins A, B, C, D, E, F (by M. anisopliae) are produced during pathogenesis and these act like poisons for the insects.
- After the death of the insects, the fungus breaks open the integument and forms aerial mycelia and sporulation on the cadavers.
- Environmental conditions particularly humidity and temperature play an important role in the infection and sporulation of entomopathogenic fungi.


Infected grasshopper climbs to final clinging place, and dies
Grasshoppe's cadaver disintegrates
Resting spore falls
And remains on or under soil
Spore germinates the same or following year, and forms a sticky conidium which it ejects into surrounding area
Conidium gets stuck on the body of a foraging grasshopper, germinates there, and penetrates the hopper's cuticle
Inside the insect
he fungus feeds on organs and
eventually forms resting spores

Cadavers


Isaria fumosorosea-Bagrada bug


Beauveria bassiana-Bagrada bug


Metarhizium brunneum-Bagrada bug


Beauveria bassiana-Lygus bug


Beauveria bassiana-GWSS


Metarhizium brunneum-GWSS


Paecilomyces sp.-Western harvester ant


Beauveria bassiana-Western harvester ant


Entomophthora sp.-Strawberry aphid

Fungal Formulation

- In India, talc based formulations of entomofungal pathogens are extensively marketed for pest management programs.
- Since the efficacy of entomofungal pathogens in the field is greatly dependent on existence of favourable climatic conditions (low temperature, 70% humidity), there is great necessity for development of suitable oil based formulations for improving the field performance.

Fungal Target	Target pests	Commercial name/status
Aschersonia aleyrodis	White flies/scale insects	Experimental
Beauveria bassiana	Colorado potato beetle, White flies, Aphids, grasshoppers, Locusts,Cotton boll worms	Boverin® (Russia) Myocotrol® WP (USA) Myocotrol® GH-ES (USA) Botanigard® (USA)
Beauveria brogniarti	Cockchafer	Experimental
Culicinomyces clavisporus	Mosquito larva	Experimental
Hirsutella sphaerospora	Mealy bug	Experimental
Laegenidium giganteum	Mosquito larvae	EPA Registered
Metarhizium anisopliae	Cockroaches , Termites,Sugarcanepyrilla, Rhinoceros beetle	Biopath® Bioblast® Bio Green® (Australia
Verticillium lecanii	Whiteflies , Aphids	Vertalec®, Mycotal®

Questions

- Write an essay on microbial insecticides.
- What are the types of microbial insecticides, give examples of each, and their properties?
- Write essay on bacterial insecticide.
- Write essay on viral insecticides.
- Write short note on fungal insecticides
- Write short note on bacterial/ viral/ fungal insecticides formulations