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# Insect Pathogens: Types, Mode of Action and Examples

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Farmers have been using chemical pesticides for the control of insect-pests for many decades; however, the effect on non-targeted organisms, residues on food crops and development of insect resistance to chemical pesticides increase the requirement for substitute methods. As a substitute for chemical pesticides, microbial control agents like insect pathogens (entomopathogens) have gained more importance due to their eco-friendly properties. The microorganisms such as bacteria, fungi, viruses, nematodes and protozoa or their by-products; used for the control of insect-pests are known as insect pathogens. The insect pathogens are grouped as ingested microbes (bacteria, viruses and protozoa) which enter the insect body along with food (like stomach insecticides) and penetrating microbes (fungi and nematodes) that enter by penetrating integument (like contact insecticide).

## 1. Entomopathogenic bacteria (EPBs)

### History

More than a century ago, the discovery of entomopathogenic bacteria began with the first isolation of the bacterium from diseased (Flacherie) silkworm larvae, *Bombyx mori* in Japan (Ishiwata, 1901). Berliner (1915) reisolated it from the Mediterranean flour moth, *Ephestia kuehniella* in Thuringia, Germany and gave the name *Bacillus thuringiensis* (Bt). Honnay (1953) first described that the parasporal inclusions in sporulating cultures of Bt were proteinaceous and might have insecticidal activity. The insecticidal activity of the bacterium is due to three proteins, crystal (Cry), cytolytic (Cyt) and vegetative insecticidal protein (Vip) produced during the bacterial infection in the larvae.

### Classification

1. Non-spore former: *Serratia entomophila* (Grass grub)
2. Spore formers

   i. Obligate: *Paenibacillus popilliae* (*B. popillae*) (Japanese beetle, *Popillia japonica*)

ii. Facultative

a) Non-crystalliferous: *B. cereus* (Coleoptera, Hymenoptera and Lepidoptera)

b) Crystalliferous: *B. thuringiensis* (var. kurstaki, galleraie, israelensis, sandiego, tenebrionis) and *Lysinibacillus sphaericus* (Mosquitoes and blackflies)

**Mode of action**

Insect ingests *B.t.* crystals and spores with plant materials (stomach poison). The parasporal inclusions (delta-endotoxins) dissolve in the alkaline pH of the insect gut and release protoxins (27 to 140 kDa) which are proteolytically converted into still smaller toxic polypeptides. These polypeptides bind to glycoprotein receptors (specific receptors) on midgut cell membrane. They generate pores in the cell membrane disturbing cellular osmotic balance and causing cells to swell and lyse through a process that has been termed as "Colloid-osmotic lysis". The leakage of ions from midgut to hemolymph results in ionic imbalance in hemolymph which leads to septicemia. Multiple toxins will bind and follow these steps until the insect is paralyzed. The insects stop feeding (starvation), and crystals cause the gut wall to break down, allowing spores and normal gut bacteria to enter the body. The insect dies as spores and gut bacteria proliferate in the body. *B.t.* toxicity depends on recognizing receptors, damage to the gut by the toxin occurs upon binding to a receptor. The *Bacillus* species with *B.t.* is the most widely used EPB (Mishra *et al.,* 2015).

**Symptoms**

Infected larvae become inactive, stop feeding, regurgitate or have watery excrement. The head capsule becomes large compared to body size. The larva becomes flaccid and dies, usually within 2-4 days. The body contents turn brownish-black as they decompose.

**Table 1: Examples of entomopathogenic bacteria**

| SN | Entomopathogenic bacteria | Target insect-pests |
|---|---|---|
| 1 | *Bacillus thuringiensis* (*B.t.*) | Lepidopteran larvae |
| 2 | *B.t.* var. *kurstaki* | |
| 3 | *B.t.* var. *israelensis* | Diptera (larval stages of mosquitoes and black flies) |
| 4 | *B.t.* var. gallerine | Lepidoptera (Wax moth) |
| 5 | *B.t.* var. tenebrionis | Coleopteran (Beetles and weevils) larvae |
| 6 | *B.t.* var. sandiego | |
| 7 | *B.t.* var. aizawai | Lepidopteran larvae |
| 8 | *Paenibacillus popilliae* | Coleoptera (Scarabaediae) "milky disease" |
| 9 | *Streptomyces* spp. (*S. albus* and *S. avermitilis*) | *Aphis gossypii, Chilo partellus, Helicoverpa armigera and Spodoptera litura.* |

| | | |
|---|---|---|
| 10 | *Burkholderia rinojensis* | Two spotted spider mite, *Tetranychus urticae* and the beet armyworm, *Spodoptera exigua* |

**2. Entomopathogenic fungus (EPFs)**

The first insect pathogens found to cause diseases in insects were fungi because of their conspicuous macroscopic growth on the surface of their hosts. Agostino Bassi found that the fungus *Beauveria bassiana* was the causal agent of the white muscardine disease of the silkworm, *Bombyx mori*. Metschnikoff (1879) and Krassiltchik (1888) produced the fungus, *Metarhizium anisopliae* to control the wheat cockchafer, *Anisoplia austriacea* and sugarbeet weevil, *Cleonus punctiventris*. Only, Fungi provide the microbial control of plant sucking insects such as aphids and whiteflies which are not susceptible to bacteria and viruses.

**Classification**

1. Ascomycetes: *Cordyceps*
2. Basidiomycetes: *Septobasidium*
3. Deuteromycetes: *Beauveria, Metarhizium, Lecanicillium, Aspergillus, Hirsutella*
4. Phycomycetes: *Coelomomyces* infecting mosquitoes and *Entomophthora* infecting aphids

**Mode of action**

When spores of the fungus come in contact with the cuticle (integument) of susceptible insects, they germinate and penetrate either by germ tube or by infection pegs form appressoria and grow directly through the cuticle to the inner body of their host. The fungus proliferates throughout the insect's body, producing toxins and draining the insect of nutrients, eventually killing it. Death is caused by tissue destruction and, occasionally, by toxins produced by the fungus. Once the fungus has killed its host, it grows back out through the softer portions of the cuticle, covering the insect with a layer of mold. This downy mold produces millions of new infective spores that are released into the environment.

**Symptoms**

The point from where the fungus has penetrated the cuticle turns black. Some behavioral symptoms are also appeared such as loss of appetite, weakness, and restlessness. As the fungal hyphae grow, the host body becomes hard and no change occurs in the shape and structure of insects. However, some Lepidopterous larvae appear flaccid with fragile integument. Insect hosts filled with Zygomycota conidia turn yellow and host insects having resting spores turn black. The conidia of Deuteromycetes fungi also produce several colors in cadavers. The cadavers that are covered with white color may infected with *Beauveria* (white muscardine) whereas *Paecilomyces* conidia infected are usually gray, yellow, or in some cases, pink or pink-gray (yellow muscardine), conidia of *Nomuraea rileyi* and *Metarhizium anisopliae* are green, (green muscardine), the infection by *Sorosporella* (Deuteromycota) is called red muscardine while *Verticillium* known as white halo fungus because of the presence of white mycelium on the edges of scale.

**Table 2 : Examples of entomopathogenic fungus**

| SN | Entomopathogenic fungi | Target insect-pests |
|---|---|---|
| 1 | *Beauveria bassiana* | Lepidopteran larvae, aphids, whiteflies, mealy bugs, grasshoppers, locusts and crickets |
| 2 | *Metarhizium anisopliae* | White grubs, coconut rhinoceros beetle and sugarcane borers |
| 3 | *Verticilium lecanii* | *Coccus viridis*, whitefly, thrips, and aphids |
| 4 | *Hirsutella thompsonii* | Phytophagous mites |
| 5 | *Nomuraca rileyi* | Green clover worm, cabbage looper, cabbage worm, armyworms, corn earworm, and tobacco budworm |
| 6 | *Purpureocillium lilacinum* | Plant parasitic nematodes in the soil |

### 3. Entomopathogenic viruses (EPVs)

Viruses are intracellular and obligate parasites hence need a host for multiplication. Baculoviruses of the Baculoviridae family are the most common viruses pathogenic to insects. These are dsDNA viruses having bacilliform or rod-shaped infecting particles (virion) that are protected by polyhedron. Symptoms of Baculovirus infections were first recognized in silkworms in the 16$^{th}$ century.

**Classification**

1. Non-Inclusion viruses (NIV): non-occluded virus particles (*Orrh*V)
2. Inclusion viruses (IV): occluded virus particles
   i. Granulosis viruses (GV)
   ii. Polyhedrosis viruses (PV)
      a) Nuclear polyhedrosis viruses (NPV)
      b) Cytoplasmic polyhedrosis viruses (CPV)

**Mode of action**

Nucleopolyhedroviral occlusion bodies (OBs) are polyhedral proteinaceous bodies, mainly comprised of crystalline polyhedrin that surrounds occlusion derived virions (ODVs). During primary infection, OBs are ingested during feeding on contaminated foliage. OBs are solubilized in the insect midgut and release ODVs that pass through the peritrophic membrane and fuse with the microvilli of midgut epithelial cells. Nucleocapsids travel to the nucleus where they release the viral genome to initiate replication. Virus replication occurs in virogenic stroma. Progeny nucleocapsids assemble and bud through the basal membrane during which they acquire an envelope containing GP64 or F fusion protein present in the virus-modified cell membrane. During the secondary phase of infection, these budded virions (BVs) disperse in the haemolymph or along the cells of the insect tranquil system (traqueoblasts) and enter cells by endocytosis and replicate in the nucleus. Newly assembled

nucleocapsids may bud out of the cell or may be enveloped to form ODVs that are occluded into OBs. At the end of the infectious cycle OBs accumulate in the nucleus. Upon death the larvae typically hang from the uppermost leaves of the host plant, the larval tegument ruptures and releases OBs that contaminate foliage for further cycles of horizontal transmission (Williams *et al.,* 2017).

**Symptoms**

Infected larvae will have a characteristic shiny-oily appearance and they stop feeding. It will become extremely fragile to the touch, and after rupturing it releases fluid filled with infective virus particles. It climbs to the top of the crop canopy, becomes limp and hangs from the upper leaves or stems, hence the common name "caterpillar wilt" or "tree top" disease is given. The NPV infected larvae (peros) may initially turn white and granular or very dark.

**Table 3 : Examples of entomopathogenic viruses**

| SN | Entomopathogenic virus | | Target insect-pests |
|---|---|---|---|
| 1 | Nuclear Polyhedrosis Virus (NPV) | *Hear*NPV | *Helicoverpa armigera* |
| | | *Spli*NPV | *Spodoptera litura* |
| 2 | Granulosis Virus (GV) | *Chin*GV | Sugarcane early shoot borer |
| | | *Chsa*GV | Sugarcane internode borer |

**Some important points**

- ✓ Dose of virus: Larval equivalent (LE), one LE (Three fully grown-up virus infected larvae)
- ✓ Indian standard specifications for baculovirus registration:
    - NPVs = $1 \times 10^9$ POBs/ml or g (Recommended dose: 250-500 LE/ha)
    - GVs = $5 \times 10^9$ capsules/ml or g

According to Kabaluk *et al.* (2010), the whole biopesticide market accounts for 60% bacterial biopesticides, followed by fungal (27%), viral (10%) and other (3%) biopesticides.

**4. Entomopathogenic nematodes (EPNs)**

Entomopathogenic nematodes are soil-inhabiting, lethal insect pathogens, that live inside the body of their host, and so they are designated endoparasites. The most commonly studied genera are the Steinernematidae (DD-136) and Heterorhabditidae. The only stage that survives outside of a host is the non-feeding third stage infective juvenile (IJs) or dauer juvenile. The IJs of *Steinernema* and *Heterorhabditis* carry bacterial symbionts of the genus *Xenorhabdus* and *Photorhabdus*, respectively in their intestinal tract.

**Host finding mechanism of EPNs**

Only the third ($3^{rd}$) instar infective juvenile (IJ) (dauer) of entomopathogenic nematodes will survive in the soil and find and penetrate the insect host. IJ finds its hosts in the soil through ambushing and cruising strategies.

1. **Ambushing:** The IJs remain in the same spot for a long period waiting for the prey to cross the boundary of their strike area. Ambushers are also capable of nictate, *i.e.*, standing on their tails with more than 75 percent of the body held straight. Ambusher species include *Steinernema carpocapsae* and *S. scapterisici*; they most effectively control insect pests that are highly mobile at the soil surface, such as cutworms, armyworms and mole crickets.
2. **Cruising**: The cruisers are highly mobile in the environment in search of hosts throughout the soil profile hence they may become prey themselves. They largely use long-range chemical cues (carbon dioxide, vibration and other volatiles released by the host) to discover the location of resources. Cruiser species include *Heterorhabditis bacteriophora* and *S. glaseri*; they most effectively control sedentary and slow-moving insect pests at various soil depths, such as white grubs and root weevils.

✓ *S. riobrave* and *S. feltiae* do a bit of both ambushing and cruising.

**Mode of action**

After locating the suitable host, the IJs invade it through natural openings such as mouth, spiracles, anus, or thin areas of the host cuticle and penetrate the host hemocoel. The IJs recover from their developmental arrestment, release the symbionts and bacteria and nematodes cooperate to overcome the host immune response. The bacteria propagate and produce a biostatic substance that rapidly kills the host and protects the cadaver from colonization by other microorganisms. The nematode starts developing, feeds on the bacteria and host tissues metabolized by the bacteria and passes through 1-3 generations. Depleting food resources in the host cadaver leads to the development of new generations of IJs that emerge from the host cadaver in search of a new host. EPNs are susceptible to desiccation and hence soil moisture must be high to achieve full benefit from their application.

**Symptoms**

Steinernematids nematodes, infected larvae turn creamy/dark brown and Heterorhabditid nematodes infected larvae will turn reddish/purplish. The sticky nature of infected cadavers is the characteristic of *H. bacteriophora* is also used in the identification. The important characteristic of the symbiotic bacteria (*Xenorhabditis luminescence*) of *Heterorhabditis* spp. is its ability to fluorescent. The entire infected insect cadaver glowed in the dark and the infected larvae can be easily detected. Insects die within 24 hours of infection.

**Table 4: Examples of entomopathogenic nematodes**

| SN | Entomopathogenic nematodes | Target insect-pests |
|---|---|---|
| 1 | *Steinernema carpocapse, S. scapterisci, S. masoodi* | Lepidoteran larvae |
| 2 | *S. feltiae* | Dipteran larvae |
| 3 | *Heterorhabditis indica* | White grub, *Galleria* larvae |

**5. Entomopathogenic protozoa**

The majority of the entomopathogenic protozoa are described from the phyla Apicomplexa and Microspora. The efficiency and control potential of protozoans is considered to be limited because of the chronic nature of infection produced in their host.

**Mode of Action**

It enters the insect body via the gut wall, spreads to various tissues and organs, and multiplies, sometimes causing tissue breakdown and septicemia**.** Protozoa kill the insects either directly or by reducing the fecundity of the adult. Their effect on the host is chronic. They prolong the larval life in the field, thus exposing the insect longer to predators and parasitoids, making them susceptible to adverse weather and other mortality factors. Hence, these are called debilitating infections. They are always associated with other pathogens. These are relatively slow acting organisms, taking days or weeks to debilitate their host.

**Symptoms**

The infected insect becomes whitish, is reduced in size, and remains in immature stages much longer than a healthy insect. The protozoal spores are present in fat, midgut and haemolymph may cause these structures to turn milky colored. The integument of infected insects remains firm and does not easily disintegrate. No toxins have been detected in protozoal infection in insects, but toxins may be produced by microsporidia that cause tumour like growth and inflammatory response in insects (Kooyman *et al.,* 2007).

**Table 5: Examples of entomopathogenic protozoans**

| SN | Entomopathogenic protozoa | Target insect-pests |
|---|---|---|
| 1 | *Paranosema locustae* | Grasshoppers, locusts and crickets |
| 2 | *Nosema melolonthae* | Chaffer beetles |
| 3 | *Vairimorpha necatrix* | Armyworms and cabbage looper |
| 4 | *Farinocystis triboli* | Red flour beetle |

**Factors affecting the efficacy of insect pathogens**

Several factors that should be considered when introducing the insect pathogen into an insect population, these includes:

1. The concentration of the insect pathogen must be high enough to produce infection in at least some of the insect hosts.
2. The host population in which the pathogen is to be applied should have a relatively high density in order to assure propagation of the pathogen and its survival from one generation to the next.
3. The insect pathogen should be applied when a susceptible stage of the host is present.

**CONCLUSION**

Insect pathogens offer effective alternatives for the control of many insect-pests. Their greatest strength is their safety, as they are non-toxic and non-pathogenic to animals and humans. Although not every insect-pests can be controlled using an insect pathogen, these products can be used successfully in place of more toxic insecticides to control many household pests and several important agricultural crop and forest insects. Because most insect pathogens are effective against only a narrow range of pests and vulnerable to rapid inactivation in the environment, users must properly identify target insect-pests and plan the most effective application. Consequently, insect pathogens are likely to become increasingly important tools in integrated pest management.

**REFERENCES**

Berliner, E. (1915). Ueber die Schlaffsucht der *Ephestia kuhniella* und *Bac. thuringiensis n. sp*. *Zeitschrift für Angewandte Entomologie*, 2(1), 29-56.

Ishiwata, S. (1901). On a kind of severe flacherie (Sotto disease). *Dainihon Sanshi Kaiho,* 114*,* 5.

Johnson, M. W. (2000). Lecture note: Biological Control of Pests. University of Hawaii at Manoa Honolulu, Hawaii, pp. 46-51.

Kabaluk, J. T.; Svircev, A. M.; Goette, M. S., & Woo, S. G. (2010). *The use and regulation of microbial pesticides in representative jurisdictions worldwide*. International Organization for Biological Control of Noxious Animals and Plants (IOBC), p. 99.

Kooyman, C.; Douro-Kpindou, O. K., & Poehling, H. M. (2007). Susceptibility of immature stages of *Schistocerca gregaria* and *Oedaleus senegalensis* to *Paranosema locustae* and its vertical transmission in infected adults. Retrieved from https://d-nb.info/986002828/34

Mishra, J., Tewari, S., Singh, S., & Arora, N. K. (2015). *Biopesticides: Where we stand?* In N. K. Arora (Ed.), *Plant microbes' symbiosis: Applied facets* (pp. 37-75). Springer.

Peng, T. L., Syazwan, S. A., & Lee, S. H. (2019). Soil-borne entomopathogenic bacteria and fungi. *Sustainability in Plant and Crop Protection,* 1(1), 23-41.

Prasad, T. V. (2019). *Handbook of Entomology (4th edition).* New Vishal Publication, New Delhi.

Sharma, L., Bohra, N., Singh, R. K., & Marques, G. (2019). Potential of entomopathogenic bacteria and fungi. *Sustainability in Plant and Crop Protection,* 1(1), 115-149.

Williams, T., Virto, C., Murillo, R., & Caballero, P. (2017). Covert infection of insects by baculoviruses. *Frontiers in Microbiology,* 8*,* 1337.