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Bacillus thuringiensis: A Microbial Biopesticide

(*Kiran Kumawat[1], Dr. Lalita Lakhran[2] and Dr. B. L. Naga[3])
[1]Department of Plant Pathology, Rajasthan College of Agriculture, MPUAT, Udaipur (Raj.)
[2]Department of Plant Pathology, Agricultural Research Station, Mandor, AU, Jodhpur (Raj.)
[3]Officer In charge, Agricultural Research Sub Station, Diggi, Tonk (SKNAU, Jobner, Jaipur, Raj.)
*Corresponding Author's email: kkumawatkiran666@gmail.com

Abstract
Biopesticides is an ecofriendly alternative to chemical pesticides, which encompasses a broad array of microbial pesticides, biochemicals derived from micro-organisms and other natural sources. *Bacillus thuringiensis* has been widely used to control insect pests important in agriculture, forestry, and medicine. In India, some of the biopesticides like *Bt*., NPV, neem-based pesticides, etc. have already been registered and are being practiced. Biopesticides are mass produced biologically based agents used for the control of plant pests. The development of a great variety of matrices for support of the spore-crystal complex enables many improvements, such as an increase in toxic activity, higher palatability to insects, or longer shelf lives. These matrices use many chemical, vegetable or animal compounds to foster contact between crystals and insect midguts, without harming humans or the environment.

Introduction
The bacterium *Bacillus thuringiensis* was discovered by Shigetane Ishiwata in 1901 and rediscovered by Berliner ten years later. The bacterium was isolated from diseased larvae of *Anagasta kuehniella*, and this finding led to the establishment of *B. thuringiensis* as microbial insecticide. *Bacillus thuringiensis* is a gram-positive spore-forming bacterium that produces crystalline proteins called delta-endotoxins during its stationary phase of growth. The crystal is released to the environment after lysis of the cell wall at the end of sporulation, and it can account for 20 to 30% of the dry weight of the sporulated cells. This bacterium is distributed worldwide. The soil has been described as its main habitat; however, it has also been isolated from foliage, water, storage grains, and dead insects, etc. Isolation of strains from dead insects has been the main source for commercially used varieties, which include toxin inserts itself into the membrane. During the intoxication process, in lepidopterans as in coleopterans, many histopathological changes have been described, including swelling and disruption of the microvilli, vacuolization of the cytoplasm, hypertrophy of epithelial cells and necrosis of the nuclei. Biopesticides fall into three major classes, microbial pesticides, biochemical pesticides and Plant-Incorporated-Protectants (PIPs). The potential benefits to agriculture and public health programmes through the use of biopesticides are considerable. The total world production of biopesticides is over 3,000 tons/year, which is increasing at a rapid rate. India has a vast potential for biopesticides. However, its adoption by farmers in India needs education for maximizing gains. The market share of biopesticides is only 2.5% of the total pesticide market. The stress on organic farming and on residue free commodities would certainly warrant increased adoption of biopesticides by the farmers. Biopesticides being target pest specific are presumed to be relatively safe to non-target organism including humans. However, in India, the registration committee requires the data on chemistry, bio

efficacy, toxicity and packaging and labelling, for registration. Pesticide Registration Committee has so far not approved any guidelines for the registration of GM biopesticides.

Microbial biopesticides

It consists of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pests.

History

1901- Dr. Ishiwata - discovered bacterium *Bacillus thuringiensis*
1911 - Insecticidal activity - Berliner (Germany)
1920s - First time use as insecticides.
1960s - Spore-based formulations prepared
1970s - Primary insecticidal activity - delta (d) endotoxins.
1977- 13 *Bt*. subspecies described - all toxic to lepidopteran larvae
1977- Discovery of subspecies toxic to dipteran insects.
1983 - Discovery of subspecies toxic to coleopteran insects.
1996- 1st commercialisation of Cotton, Maize and Potato

Scientific classification of *Bacillus thuringiensis*

Kingdom	:	Eubacteria
Phylum	:	Firmicutes
Class	:	Bacilli
Order	:	Bacillales
Family	:	Bacillaceae
Genus	:	*Bacillus*
Species	:	*thuringiensis*

Characteristics of *Bacillus thuringiensis*

- *Bacillus thuringiensis* is a gram-positive spore-forming bacterium that produces crystalline proteins called delta-endotoxins during its stationary phase of growth. The bacterium was isolated from diseased larvae of *Anagasta kuehniella*, and established that *B. thuringiensis* as microbial insecticide. The crystal is released to the environment after lysis of the cell wall at the end of sporulation.
- During the spore form, this microbe can produce delta endotoxin, also known as crystal proteins (CRY proteins).
- The genes encoding the *Bt*. production of CRY proteins have been characterised.
- These genes have also been modified to enhance the selectivity of the insecticide.
- As a result of these characterisation and isolation of these genes, various techniques have been utilised to introduce these CRY genes into the plant genome.
- These CRY proteins are selective for the insects.
- After eaten by the insect, these CRY proteins form an active and effective insecticidal compound.

Features of the Cry toxin

Protein	Crystal shape	Size (kDa)	Target Pests
Cry 1	Bipyramidal	130-135	Lepidoptera larvae
Cry 2	Cuboidal	70	Lepidoptera & Diptera
Cry 3	Flat/Irregular	74	Coleoptera
Cry 4	Bipyramidal	70-133	Diptera
Cry 5	Multiple	33-130	Un-defined

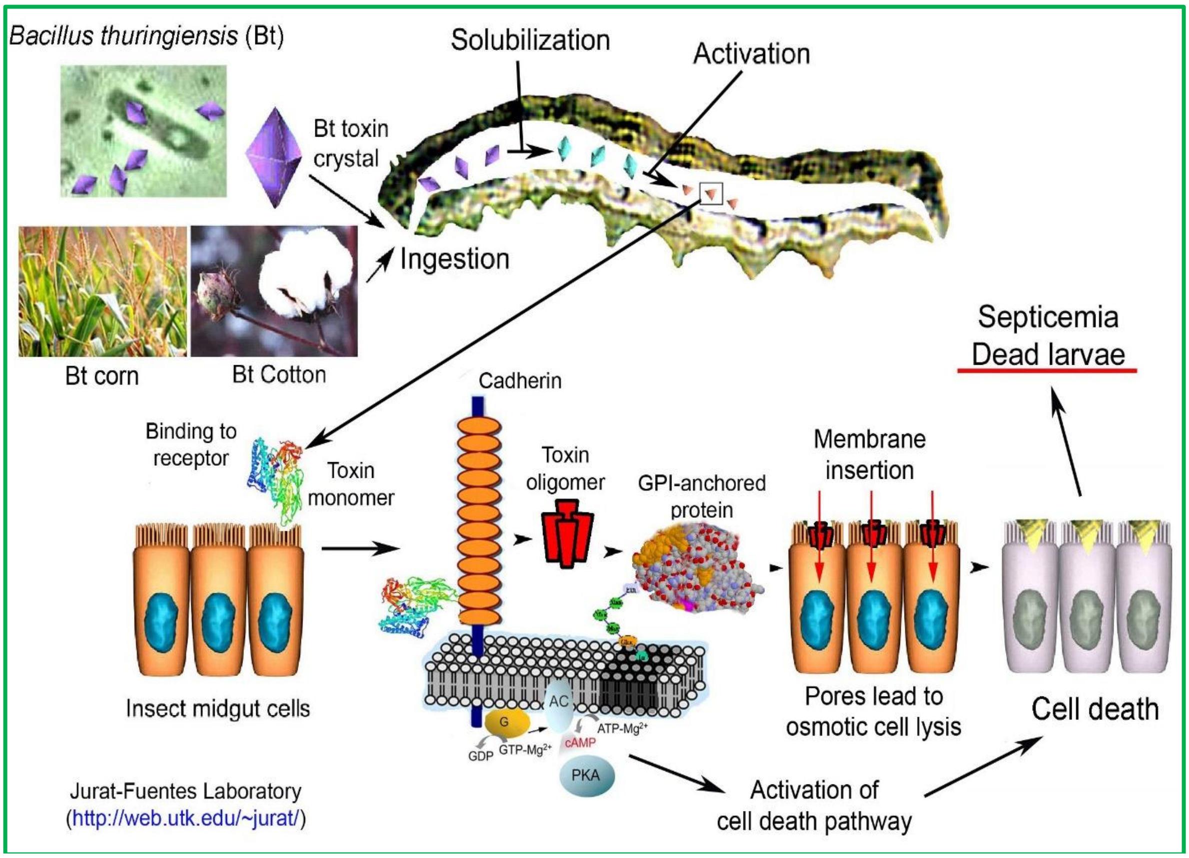
VIP 3 & 5 (Vegetative Insecticide Protein)	--	--	Lepidoptera larvae

Mode of Action of *Bacillus thuringiensis*

- It is highly specific for the control of insect species typically i) Lepidoptera (butterflies/moths), ii) Coleoptera (beetles), and iii) Diptera (flies/mosquitos)
- The *Bt*. is effective when eaten in sufficient quantity by the specific insects with an alkaline gut pH (typically butterflies, moths, beetles, flies and mosquitoes)
- After ingestion, the spores feed on intestinal flora and later burst releasing the protein toxin (Crystalline protein) damaging the gut lining
- Affected insects stop feeding and die from the combined effects of starvation, tissue damage and gastrointestinal infections by other pathogens like bacteria and fungi.
- The crystal is short lived as it breaks down after exposure to sunlight so it is appropriate to spray it in cloudy days.
- *Bt* is usually formulated with insect attractants to increase the probability that the target insect will ingest the toxin.

Effects on Insects

- Immediate cessation of feeding and general paralysis of the larval midgut.
- Subsequent symptoms include vomiting, diarrhea, general sluggishness, and general paralysis.
- Death may follow immediately or in an hour to a few days.
- Sublethal effects may include: reduced feeding, reduced larval and adult longevity, reduced fecundity, reduced larval and adult body weight.



Mode of Action

Commercial Products

- *Bt*. effective against lepidopteran pests hence can be applied for the control of cotton bollworms @1-2 kg/ha or 1 l/ha. ***Bt.*** products represent about 1% of the total 'agrochemical' market (fungicides, herbicides and insecticides) across the world. The commercial *Bt*. products are powders containing a mixture of dried spores and toxin crystals. They are applied to leaves or other environments where the insect larvae feed.
- Biobit WP, Dipel ES, Javelin, Thuricide 32B and Thuricide 32LV are some commercial formulations.

Some Commonly Available *Bt.* Varieties and Target Pests

***Bacillus thuringiensis* var. *tenebrionis*-** Colorado potato beetle & elm leaf beetle larvae
***Bacillus thuringiensis* var. *kurstak*i-** Caterpillars
***Bacillus thuringiensis* var. *israliensis*-** Mosquito, black fly & fungus gnat larvae.
***Bacillus thuringiensis* var. *aizawai*-** Wax moth larvae & various caterpillars especial DBM caterpillar.

Applications of Bacillus thuringiensis

- Transgenic plants development using *cry* genes
- Insecticidal activity against Lepidoptera, Coleoptera, Diptera, Hemiptera, Hymenoptera pests
- Other activities- Cytocidal activities, anti-cancerous, antifungal, antibacterial, anti-nematode, reduction activity and nanoparticles synthesis
- PGPR & Biofertilizers
- Indirect growth effect – antagonism, competition and nutrition

Advantages

- **Performance:** although each kilogram is more expensive, only a few grams per hectare are needed compared to 4 kg of chemical insecticides.
- **High toxicity:** a small amount is needed to kill pests.
- **Specificity:** it only kills the target organism.
- **It does not produce infections**: it is demonstrated that an infected larva does not harm other insects, animals, or even humans.
- **Limited time of permanence in the environment:** after 3 or 4 weeks of application, traces of the bioinsecticide are no longer found.
- **Few cases of resistance:** there are few cases reported, and only in extraordinary conditions there are certain degrees of resistance

Disadvantages

- Application with difficulty
- It is not easy to produce it
- Little diffusion and acceptance by producers
- Its quality could not be controlled. Sometimes it works, and sometimes it does not
- Variability in insect resistance
- Its use may be limited to faunas of a certain region

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