Effect of *Bacillus thuringiensis* (Bt) Toxin on Insects and Non-Target Animal Species: A Comprehensive Review

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Abstract: ***The increasing demand for sustainable pest management has led to widespread use of biological control agents such as Bacillus thuringiensis (Bt). Bt is a Gram-positive, spore-forming bacterium that produces insecticidal crystal proteins (Cry toxins) and cytolytic proteins (Cyt toxins), which exhibit high specificity toward several insect pests belonging mainly to Lepidoptera, Diptera, and Coleoptera. Bt-based bioinsecticides and genetically engineered Bt crops have become essential tools in integrated pest management programs worldwide. Despite their environmental advantages compared with synthetic pesticides, concerns persist regarding their ecological effects, especially on non-target organisms including beneficial insects, aquatic organisms, soil fauna, and vertebrates. This review provides a detailed synthesis of current knowledge on Bt toxins, including their origin, molecular structure, mode of action in target insects, and ecological effects on non-target species. Evidence from laboratory and field studies indicates that Bt toxins generally display high target specificity and minimal toxicity toward vertebrates and most non-target organisms. However, indirect ecological effects such as alterations in food webs, trophic interactions, and resistance development in pests remain important considerations. Continuous monitoring, ecological risk assessment, and responsible deployment of Bt technologies are essential to ensure their long-term sustainability and environmental safety.***

Keywords: *Bacillus thuringiensis*, Cry toxin, Bt crops, non-target organisms, ecological risk assessment, biological pest control

1. Introduction

Agricultural productivity worldwide is severely affected by insect pests, which are responsible for significant crop losses annually. Traditionally, chemical pesticides have been widely used to control these pests. However, excessive use of chemical insecticides has resulted in several environmental and ecological problems including pesticide resistance, contamination of soil and water resources, toxicity to non-target organisms, and negative impacts on human health (Romeis et al., 2006).

To address these concerns, researchers have focused on developing environmentally friendly pest management strategies. Biological control agents have emerged as promising alternatives to synthetic pesticides. Among these agents, *Bacillus thuringiensis* (Bt) has become one of the most widely used microbial insecticides in the world (Bravo et al., 2011).

Bt was first discovered in 1901 by the Japanese scientist Ishiwata Shigetane while investigating the cause of a disease affecting silkworms. Later, in 1911, the German microbiologist Ernst Berliner isolated the bacterium from diseased flour moth larvae and named it *Bacillus thuringiensis* after the German region of Thuringia (Schnepf et al., 1998).

The bacterium produces insecticidal proteins that are highly toxic to specific insect groups but relatively harmless to vertebrates and most beneficial organisms. These characteristics have made Bt an important component of integrated pest management (IPM) systems.

The discovery of Bt toxin genes led to the development of genetically modified crops expressing Cry proteins, commonly known as Bt crops. These crops include Bt cotton, Bt maize, and Bt brinjal, which provide protection against major insect pests throughout the growing season (James, 2017).

Despite their advantages, the large-scale adoption of Bt crops and bioinsecticides has raised concerns regarding their ecological safety. Questions have been raised about potential impacts on non-target organisms, biodiversity, and ecosystem functioning (Hilbeck & Otto, 2015).

This review aims to synthesize current knowledge on the effects of Bt toxins on target insects and non-target organisms, highlighting both benefits and potential ecological risks.

2. Biology and Classification of *Bacillus thuringiensis*

2.1 Taxonomy and Characteristics

Bacillus thuringiensis belongs to:
- Domain: Bacteria
- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Bacillaceae

Bt is closely related to *Bacillus cereus* and *Bacillus anthracis*. These bacteria share many genetic similarities but differ in their ecological roles and pathogenicity (Schnepf et al., 1998).

Bt is characterized by the production of parasporal crystalline inclusions during sporulation. These crystals contain insecticidal proteins known as δ-endotoxins.

2.2 Types of Bt Toxins

Bt produces several types of insecticidal proteins.

Table 1: Major Bt Toxin Types and Their Target Insects

Toxin Type	Stage Produced	Target Insects	Example
Cry toxins	Sporulation	Lepidoptera, Diptera, Coleoptera	Cry1, Cry2
Cyt toxins	Sporulation	Diptera	Cyt1A
Vip proteins	Vegetative growth	Lepidoptera	Vip3A
Sip proteins	Vegetative growth	Coleoptera	Sip1A

Cry proteins are the most widely studied and used toxins in Bt-based pest control.

3. Molecular Structure of Bt Cry Toxins

Cry toxins are protein molecules consisting of three functional domains:

1) **Domain I** – Responsible for pore formation in insect gut membranes
2) **Domain II** – Responsible for receptor binding
3) **Domain III** – Responsible for toxin specificity and stability

These domains play a crucial role in determining the insecticidal activity and specificity of Bt toxins (Bravo et al., 2007).

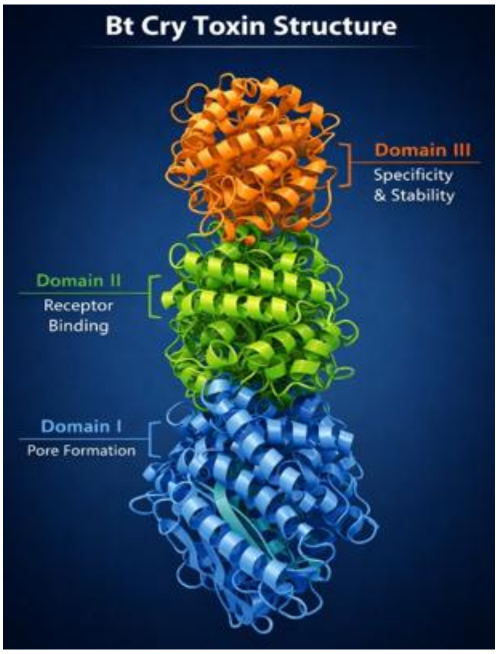

Figure 1: Structure of Bt Cry Toxin (Conceptual Diagram)

4. Mechanism of Action of Bt Toxins in Target Insects

The insecticidal action of Bt toxins involves several sequential steps.

Step 1: Ingestion
Insect larvae ingest Bt spores and crystalline protoxins while feeding on treated plants.

Step 2: Solubilization
The alkaline pH (pH 9–11) of the insect midgut dissolves the crystal proteins.

Step 3: Activation
Digestive enzymes convert protoxins into active toxins.

Step 4: Receptor Binding
Activated toxins bind to specific receptors located on epithelial cells of the insect midgut.

Step 5: Pore Formation
The toxin inserts into the cell membrane and forms pores.

Step 6: Cell Lysis and Death
The disruption of osmotic balance causes cell lysis, gut paralysis, and insect death (Soberón et al., 2009).

5. Effects of Bt Toxin on Target Insects

Bt toxins are effective against many agricultural pests.

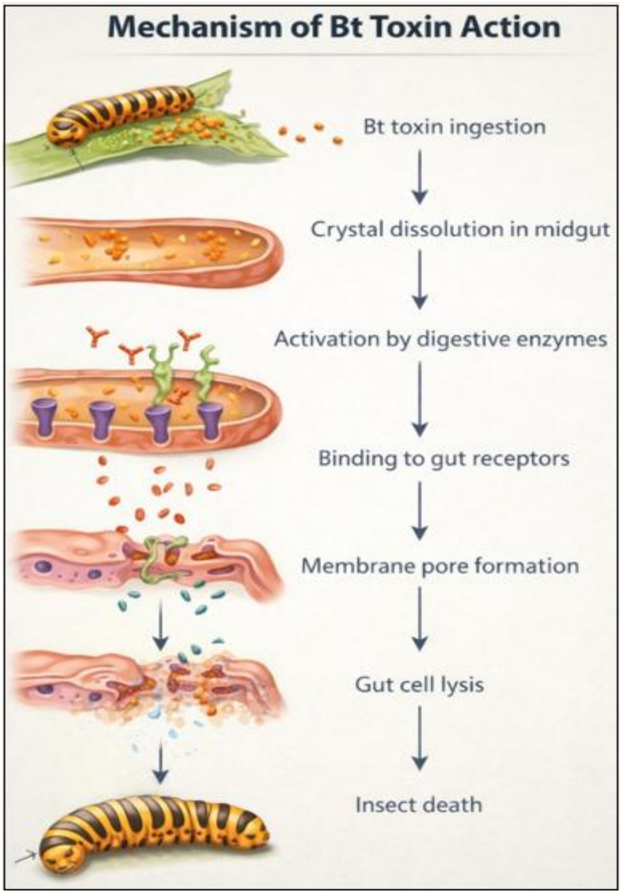

Figure 2: Mode of Action of Bt Toxin

Table 2: Major Agricultural Pests Controlled by Bt

Insect Species	Order	Crop Affected
Helicoverpa armigera	Lepidoptera	Cotton
Spodoptera litura	Lepidoptera	Vegetables
Ostrinia nubilalis	Lepidoptera	Maize
Aedes aegypti	Diptera	Mosquito
Leptinotarsa decemlineata	Coleoptera	Potato

Bt crops have significantly reduced pesticide use and increased crop productivity worldwide (Tabashnik et al., 2013).

6. Effects on Non-Target Insects

Although Bt toxins are highly specific, some studies have examined their effects on non-target insects.

One well-known example involved the monarch butterfly (*Danaus plexippus*). Laboratory experiments showed that Bt corn pollen could harm monarch larvae feeding on milkweed leaves (Losey et al., 1999). However, later field studies demonstrated that real-world exposure levels were much lower and unlikely to significantly affect monarch populations (Sears et al., 2001).
Other studies on insects such as bees, beetles, and flies have generally shown minimal adverse effects.

7. Effects on Beneficial Insects (Predators and Parasitoids)

Beneficial insects are essential components of agro-ecosystems.

Examples include:
- Lady beetles (*Coccinellidae*)
- Lacewings (*Chrysoperla spp.*)
- Parasitoid wasps (*Trichogramma spp.*)

Most studies indicate that Bt toxins have little direct toxicity to these beneficial organisms because they lack the specific gut receptors required for toxin binding (Romeis et al., 2006).

However, indirect effects may occur if predators consume prey that have ingested Bt toxins.

8. Effects on Aquatic Organisms

Bt formulations used for mosquito control can enter aquatic ecosystems.

Bt var. *israelensis* specifically targets mosquito larvae. Its toxins damage the gut epithelium of mosquito larvae, leading to rapid mortality (Lacey, 2007).

Most aquatic vertebrates such as fish and amphibians are not affected by Bt toxins due to the absence of suitable receptors. However, some aquatic insects may be sensitive at high concentrations.

9. Effects on Soil Organisms

Bt spores can persist in soil environments.

Studies examining soil fauna such as:
- Earthworms
- Nematodes
- Soil arthropods

generally show minimal negative effects.

Bt proteins degrade gradually through microbial activity and environmental factors.

10. Effects on Vertebrates

Extensive toxicological studies have demonstrated that Bt toxins are safe for vertebrates.

Reasons include:
- Vertebrates lack alkaline midgut conditions.
- They lack specific toxin receptors.
- Digestive enzymes rapidly degrade Bt proteins.

Therefore, Bt toxins pose minimal risk to humans, livestock, birds, and fish (Betz et al., 2000).

11. Ecological Implications

Although Bt technology has many environmental benefits, several ecological concerns remain.

11.1 Resistance Development

Continuous exposure to Bt toxins may lead to the evolution of resistant insect populations.
Management strategies include:
- Refuge planting
- Gene pyramiding
- Crop rotation

11.2 Food Web Effects

Bt toxins may move through food chains when predators consume herbivorous insects that fed on Bt plants.

However, the ecological significance of such trophic transfer remains unclear.

12. Advantages of Bt Technology

Major advantages include:
- Reduced chemical pesticide use
- High specificity toward pests
- Environmentally safe
- Compatible with IPM programs
- Increased agricultural productivity

13. Limitations and Concerns

Some limitations include:
- Evolution of resistant pest populations
- Potential effects on non-target insects
- Public concerns regarding genetically modified crops

14. Future Research Directions

Future research should focus on:
- Long-term ecological monitoring
- Improved resistance management strategies
- Development of next-generation bioinsecticides
- Integration with sustainable agricultural practices

15. Conclusion

Bacillus thuringiensis toxins have revolutionized biological pest control and represent one of the most successful examples of microbial biotechnology in agriculture. Their specificity toward insect pests, environmental compatibility, and safety for vertebrates make them valuable alternatives to chemical insecticides.

Although most studies indicate minimal risk to non-target organisms, continuous ecological monitoring and responsible deployment are necessary to ensure long-term sustainability.

Bt-based technologies will continue to play an important role in sustainable agriculture and integrated pest management systems.

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