Vol. 4 Issue- 10, June 2024 (e-ISSN: 2582-8223)

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multidisciplinary e-Newsletter

Eco-Friendly *Bt*-Based Bioinsecticide for Sustainable Pest Management

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ARTICLE ID: 18

Introduction:

Chemical insecticides have long been used to control insect pests in agriculture and combat insect-borne diseases. However, their widespread use has led to environmental pollution and adverse health effects. Additionally, the emergence of insect resistance has resulted in outbreaks of secondary pests. While microbial insecticides like *Bacillus thuringiensis* (*Bt*) have been proposed as alternatives, their limited spectrum of activity and low persistence hinder their efficacy. *Bt* stands out as a successful insect pathogen, constituting a significant portion of the insecticidal market. Its effectiveness lies in its ability to disrupt insect larval stages' midgut tissue, leading to septicemia. *Bt* produces insecticidal crystal inclusions containing toxins like *Cry* and *Cyt*, which selectively target specific insect species. These toxins function as pore-forming proteins that insert into the host membrane, ultimately causing insect death. The introduction of *Cry* toxins into transgenic crops has revolutionized pest control in agriculture, reducing reliance on chemical insecticides. Recent research has delved into the mode of action of *Cry* toxins across different insect orders, identifying insect midgut proteins that bind to these toxins and mediate their toxicity and specificity. Genetic studies have also explored mechanisms of resistance to *Cry* toxins and their practical applications in pest management. Overall, *Bt* represents a promising avenue for sustainable pest control, offering targeted and effective solutions while minimizing environmental and health risks associated with chemical pesticides.

History:

The historical perspective of *Bacillus thuringiensis* (*Bt*) dates back to ancient Egypt, where *Bt* spores may have been used. In the modern era, *Bt* was first discovered in 1901 by Ishiwata Shigetane as the cause of silkworm larva death. Ten years later, Ernst Berliner identified a similar bacterium from Mediterranean flour moth larvae, naming it *Bt* Berliner. Berliner observed crystal inclusion bodies within sporulated cells but did not initially link them to

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pathogenicity. However, subsequent studies confirmed these crystals' insecticidal activity. Commercial *Bt* insecticides emerged in 1938, gaining traction in the 1950s with large-scale fermentation studies. Edward Steinhaus pioneered commercial *Bt* insecticides in the USA in 1958. By 1961, *Bt* products like Thuricide and Dipel were in the market, targeting lepidopteran pests. Despite initial challenges, strain improvement efforts in the 1960s led to more potent *Bt* products. Today, *Bt* insecticides, primarily derived from *kurstaki* HD1, continue to evolve, aided by advancements in biotechnology since the 1980s.

Classification:

Bacillus thuringiensis (*Bt*) strains produce two types of toxins: *Cry* and *Cyt* proteins, also known as delta-endotoxins. Additionally, certain toxins, such as secreted insecticidal protein (Sip), are produced during the vegetative phase. The *Cry* genes encode intestinal cry protein (ICP) toxins with a broad spectrum of insecticidal activity. There are over 800 *Cry* genes classified into 75 families, along with *Cyt* and *Vip* genes grouped into different families. Initially categorized into four classes based on their amino acid sequence homology and insecticidal properties, these toxins have since been further classified based on their biological activity and amino acid sequence similarity. Another classification method by the *Bt* toxin Nomenclature Committee uses phylogenetics to group toxins into three-domain groups, bin groups, and epsilon toxin (ETX) groups. The *Cyt* genes encode non-specific cytolytic factor toxins that enhance the insecticidal properties of *Cry* toxins, particularly against dipterans, helping to decrease insect resistance.

Mode of Action:

In 1956, Tom Angus identified *Cry* proteins as responsible for the insecticidal action of *Bt*. Further research revealed varying activity spectra within and between insect orders, prompting investigations into the genetic basis of these differences. The mechanism of toxicity involves *Cry* proteins acting as stomach poisons, causing lysis of epithelial cells. After ingestion, the crystals dissolve in the intestinal tract, activated by gut pH. Proteolytic cleavage by insect midgut proteases activates protoxin molecules, reducing their molecular weight. Activated *Cry* toxins pass through the peritrophic membrane and bind to specific receptors on the midgut microvilli, leading to irreversible insertion into the cell membrane and epithelial cell lysis. This paralysis of the insect's digestive system stops feeding, potentially leading to death.

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Additionally, ingestion of *Bt* spores along with crystals may enhance the toxic effect by causing septicemia due to bacterial development.

Applications:

- **Transgenic Crop:** *Bt* toxin genes have been extensively utilized to confer resistance to pests in crops. The adoption of *Bt* crops has led to a significant reduction in pesticide application. Various *cry* (*cry1Ab*, *cry1F*, and *cry1Ac*) and *vip* (*vip3A*) genes have been employed to enhance resistance to lepidopteran pests, with some *Bt* crops incorporating multiple genes to delay potential pest resistance. *Bt* crops also utilize anti-coleopteran genes (*cry3A* and *cry34Ab1*–*cry35Ab1*) to combat coleopteran pests.
- **Nematicide:** Studies have demonstrated the efficacy of *Bt* against various nematode species, including *Meloidogyne incognita*, which causes substantial damage to crops like tomatoes and potatoes. *Bt* strains produce crystal proteins with potent nematicidal effects, offering promising solutions for nematode management.
- **Acaricide:** Although research on the acaricidal activity of *Bt* is limited, notable findings have been reported. For instance, studies have demonstrated the efficacy of *Bt* strains such as var. *kurstaki* and var. *israelensis* against tick species like *Argas persicus* and *Hyalomma dromedarii*.
- **Plant Growth-Promoter:** Certain strains of *Bt* exhibit plant growth-promoting characteristics, offering additional benefits beyond pest resistance. For example, *Bt* strain NEB17 has been shown to significantly enhance soybean nodulation, growth, and yield parameters when co-inoculated with *Bradyrhizobium japonicum* onto soybean plants. Moreover, *Bt* strains have been found to produce metabolites like indole-3-acetic acid (IAA) and phosphate-solubilizing enzymes, which contribute to improved plant growth and stress tolerance.
- **Antagonist of Plant Pathogenic Fungi:** While Cry proteins synthesized by *Bt* do not exhibit antifungal activity, some *Bt* strains produce compounds like cell wall-degrading enzymes, lipopeptide fengycin, volatile organic compounds (VOCs), and signalling molecules that induce systemic resistance in plants against fungal pathogens. *Bt* strains have shown efficacy against various plant pathogenic fungi, including Fusarium, Sclerotium, Colletotrichum, Rhizoctonia, and Botrytis. For instance, chitinase-producing

Bt strains demonstrated high antifungal activities against *Fusarium* spp., *Sclerotium* spp., *Penicillium chrysogenum* (causal agent of human disease), and *Botrytis cinerea*.

- **Antagonist of Pathogenic Bacteria:** Some *Bt* strains produce bacteriocins and N-acylhomoserine lactone (AHL)-degrading enzymes, which can combat plant and human pathogenic bacteria. *Bt*-derived bacteriocins have been effective against plant pathogenic bacteria like *Agrobacterium tumefaciens* and *Pseudomonas* spp. Moreover, the presence of *Bt* in mixtures with other antagonists improved control against bacteria like *Ralstonia solanacearum* in various crops.
- **Biosynthesis of Metal Nanoparticles:** Certain *Bt* strains have been utilized for the green synthesis of metal nanoparticles, including silver and cobalt nanoparticles, which have shown larvicidal activities against vector mosquitoes. *Bt* strains have been employed to synthesize silver nanoparticles with high toxicity against multi-drug-resistant human pathogenic bacteria. Similarly, cobalt nanoparticles synthesized by *Bt* exhibited larvicidal activities against malaria and dengue vectors.
- **Bioremediation of Heavy Metals and Pollutants:** Some *Bt* strains possess the ability to degrade or mineralize toxic heavy metals, pesticides, herbicides, and petroleum derivatives, offering potential for environmental cleanup. *Bt*-based bioremediation has been reported for various pollutants, including heavy metals like arsenic, cadmium, lead, pesticides like chlorpyrifos, herbicides, petroleum pollutants, and organic wastes.
- **Anticancer Characteristics:** Certain *Bt* Cry proteins known as parasporins exhibit cytocidal activity against human cancer cells without affecting normal cells, making them potential candidates for targeted anticancer therapy. Parasporins have demonstrated anticancer activities against a wide range of cancer cell lines, including cervical, leukemia, prostate, breast, and colon cancer cells, suggesting their potential as future anticancer pharmaceuticals.

Conclusion and Future Directions:

The future of *Bacillus thuringiensis* (*Bt*) in agriculture faces both promise and challenges. Despite opposition, the adoption of transgenic plants with *Bt* toxins by farmers is remarkable. However, insect resistance to *Bt* toxins poses a threat, requiring reinforced precautions and diversified pest control methods. Future refinements may involve using site-specific promoters and incorporating multiple toxin genes in transgenic plants. Integrated pest control programs

(IPMs) incorporating *Bt* toxins are likely to increase, supported by investment shifts. The search for new *Bt* strains/toxins aims for greater efficacy and reduced application rates. Formulation improvements offer potential for enhanced cost-effectiveness. Research will continue to focus on microbiology, genetics, and ecology to advance our understanding of *Bt* applications. Overall, *Bt* remains a pioneering microbial control agent in sustainable agriculture.

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