

AGRO-BENEFICIAL EFFECTS OF BACILLUS THURINGIENSIS AGAINST INSECT PESTS

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Abstract

Bacillus thuringiensis is a biocontrol agent frequently employed in the management of insects in agricultural and forest ecosystems. The toxins of *B. thuringiensis* are highly selective for parasites, making them safer for the environment. They are frequently used as insecticides in organic cultivation to manage insect infestations. To naturally provide resistance to various insect predators, insecticidal genes are used to genetically modify consumable crops. Due to its insecticidal proteins, which render it an environmentally benign and valuable biopesticide, it has been used in agriculture for an extended period. *B. thuringiensis* is an insecticide that is particularly effective against a wide range of insect pests in the agricultural and medical sectors. In addition, insect predator resistance of genetically modified crops has been improved through the successful implementation of the *B. thuringiensis* toxins genes. Its potential as a natural fertilizer for enhancing crop growth, the development of transgenic plants, and other environmentally friendly endeavors. The insecticidal property of the bacterium is attributed to the presence of δ -endotoxins, specifically cry proteins, which are toxic proteins that control the insect population. Additionally, the toxic properties of specific vegetative and expressed insecticide proteins are demonstrated in relation to specific species. This review examines the most recent developments in *B. thuringiensis* toxins, including their functions, insecticidal activity, and application in agriculture.

Keywords: *Bacillus thuringiensis*, Insecticides, Biocontrol agents and Toxic proteins.

INTRODUCTION

Bacillus thuringiensis is a facultative anaerobic gram-positive bacterium commonly found in water, detritus, and plant surfaces. Crystal toxins, specifically δ -endotoxin, are classified into two distinct classes based on their structural differences.

B. thuringiensis was initially identified as an insect disease. The insecticidal properties of the substance are thought to be primarily or entirely attributed to its parasporal crystals, depending on the species of the insect. The objective of these bioinsecticides was to suppress specific insect species from the Coleoptera, Diptera, and Lepidoptera orders. Insecticides derived from the ubiquitous soil bacteria *B. thuringiensis* are increasingly being used in pest management. *B. thuringiensis* generates β -endotoxins that are also known as insecticidal crystal proteins (Abbas, 2018)¹. They are highly toxic to specific pests but do not pose a threat to humans, the preponderance of beneficial insects, or other nontarget species. After activation by proteases in the insect midgut, toxins from *B. thuringiensis* adhere to the outer brush membrane of the epithelium. Technological advancements, including the expression of *B. thuringiensis* toxin genes in recombinant microorganisms and cereal plants, should be implemented to increase the utility of *B. thuringiensis*. In the future, products were created that encapsulated *B. thuringiensis* toxin crystals is a distinct bacterial strain can produce *B. thuringiensis* toxins by encapsulating them within the cells. *B. thuringiensis* foliar applications are still necessary in the context of integrated pest management techniques, particularly for low commodity volumes. A protein expression system based on *B. thuringiensis* spores was recently described *B. thuringiensis* does not appear to have any unique advantages over

these species and the spores are currently being investigated as probiotics and the potential of *B. thuringiensis* toxin as an antitumor remedy (Bandopadhyay, 2020)².

Insect Pests in Agriculture

B. thuringiensis is frequently used as an alternative to chemical remedies in crop protection techniques because of its exceptional non-toxicity to human. It is highly toxic to blackfly and mosquito larvae, which are transmitters of tropical diseases such as malaria, onchocercosis, and dengue fever. *B. thuringiensis* may create new commercial opportunities. Pesticide regulations prohibit the use of non-target pesticides on specific categories of crops (Avinash et al., 2024)³. In 2001, the United States cultivated more than 20,000 hectares of tomato and brassica harvests, which constituted 60 % of the total area according to tomato plants and 40 % of the area according to brassica, respectively. Furthermore, 23,000 hectares of apple orchards and 35,000 hectares of almond orchards accounted for 18 % and 13 % of the total area under these trees, respectively, while 40,000 hectares of vines accounted for 10 % of the total area under vines. *B. thuringiensis* was administered to nearly 90 % of the 170,000 hectares of potential larval reproductive sites from 1988 to 1999. Insects have an extraordinary ability to develop resistance to a wide variety of insecticidal substances. The growth parameters of *B. thuringiensis* were meticulously optimized to optimize the yields of its toxins. These plants are economically valuable and serve as effective biological control agents against hazardous insect invaders. Scouting pests and determining appropriate insect pest infestation levels are viable alternatives (Vedha et al., 2023)⁴.

Bacillus thuringiensis as an Insecticide

The primary mechanism of *B. thuringiensis* is the disruption of epidermal cells in the midgut. It does not penetrate the cytoplasm and columnar cells exhibit enlargement and blabbing by disintegration. The metabolic degradation process is initiated by intestine cells as the concentrations of leucine and glucose decrease. After 30 min of lysis, cells begin to separate from the basement membrane. The entire body is rendered incapacitated within 1-7 h. Within 1-3 days, malnutrition results in mortality (Du et al., 2005)⁵.

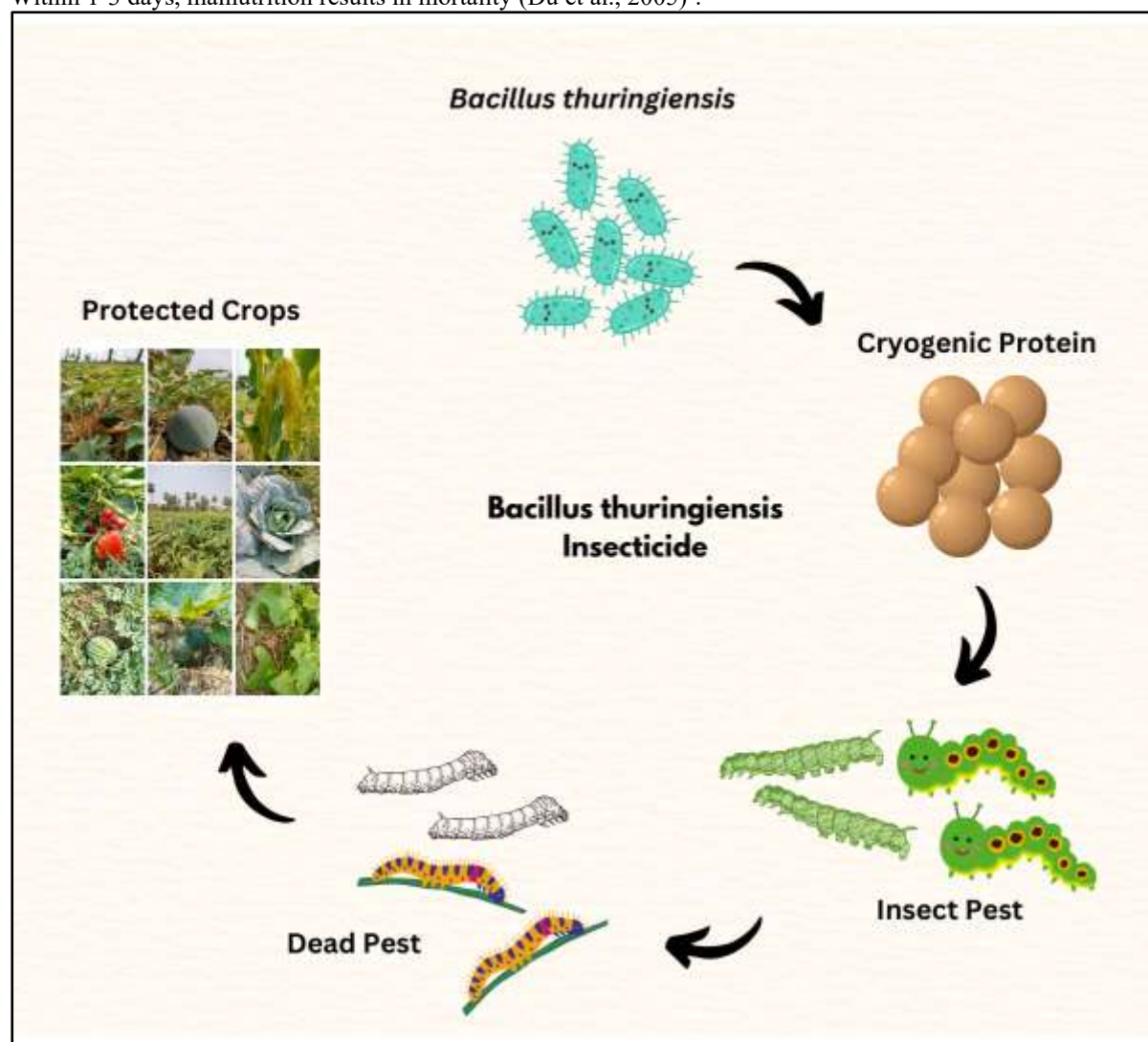


Figure 1: *Bacillus thuringiensis* as an Insecticides to Control Insect Pests

The Role of *Bacillus thuringiensis* in Agriculture

B. thuringiensis toxins exert selective pressure on target populations by binding to specific receptors in midgut epithelial cells, thereby enabling the development of parasite resistance. Over the past 15 years, the area of land allocated for the production of *B. thuringiensis* crops has consistently increased, and an increasing number of countries have adopted them. Europe continues to implement an exceedingly convoluted response to genetically modified crops, although *B. thuringiensis* agriculture is expanding to every other continent. In 2009, *B. thuringiensis* was used in the cultivation of more than 50 million hectares, accounting for 36 % of all transgenic commodities. The comparative binding of brush borders and membrane-associated proteins to the *B. thuringiensis* compounds Cry1Ab and Cry1F through midgut tissues (Saranraj et al., 2024)⁶. *B. thuringiensis* crops had become substantially more widespread by 1998 because of the availability of evidence on the advantageous effects of *B. thuringiensis* transgenic technology on agriculture and ecology. In 2001, the Environmental Protection Agency (EPA) authorized the use of a tomato line that expresses Cry1Ac. Yield Gard Rootworm, which produced an artificial variant of the cry3Bb1 gene from *B. thuringiensis* and Bollgard II cotton from Monsanto, which expressed two *B. thuringiensis* toxins, Cry1Ac and Cry2Ab, were approved in 2002, marking a significant milestone (Hong et al., 2005)⁷.

***Bacillus thuringiensis* in Genetically Modified Plants**

B. thuringiensis toxins exert selective pressure on target populations by binding to specific receptors in midgut epithelial cells. Over the past 15 years, the area of land allocated for the production of *B. thuringiensis* crops has consistently increased, and an increasing number of countries have adopted them. Europe continues to implement an exceedingly convoluted response to all genetically modified crops, despite the fact that *B. thuringiensis* agriculture is expanding to every other continent. In 2009, *B. thuringiensis* was utilized in the cultivation of more than 50 million hectares, accounting for 36 % of all transgenic commodities. This included 21.7 million hectares of cereals exclusively produced with *B. thuringiensis* and 28.7 million hectares of cereals overlaid with herbicide tolerance. The comparative binding of brush borders and membrane-associated proteins to *B. thuringiensis* compounds Cry1Ab and Cry1F through the midgut tissue of *Ostrinia nubilalis*, *Ostrinia furnacalis*, and *Diatraea saccharalis* (Tharani et al., 2024)⁸.

B. thuringiensis crops had become substantially more widespread by 1998 because of the availability of evidence regarding the advantageous effects of *B. thuringiensis* Transgenic technology on Agriculture and Ecology. In 2001, the Department of the Environment (EPA) approved the Herculex maize variety, which was developed in a partnership between Pioneer Hi-Bred (Johnston, IA) and Dow AgroSciences (Indianapolis, IN). It protected plants from the European maize borer, autumn armyworm (*Spodoptera frugiperda*), and black cutworm (*Agrotis ipsilon*) by expressing Cry1F. In 1998, the Environmental Protection Agency (EPA) authorized the use of a resistant insect tomato line expressing Cry1Ac (James, 2010)⁹. Yield Gard Rootworm, which produced an artificial variant of the cry3Bb1 gene from *B. thuringiensis* subsp., and Bollgard II cotton from Monsanto, which expressed two *B. thuringiensis* toxins, Cry1Ac and Cry2Ab, were approved in 2002, marking a significant milestone (Yuvalakshmi et al., 2024)¹⁰.

***Bacillus thuringiensis* as a Biopesticide**

The most recent developments in contemporary biotechnology, particularly in the agricultural sector are currently being watched worldwide. In the last 22 years, the global average area of transgenic cereals has increased to 191.7 million hectares. Due to their commercialization, these commodities have been introduced to 70 countries, making them the most rapidly adopted (Dinesh et al., 2024)¹¹. Herbicide capability and insect resistance are the most frequently implemented traits of *B. thuringiensis* in plant genetic engineering. To improve their pest-resistant properties, numerous cultivars incorporate the toxin DNA of *B. thuringiensis*. Potato, soybean, corn, maize, aubergine, cotton, rice, poplar, tomato, and soybean are among the approximately 198 varieties of *B. thuringiensis* cultivars that have been developed since the commercialization of these commodities (Jouzani et al., 2017)¹².

During the sporulation process, *B. thuringiensis* produces aggregated crystals, known as cryotoxin. A parasporal substance and an insecticide amino acid with a crystal-like structure are the components of these crystals. According to Mishra et al.,¹³ these compounds have a substantial effect on specific species, such as Nematoda, Lepidoptera, Coleoptera, Diptera, and Hymenoptera. In cultivars genetically modified (GM), one or more VIP or cry genes are present. There are 42, 115, and 30 varieties of cotton, maize, and potatoes, respectively, that are the most extensively authorized *B. thuringiensis*-GM commodities (Subhalakshmi et al., 2024)¹⁴.

As of now, there are 111 *B. thuringiensis* anti-coleopteran gene variants that have been approved, some of which target noxious lepidopterans. A total of approximately 34 and 60 genetically modified cultivars that are resistant to coleopteran parasites are developed using the two genes cry34Ab1–cry35Ab1 and cry3A, respectively (Muddanuru et al., 2019)¹⁵. Cry1Aa inhibits lepidopteran insect invasions. The CryII class of toxins produced by *B. thuringiensis* is by its dual selectivity for Coleopteran and Lepidopteran insects. The toxicity of the CryIIa protein from an Argentinian strain of *B. thuringiensis* against agricultural insects belonging to the Noctuidae, Curculionidae, Tenebrionidae, and Tortricidae families (Nelson, 2001)¹⁶.

The protein did not affect *Anthonomus grandis*, *Spodoptera frugiperda*, or *Alphitobius diaperinus*. It affected *Cydia pomonella*. Jayapriya et al., also demonstrated that the cryogel protein, which was extracted from *B. thuringiensis* can impede the proliferation of *S. litura*, *G. malonella*, *B. cucurbitae* and *C. pipens* larvae (Jayapriya et al., 2024)¹⁷. Plant breeders may possess the capacity to create cultivars that are resistant to these pathogens, as indicated by the above-mentioned information. It has been observed that cry genes that demonstrate resistance to nematodes and other invertebrates have not been commercialized in recent years. However, GM agriculture products also present potential health hazards to human and animals, despite their advantages (Paris et al., 2012)¹⁸. The consumption of genetically modified crops may increase the likelihood of developing of immune-resistant diseases. However, no reports of long-term effects or hazards have been published (Qi et al., 2016)¹⁹. Furthermore, the insecticide genes and amino acids of *B. thuringiensis* are used to generate spore-crystal complexes that generate a wide variety of Genetically Modified Crops. However, it is important to pursue toxins with a broad spectrum of action, despite the heterogeneity of the cryogel proteins, to have a diverse array of options for addressing insect resistance.

Application of *Bacillus thuringiensis*

Among the numerous bacterial species that are widely recognized for their capacity to promote plant growth are *Klebsiella* sp., *Pseudomonas* sp., *Rhizobium* sp., *Caulobacter* sp. and *Azotobacter* sp. *B. thuringiensis* is recognized for its insecticidal properties and ability to promote development of plants. The indirect effect is evident in the reduction of insect infection; however, certain studies suggest that the direct effect is accomplished through legume colonization, which results in an increase in nodulation and, as a consequence, the development of plants (Sahin and Ozturk, 2005)²⁰. This is advantageous because the mineral is abundant in soil; however, its assimilation is limited by its undissolved state. Similarly, the plant utilizes the siderophore-iron complex to assimilate iron, which is also difficult to dissolve. Siderophores, which are produced by microorganisms and are capable of binding to iron, facilitate iron assimilation. *B. thuringiensis* strain ATCC 33679 generates catecholate-type siderophore that exhibit a high affinity for iron, increasing its availability in plants (Saranraj et al., 2024)²¹. Tan et al.,²² evaluated the potential of *B. thuringiensis* to improve plant growth by using a charcoal-based biofertilizer on *Abelmoschus esculentus* in a two-year study. The nutritious and physiological features of the plants were considered during the investigation. They observed a significant increase in the length of the roots, weight of both the fruit and seed, diameter of the leaf, and height of the branch. Additionally, the protein content of the capsule increased by nearly 30 %, the protein content of the leaf increased by nearly 70 % and the soluble sugar content increased by over 60 % as a consequence of the use of *B. thuringiensis* as a plant fertilizer. These studies illustrate that *B. thuringiensis* is also an effective insecticide and can function as a biofertilizer that is beneficial for plant growth. Singh et al.,²³ demonstrated that coinciding with *B. thuringiensis* with *Rhizobium leguminosarum* resulted in an increase in the desiccated weight of pea plants and an improvement in nodulation. The co-inoculation of mycorrhiza with *Lavandula* plants resulted in increased oxidative metabolism and improved drought tolerance.

Sprays

Formulations of *B. thuringiensis* aerosols contain viable bacterial spores as active ingredients. The primary cause of insect mortality is the presence of Cry and Cyt ICPs. Insects with limited cryogenic protein sensitivity may also be affected by the inclusion of additional substances in *B. thuringiensis* insecticides. *B. thuringiensis* spores are essential for the lethality of parasites such as cotton bollworm, beet armyworm, and gypsy moth larvae. The production of additional insecticidal toxins and synergists is a consequence of the germination of *B. thuringiensis* spores, which initiate a vegetative growth cycle in response to intestinal injury (Saranraj et al., 2022)²⁴. A few examples of these include chitinase, phospholipases, β -exotoxin, zwittermicin A and Vegetative Insecticidal Proteins (VIPs). Around 1-2% of the \$8 billion annual pesticide spray market in the United States comprises sprays that contain *B. thuringiensis*. *B. thuringiensis* pesticides were previously expected to generate \$100 million in annual revenue; however, the introduction of Genetically Modified Organisms (GMO) containing ICP genes has resulted in a decline in sales to \$40 million (Marimuthu et al., 2024)²⁵. Half of the present sales are used to manage gypsy moths, spruce budworm, and other lepidopteran pests in Canadian woods. Sprays containing *B. thuringiensis* are administered sparingly and to limited areas. Vegetables, tree fruits, artichokes and berries are treated with conventional *B. thuringiensis* strains. Organic producers choose sprays that consist exclusively of nonsynthetic ingredients. *B. thuringiensis* is also used to prevent insect infestations in stored products, including wheat (Walker et al., 2003)²⁶.

Transgenic Plants

Approximately 12 million hectares of Genetically Modified Crops with *B. thuringiensis* ICPs are cultivated annually on a global scale to protect them from insects. These commodities are primarily grown in the United States. This trend is expected to continue as the average value of other transgenic commodities increases. In 2001, it is estimated that over 5 million producers worldwide were cultivating genetically modified crops, and the global area planted with transgenic crops is anticipated to have increased by at least 10 % since 2000. In March 2002, the Indian government announced that it would license *B. thuringiensis* cotton, thereby becoming one of the

countries to authorize the commercial use of genetically modified commodities (Wilson et al., 2006)²⁷. Currently, *B. thuringiensis* crops are genetically modified to generate a single ICP throughout the plant. The ICP gene's codon preference is regulated by a highly active plant promoter that is optimized for plant expression (Sujithra et al., 2024)²⁸. *B. thuringiensis* corn, which is cultivated in the United States, China, Argentina, Canada, for example, South Africa, Spain, Germany, and France, and *B. thuringiensis* cotton fibers, which are cultivated in the United States, China, Australia, Argentina, South Africa, and Mexico, are the primary transgenic crops that express the ICP genes. In the near future, *B. thuringiensis* crops, such as rice, canola, and a variety of vegetables and fruits, will be disseminated (Zhang et al., 2019)²⁹.

CONCLUSION

B. thuringiensis, a unique bacterium with substantial potential for agricultural development, is employed as a potential biopesticide to control pests in agriculture because of its unique insecticidal proteins. *B. thuringiensis* is the source of its toxic insecticides. The development of *B. thuringiensis*-GM crops, which are resistant to insect and plant pests but do not damage humans, the environment, or beneficial organisms, is a direct consequence of technological advancements. *B. thuringiensis* formulations are increasingly used in liquid suspensions and aerosols for commercial purposes. The extended production time of the contaminant renders it more cost-effective and efficient than chemical pesticides. This capacity to partially or completely replace a few most poisonous insecticides with chemical compounds is one of the primary advantages of microbial control agents. Additionally, the implementation of these biodegradable and safer biological pest control substances provides a variety of ecological advantages. The high level of specialization is one of these benefits, as the spreading or fatal consequences of these species are limited to some species. To augment agricultural productivity, *B. thuringiensis* has been used as a bacterium to facilitate plant growth. Application dosages, nitrogen content and stability specific to each crop are all equally important in ensuring the safety of sustainable agriculture. Environmentally preferable, cryotoxin is an exceptional insecticide that effectively combats various pests. It is relatively uncommon for resistance to develop. The use of agricultural products and hazardous chemical insecticides. In spite of this, the cryotoxins that have been identified thus far are ineffective in the management of specific insect infestations. If resistance develops, the cry genes of *B. thuringiensis* can be employed to produce proteins with increased toxicity against a variety of parasites and function as storage devices for cry toxins.

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