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Review Paper

"Bacillus thuringiensis": Role of its Toxin Gene in Agriculture

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Abstract
Presently, a number of approaches to pest control via genetic engineering have been developed and genetically engineered crops expressing insecticidal characteristics are under cultivation for the last 15 years.. One of themost notable examples, is the introduction of transgenic crops that are engineered to express a *Bacillus thuringiensis* endotoxins toxin that confers resistance to insect predation., Investments in agriculture helps millions earn a better living, grow more nutritious food, and build better futures. Agriculture is a proven pathway out of poverty with unbelievable potential for the future. .Bt is the most common environmentally-friendly insecticide used and is the basis of over 90% of the pesticides available in the market today. The *Bacillus thuringiensis* toxin is species-specific and non-pathogenic to humans, a concern which remains with chemical insecticides . Today versions of Bt cotton , Btcorn, Btpotatoes, BtBrinjal, Bt maize and many more are being grown in the United states, Canada ,Argentina, South Africa ,France and Spain. This review deals with the advantages of using *Bt* Products over chemical agents in agricultural field is any threat to the continuous use of *Bt* as Biological Control Agent in agricultural practices and the use of synergism between Bt Products or between Bt products and other substances. Finally the methods of application of *Bt* and its products in agriculture are also discussed.

Key words: *Bacillus thuringiensis, Crop plants, Endotoxins , Biological control, Synergism.*

Introduction
*Bacillus thuringiensis*was originally discovered in 1902 by a Japanese biologist Shigetane Ishiwatari who isolated it from diseased silkworm .In 1911. *Bacillus thuringiensis* was rediscovered in Germany by Ernst Berliner who isolated it as the cause of a disease called *Scahlaffsucht* in flour moth caterpillars.

It is a gram-positive, soil-dwelling, spore-forming, rod-shaped bacteria grouped into *Bacillus cereus* group of bacilli. It is approximately 1 µm in width and 5 µm in length and its life cycle consists of two phases as vegetative phase and sporulation phase.It is during in the sporulation phase ,Bt synthesizes intracellular parasporalcrystals of toxic glycoproteins(δ-Endotoxins),which is the distinctive feature between it and other members of the *Bacillus cereus* group. These δ-Endotoxins are responsible for making *Bt*an effective insect pathogen. Following ingestion, the alkaline environment of the insect midgut causes the crystals to dissolve and release their constituent protoxins. The protoxins are subsequently trimmed by gut proteases to an N-terminal, 60-65 kDa biologically active core toxins. The toxin binds to cadherinreceptors on the cell membranes of the midgut epithelial cells inserts itself into the membrane, and forms pores that kill the epithelial cells (and eventually the insect) by colloid osmotic lysis. In some insects the ingested endospores germinate, and bacterial growth is a factor of insect's death.Certain other bacteria closely associated with plant roots have been genetically engineered to contain this toxin and kill insects feeding on plants. .According to Pigott and Ellar (2007), most steps of toxin activation are still under discussion.

It has a circular chromosome and a GC-content of approximately 32%~35%. It has a genome size of between 5.2–5.8 Megabases. It is a facultative anaerobic organism (an organism that makes ATP by aerobic respiration if oxygen is present but can also switch to fermentation). It has many plasmids and Bt's strains harbors a diverse range of plasmids that vary in number and in size (2–200kb). The self-replicating plasmids are important to the organism's lineage and lifestyle

The insecticidal proteins produced in the crystal form constitute two different families, *Cry* and *Cyt*, which have been further classified on the basis of amino acid identity into about 300 *Cry*geneslik*e* cry1A,cry2A,cry3A,cry1c etc; which exist as cluster in operonsand 22 *Cyt*sub-groups. It has been reported that characteristic insecticidal *Cry* proteins were deposited in the form of crystal in the mother cell which starts from the onset of sporulation. Moreover *Cry* toxins *Bt* produces additional virulence factors including phospholipase C , proteases and hemolysins .These virulence factors are controlled by the pleiotropic regulator PlcR and it has been demonstrated that cytotoxicity of *Bt*is PlcR dependent . Because deletion of the *plcR* gene result in a drastic reduction in the virulence of *Bt*in orally infected insects. Due to this virulence property it is named as an insect pathogen. The Crytoxins acquired the scientific name Cry from the fact that they are found in the crystal while the Cyt-toxins acquired the scientific name Cyt because of their in vitro cytolyticactivity .*Cry* toxins have specific activities against insect species of the orders Lepidoptera(moths and butterflies) , Diptera (flies and mosquitoes) Coleoptera (beetles) Homoptera (wasps, bees, ants and sawflies) and nematodes. Thus *Bacillus thuringiensis*play an important reservoir of *cry* toxins for the production of biological insecticides andinsect resistant genetically modified crops.Spores and crystalline insecticidal proteins produced by *Bacillus thuringiensis* have been used to control insect pests since 1920. They are now used as specific insecticides under trade names such as Dipel and Thuricide.

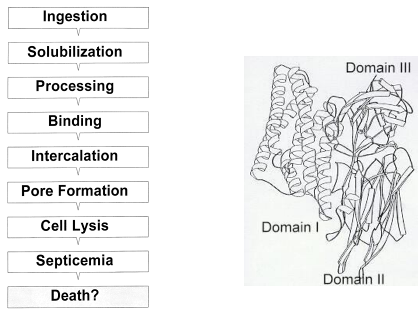

Figure 1: Different domains involved in the toxicity of B. thuringiensis toxin in the mid-gut of targeted insect. Source: Sharma et al., 2000

Commercial releases of *Bacillus thuringiensis* crops

Potato In 1995, potato plants were producing Bt toxin were approved safe by Environmental protection agency making it the first pesticide producing crop to be approved in USA

Cotton

In 1996, *Bt*-cotton was released to protect against tobacco budworm, and to a lesser extent, cotton bollworm and pink bollworm. In 1997, *Bt*-cotton was reported to give an average yield increase of 14%, with a reduction in insecticide use of 300 000 gallons. Approval for release has been granted in Australia, China, Mexico, South Africa and the USA.

Maize

Several companies have developed *Bt*-maize since1996 by licensing it to seed companies. Approval for release has beengranted in Argentina, Canada, Japan, the USA and within the European Community.. By 2009, these crops had been planted on over 334 million acres (135x106 hectares) worldwide. In the United States over 70% of the cotton planted and 63% of the corn planted is now with transgenic varieties

Brinjal

The Bt brinjal is a suite of transgenic brinjals (also known as an eggplant or aubergine) created by inserting a crystal protein gene (Cry1Ac) from the soil bacterium *Bacillus thuringiensis*.. The Bt brinjal has been developed to give resistance against lepidopteron insects, in particular the Brinjal Fruit and Shoot Borer (Leucinodesorbonalis)(FSB). Mahyco, an Indian Seed Company based in Jalna, Maharashtra has developed the Btbrinjal. The genetically modified brinjal event is termed Event EE 1 and Mahyco have also applied for approval of two brinjal hybrids. Some of the cultivars of brinjal include: Malpur local, Manjarigota, Kudachi local, Udupi local,, and Pabkavi local. It was approved for commercialization in India in 2009, but after a public outcry the Indian government applied a moratorium on its release.

Advantages of Using *Bt* Products Over Chemical Agents in Agricultural Practices.
Bacillus thuringiensis (Bt) has been used as a biopesticide in agriculture, forestry and mosquito control because of its advantages of specific toxicity against target insects, lack of polluting residues and safety to non-target organisms. In the past indiscriminate use of chemicals results in attack of Non-target organisms, including predators and parasites of pests, are highly dangerous to humans and animals in their concentrated forms. However a few studies have been done in order to find out the advantages of Bt products over chemicals. It has been found that modern genetic engineering allows the genes for Bt proteins to be inserted directly into crops, so that the plants themselves produce the insecticidal proteins. Unlike chemicals Bt insecticides are biodegradable and non-toxic, an improvement over strong chemical pesticides. These spores are approved for use in organic agriculture as natural pesticides.

Researchers have found Genetically engineered Bt crops have the increased advantages of :

- requiring less insecticide application with machines, leading to reduced labor and fuel costs;
- greater effectiveness in insect control because the crops continuously produce the Btproteins within their own tissues ;
- control of molds and fungi that can infect holes in the crops left by burrowing insects.

According to most published reports,Bt plants are one of the dominant genetically engineered crops grown on a large scale and in many regions of the world and they are being grown under various regional and climatic conditions. Thus the increased popularity of biological control agents over synthetic chemicals is because of the non-selective lethal effect of the latter agents. Various research have been carried out to check for the safety of *Bt*toxins from sprays or transgenic plants to non-target species in the environment and it has been shown to be mostly environmentally friendly without significant adverse effects.

B.t. is less toxic to mammals and shows fewer environmental effects than many synthetic insecticides. However, this is no reason to use it indiscriminately. Its environmental and health effects as well as those of all other alternatives must be thoroughly considered before use. B.t. should be used only when necessary, and in the smallest quantities possible. It should always be used as part of a sustainable management program.

Genetically modified organisms (GMO)

The first diagram represents the Non transgenic peanut leaves showing extensive damage from European corn borer larvae and the second diagram shows the Peanut leaves genetically engineered to produce Bt toxins are protected from herbivory damage. Here the genetic material from *Bacillus thuringiensis* (Bt) has been inserted so that the pea plants produce toxins effective against arthropod pests.

Threat to Continuous Use of *Bt*as Biological Control Agent

Plants expressing *Bt*toxins were among the first plant biotechnology products to be approved for commercial use. However, opposition against the use of transgenic *Bt*-plants arose due to the continuous use. The most important threats are:

- Toxins could negatively affect non-target animals such as predators or parasites that feed on the pest. There are also concerns that non-target organisms, such as pollinators and natural enemies, could be poisoned by accidental ingestion of Bt-producing plants. A recent study testing the effects of Bt corn pollen dusting nearby milkweed plants on larval feeding of the monarch butterfly found that the threat to populations of the monarch was low. . According to Griffitts et al. (2005), only non-vertebrates can be seen as potential target organisms for Bt endotoxins. However, Huffmann et al. (2004) raise questions beyond receptor-specific activity of Bt toxins also being relevant for vertebrates. In addition, Ito et al. (2004) show cytocidal activity on human cells. Taking into accord the question of certain factors influencing the toxicity of Bt toxins in non-target organism such as mammals, it is interesting that Thomas and Ellar (1983) show that the effect of certain Bt toxins (from B. thuringiensis var. israelensis), which, in their native (crystallized) form, show no toxicity in mammalians, can become highly toxic in an alkali-solubilized form (if being administered parenteral).

- In a purified form, some of the proteins produced by B.t. are acutely toxic to mammals. However, in their natural form, acute toxicity of commonly-used B.t. varieties is limited to caterpillars, mosquito larvae, and beetle larvae. B.t. is closely related to B. cereus, a bacteria that causes food poisoning and to B. anthracis, the agent of the disease anthrax. Few studies have been conducted on the chronic health effects, carcinogenicity, or mutagenicity of B.t. People exposed to B.t. have complained of respiratory, eye, and skin irritation, and one corneal ulcer has occurred after direct contact with a B.t. formulation. People also suffer from allergies to the `inert'' (secret) ingredients. People with compromised immune systems may be particularly susceptible to B.t. The earliest tests done regarding B.t.'s toxicity were conducted using B.t. varthuringiensis, a B.t. strain known to contain a second toxin called beta-exotoxin.This study found out that the beta-exotoxin is toxic to vertebrates, with an LD50 (median lethal dose; the dose that kills 50 percent of a population of test animals) of 13-18 milligrams per kilogram of body weight (mg/kg).

- Continuous exposure of pest insects to the *Bt*toxin would create a high selection pressure for the development of resistance against the toxin. It has been reported that viable Bt spores are known to exist for up to one year following application. Insect resistance to B.t. has been well documented. Genetic engineering may greatly expand use of Bt., speeding up the development of more resistance. Insects are highly adaptable and have evolved resistance to many chemical insecticides. In this context, *Bt*toxins are not expected to be different from other insecticides.

Laboratory studies have shown that resistance that is already present in the gene pool of a population can be selected for with purified toxins or *Bt* formulations in several insect species and for several different toxins. The occurrence of resistance in field populations in response to extensive applications of *Bt* sprays are rare, but it has been reported. Resistance in response to *Bt*-plants has not been reported to date, but of course this may be attributed to the fact that *Bt*-plants have not been around for very long. However, there is little doubt that the genetic potential for resistance is present. Many scientists, as well as members of environmental pressure groups, believe that continuous exposure to *Bt*-plants will lead to selection for resistance, and that the large scale introduction of *Bt*-crops endangers the durability of *Bt* as an insecticide, both in crops and in sprays. This would have an impact on the growers of transgenic *Bt*-crops as well as on organic, conventional and IPM farmers who use *Bt* spray.

There have been reports of insect populations resistant to a particular toxin showing resistance to other toxins to which they have not previously been exposed, a term known as 'cross-resistance' (Pereira *et al.* 2008; Sayyed *et al.* 2008; Gong *et al.* 2010; Xu *et al.* 2010). There have been a number of proposed modes of resistance of insect pests to *Bt* toxins including reduction of binding of toxins to receptors in the midgut of insects reduced solubilisation of protoxin, alteration of proteolytic processing of protoxins and toxin degration and or precipitation by proteases (Bruce et al. 2007).

- A particular concern with Bt crops is that the Bt genes could be spread to other plants through open pollination, which is known as "gene flow". Open pollination is when pollen is spread by the wind to any nearby plants, where insect resistance in these plants could then develop. The potential environmental impacts have yet to be fully explained, and research is ongoing. In the U.S., the Environmental Protection Agency requires a certain distance between Bt crops and areas with wild plants to limit gene flow.
- Bt crops can, however, result in genetic contamination of native plant species through hybridization. This could lead to increased weediness of the plant or the extinction of the native species. In addition, the transgenic plant itself may become a weed if the modification improves its fitness in a given environment. Large-scale applications of B.t. can have far reaching ecological impacts. B.t. can reduce dramatically the number and variety of moth and butterfly species, which in turn impacts birds and mammals that feed on caterpillars. In addition, a number of beneficial insects are adversely impacted by B.t.

Use of Synergism between Bt Products or Between Bt Products and Other Substances

Published literatures shows that most of the Bt toxins cloned have narrow spectrum activity**.** Studies have shown that some expressed toxins like Cyt1Aa show a weak toxicity to mosquitoes on their own but show synergistic activity when combined with other toxins like Cry4Ba and Cry11Aa .The reporters found that this is due to the broad range of extrinsic factors are able to influence the selectivity and or efficacy of Bt toxins. These extrinsic factors are various and include other Bt toxins or parts from the spore of *Bacillus thuringiensis* as well as certain enzymes, environmental stress, non-pathogenic microorganisms, and infectious diseases..There are several other factors that can influence the toxicity and selectivity of Bt proteins, such as a combination of biotic and abiotic stress factors (Koppenhöfer and Kaya 1997; Kramarz et al. 2007), infectious diseases (Dubois and Dean 1995), normal gut bacteria (Broderick et al. 2006 and 2009), interactivity with other Bt toxins and/or the spore part of the toxins (Lee et al. 1996; Liu et al. 1998; Perez et al. 2005; Schnepf et al. 1998; Sharma et al. 2004). In this context, it also should be reflected that the current theory, which explains toxicity of Bt toxins such as Cry1Ab in target organisms (de Maagd et al. 2001), leaves room for several open questions (Crickmore 2005; Gilliland et al. 2002) and even contradicting explanations (Zhang et al. 2005, 2006; Broderick et al. 2006; Soberon et al. 2007). In general, it seems premature to rely on the assumed selectivity and a linear dose–response relationship as suggested by Monsanto (2007). On the contrary, the issue of synergism, efficacy, and selectivity remains a gap in current risk assessment. Several strategies that should prevent or delay the rapid development of resistance to *Bt*-plants have been proposed and compared.

The cadherin Bt-R_1 is a binding protein for Bt Cry1A toxins in midgut epithelia of tobacco hornworm (*Manducasexta*). Researchers previously identified the Bt-R_1 region most proximal to the cell membrane (CR12-MPED) as the essential binding region required for Cry1Ab-mediated cytotoxicity. It was been reported that peptide containing this region expressed in *Escherichia coli* functions as a synergist of Cry1A toxicity against lepidopteran larvae.It has been found that mixtures of low Bt toxin dose and CR12-MPED peptide effectively control target insect pests. So it has been proved that important implications related to the use of this peptide to enhance insecticidal activity of Bt toxin-based biopesticide and transgenic Bt crops.

Bacillus thuringiensis (Bt) Cry1A proteins are pore-forming toxins that are specifically toxic to insect larvae in the order Lepidoptera. There are two issues on the deployment of Bt crops are the evolution of resistance in target pests and the lower level of control of specific target pests. Although insect resistance to Bt cotton has not caused a control failure in the field, there is a natural difference in lepidopteran susceptibility that effects insect control. For example, Bt cotton expressing only a Cry1A protein is highly effective in controlling tobacco budworm (Heliothisvirescens) populations, whereas control of cotton bollworm (Helicoverpazea) larvae is only achieved after additional insecticide treatment .

The methods of application of *Bt*and its products in agriculture

The greatest use of Bt involves the *kurstaki strain* used as a spray to control caterpillars on vegetable Crops. In addition, Bt is used in agriculture as a liquid applied through overhead irrigation systems or in a granular form for control of European corn borer. The treatments funnel down the corn whorl to where the feeding larvae occur. Many formulations (but not all) are exempt from pesticide tolerance restrictions and may be used up to harvest on a wide variety of Crops. This also makes Bt useful in applications where pesticide drift onto Gardens is likely to occur, such as treating trees and shrubs. The exceptional safety of Bt products also makes them useful where exposure to pesticides is likely during mixing and application.

To control mosquito larvae, formulations containing the *israelensis*strain are placed into the standing water of mosquito breeding sites. For these applications, Bt usually is formulated as granules or solid, slow-release rings or brickettes to increase persistence. Rates of use are determined by the size of the water body. Make applications shortly after insect eggs are expected to hatch, such as after flooding due to rain or irrigation. Bt persistence in water is longer than on sun-exposed leaf surfaces, but reapply if favorable mosquito breeding conditions last for several weeks. Although the *israelensis*strain is quite specific in its activity, some types of nonbiting midges, which serve as food for fish and wildlife, also are susceptible and may be affected. Use of Bt*(israelensis*) for control of fungus gnat larvae involves drenching the soil. Bt applied for control of elm leaf beetle or Colorado potato beetle (san diego/tenebrionis strain) is sprayed onto leaves in a manner similar to the formulations used for caterpillars. Bt does not control shore flies, another common fly found in greenhouses.

Bt products are available in garden shops are commonly sold as wettable powders that can be mixed with water and sprayed on plants with conventional garden spray equipment. A wettable powder goes into the solution easily and has along shelf life .Bt is a ctually a living organism,pH of the water used to make the solution is important.AlthoughBt has a reasonable shelf life in the dry form,keep in mind that the dry powder is a composed of living spores.so we should follow all the safety directions on the label regarding mixing,using and cleaning up afterward.

Findings and Conclusion

Unlike most insecticides, Bt insecticides do not have a broad spectrum of activity, so they do not kill beneficial insects. They are now used as specific insecticides under trade names such as Dipel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators, and most other beneficial insects and are used in Organic farming. *Bacillusthuringiensis* serovar *israelensis*, a strain of *B.thuringiensis* is widely used as a larvicide against mosquito larvae, where it is also considered an environmentally friendly method of mosquito control. Today, *Bt*-based insecticides are frequently used in intensive agriculture, either in conjunction with conventional insecticides as a backup for control failure, or, as a last resort once resistance to other registered insecticides has occurred. Many insect pests, therefore, are already adapted to mixtures of endotoxins.

Thus *Bacillus thuringiensis* (Bt) is an insecticide with unusual properties that make it useful for pest control in certain situations. Bt is a naturally occurring bacterium common in soils throughout the world. Several strains can infect and kill insects. Because of this property, Bt has been developed for insect control. At present Bt is the only "microbial insecticide" in widespread use.

Thus toxin from the *Bacillus thuringiensis* can be used in various agricultural applications with narrow spectrum activity. *Bacillus thuringiensis* produces cry crystal toxin that attacks the gut of pests and kills them internally.These cry toxins are good agricultural tools for growing plants .Instead of using chemicals that may have adverse effect on humans ,researchers integrate then *Bt* toxin into the plants genome. These *Bt* cry toxins are safe for humans and kill off species of pests that are susceptible to the *Bacillus thuringiensis* endospre.

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