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Biosafety of Bt-crop in soil ecosystem: A review

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Abstract
Transgenic crops, a new product of agricultural biotechnology has its own share of environmental risks and benefits. Present agricultural management practices and new ecosystems have their own impacts on the environment and further any additional negative effect of transgenic crops may mitigate their positive impacts as well as increase the background value of negative impacts due to new agriculture practices. Several risk assessment experiments on transgenic plants reported observations on changes in their respective aboveground environment and their biota. Very few study reports are available on the effect of transgenic plants and their products (released in the soil) on soil biota (both invertebrates and microorganisms) and soil processes mediated by them. Moreover, observation of these studies does not indicate anything conclusively and create a confusion regarding impact of transgenic plants on soil flora, fauna and processes. We aim to concisely review the impact of the transgenic crop on soil ecosystem.

Keywords: *Bacillus thuringiensis*, soil microorganism, invertebrates, pest resistance, non-target effects

1. Introduction
Bacillus thuringiensis (*Bt*) forms parasporal crystalline protein inclusions that exhibit larvicidal activity towards Lepidoptera, Diptera and Coleoptera, as well as lethality against members of other animal phyla. The δ-endotoxin produced by *B. thuringiensis* subsp. *kurstaki* is contained within a very large structure called the parasporal crystal, which is synthesized during bacterial sporulation (Hanny *et al.*, 1955) [68]. The parasporal crystal comprises approximately 20-30% of dry weight of the sporulated culture and usually consists mainly of protein (95%) and a small amount of carbohydrate (5%) (Yamamoto *et al.*, 1983) [173]. The crystal is an aggregate of protein that can generally be dissociated by mild alkali treatment into subunits (Heimpel *et al.*, 1997; Lee *et al*., 1997) [100]. The subunits can be further dissociated *in vitro* by treatment with β-mercaptoethanol, which reduces disufide linkages (Faust *et al*., 1968; Miller, 1983) [43, 118]. A protein is released when the parasporal is solubilized. The protoxin of the CryI toxin group has a molecular mass of approximately 130 kDa (Oh *et al*., 1985) [125]. The parasporal crystal is the active component in the formulation of bioinsecticides against larvae of several insects. It was observed that very low mortality levels (<10%) were obtained with crystals or endospores alone. In contrast, when both *B. thuringiensis* endospores and crystals were present, a strong increase in mortality (70%) was found, demonstrating a synergism between them, and hence the importance of endospore concentration in *B. thuringiensis* formulae (Bulla *et al*., 1980; Bravo *et al.*, 1993; Salamitou *et al*., 2000) [25, 22, 138]. It has been well documented that the insecticidal potency is closely related to the amount of parasporal crystal or solubilized protoxin (Yamamoto *et al.*, 1983) [173].

The *B. thuringiensis* genome
B. thuringiensis strains have a genome size of 2.4 to 5.7 million bp. (Carlson *et al*., 1994) [30]. Physical maps have been constructed for two *B. thuringiensis* strains (Carlson *et al*., 1996; Carlson *et al*., 1993) [31, 29]. Comparison with *B. cereus* chromosomal maps suggests that all of these chromosomes have a similar organization in the half, near the replication origin while displaying greater variability in the terminal half (Carlson *et al*., 1996) [31]. Most *B. thuringiensis* isolates have several extra chromosomal elements, some of them circular and others linear (Carlson *et al*., 1994) [30].

It has long been recognized that the proteins comprising the parasporal crystal are generally encoded by large plasmids (Gonza´lez *et al*., 1981) [59]. Sequences hybridizing to *cry* gene probes occur commonly among *B. thuringiensis* chromosomes as well (Carlson *et al*., 1993) [29]. Although, it is unclear to what degree these chromosomal homologs contribute to production of the crystal.

The transposable elements of *B. thuringiensis*

The *B. thuringiensis* species harbors a large variety of transposable elements, including insertion sequences and transposons. The *B. thuringiensis* transposable elements are associated with the *cry* genes. It is postulated that the *B. thuringiensis* transposable elements are involved in the amplification of the *cry* genes in the bacterial cell, but this hypothesis has not been clearly tested. A second possible role is one of mediating the transfer of plasmids by a conduction process involving the formation of cointegrate structures between self-conjugative plasmids and chromosomal DNA or nonconjugative plasmids. Indeed, conjugation experiments suggest that Tn 4430 mediates the transfer of nonconjugative plasmids by a conduction process (Green *et al*., 1989) [61]. Thus, a major adaptive function for these transposable elements may be the horizontal dissemination of genetic material, including *cry* genes, within the *B. cereus-B. thuringiensis* species (Schnepf *et al*., 1998) [143].

***Cry* gene expression**

A common characteristic of the *cry* genes is their expression during the stationary phase. Their products generally accumulate in the mother cell compartment to form a crystal inclusion that can account for 20-30% of the dry weight of the sporulated cells. The very high level of crystal protein synthesis in *B. thuringiensis* and its coordination with the stationary phase are controlled by a variety of mechanisms occurring at the transcriptional, post-transcriptional, and Post-translational levels (Agaisse *et al*., 1995; Baum *et al*., 1995) [5, 20].

Classification of *Bt*-endotoxin

Bt δ-endotoxins in turn are classified by the sequence homology of their genes and insect specifity. Every *Bt* strain can have a variable number of plasmids responsible for the synthesis of different endotoxins. Plasmids can bear several, usually identical, toxin genes. *Bt* strains can easily exchange their plasmids via a conjugation-like process, as has been demonstrated in the larval gut. In this way *Bt* strains can also exchange plasmids containing δ-endotoxin genes and so express different activity patterns in different lepidopterous species. There are five major classes of cry toxins with specific insecticidal activity, namely Cry1 (Lepidoptera), Cry2 (Lepidoptera and dipteral), Cry3 (Coleoptera), Cry4 (Diptera) and Cry5 (Lepidoptera and coleoptera) (Crickmore *et al*., 1998) [38].

More than 150 different Cry toxins have been cloned and tested for their toxicity on various insect species till date. In an attempt to accommodate the growing list of new toxin genes/proteins, a new nomenclature has been formulated, wherein each toxin gene/protein will be having four-letter code, according to their amino acid sequence identity among them (Crickmore *et al*., 1998) [38]. Biocontrol of insect and invertebrate pests by these insecticidal crystal proteins (ICPs) represents one of the most successful uses of a biological control agent and it is an important alternative to the use of chemically synthesized insecticides (Hofte *et al*., 1989; Schnepf *et al*., 1998) [77, 143].

3-D structure of the *Bt* endotoxin

Bt δ-endotoxins are globular protein molecules, which accumulate as protoxins in crystalline form during late stage of the sporulation. Protoxins are liberated in the midgut after solubilization and is cleaved off at C-terminal part to release ~66 kDa active N-terminal toxic molecule. The protoxin contains well-conserved cysteine residues (as many as 16 in Cry1Ac), which helps in bridging the protoxin molecules through intermolecular disulphide bonds and thereby crystal formation. Primary amino acid composition determines the final structure of a protein, closely related proteins, Cry1Aa and Cry3A, with 36% amino acid sequence identity showed super imposable structure with similar mode of action, whereas Cyt2A protein, which shares less than 20% amino acid sequence identity, is made of single domain with different functional properties (Schnepf *et al*., 1998) [143].

The tertiary structure of δ-endotoxins is comprised of three distinct functional domains connected by a short conserved sequence. Each domain of δ-endotoxin has independent and inter-related functions in the larval midgut, which brings out colloid osmotic lysis (Knowles, 1994) [94]. The nature of each domain was predicted from X-ray crystallography (Grochulski *et al*., 1995; Li *et al*., 1991) [103]. Domain I is made up of seven α-helices, domain II comprises three antiparallel β sheets, which are folded into loops and domain III is made of a β sandwich of two antiparallel β strands (Saraswathy *et al*., 2004). Each domain of δ-endotoxin has independent and inter-related functions in the larval midgut, which brings out colloid osmotic lysis (Knowles, 1994) [95].

Molecular studies on the structure and functional properties of different δ-endotoxins revealed that the domain I by virtue of its membrane spanning hydrophobic and amphipathic α-helices is capable of forming pores in the cell membranes of the larval midgut. Domain II being hyper variable in nature determines the insecticidal specificity of a toxin and domain III is involved in varied functions like structural stability, ion channel gating, binding to Brush Border Membrane Vesicles (BBMV) and insecticidal specificity. Three domains interact closely to bring about the insecticidal activity of *Bt* (Nachimuthu *et al*., 2004) [123]. (Fig.1.1).

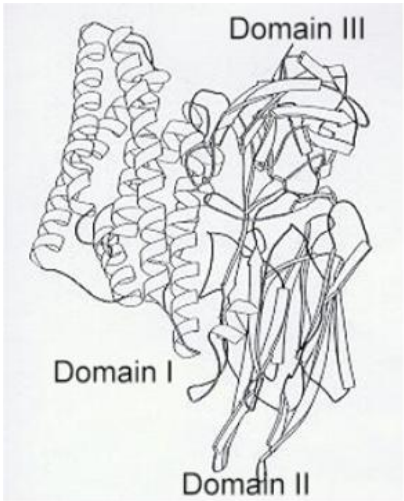

Fig.1.1: Structure of Bt δ-endotoxin Cry3Aa depicting the three domains. (Li *et al*., 1991) [103].

Biochemistry of *Bt*-endotoxin

The protein engineering studies conducted on different δ-endotoxins which led to the result that most of the mutants created in domain I resulted in low or no toxicity on tested insects. This might be due to domain I being the most conserved among three domains and it is involved in the basic function of the δ-endotoxins viz., ion channel formation. Domain II and III mutations resulted in altered/enhanced or decreased specificity and altered receptor binding (in case of Domain III substitutions). Variable and hyper variable regions confer differential specificity and differential receptor binding in the target cells (Nachimuthu *et al*., 2004) [123].

Quantification of insecticidal potency of *Bt*-endotoxin

The traditional quantitative analysis method for insecticidal potency is spore counting (Yamamoto *et al*., 1983) [173]. However, recent investigations have found that the number of spores is sometimes not representative of the amount of parasporal crystal (Lee et. al., 1997) [100]. Also, some laboratories are using bioassay to assess insecticidal activity, but this method is time-consuming and lacks accuracy. Yamamoto *et al*., (1983) [173] used HPLC to assess the concentration of protoxin and its digested peptides, and found that HPLC is very useful in characterizing these proteins. However, there are some drawbacks in using HPLC for δ-endotoxin determination. For example, the HPLC column is expensive and the procedure is time-consuming as well. Capillary electrophoresis (CE) is a new analytical technique, which provides a simple and rapid analysis with high resolution separation. This new technique has been applied for quantitative analysis for δ-exotoxin (Liu *et al*., 1988) [105]. The results indicated that the CE method is more accurate and rapid than that of HPLC. The present CE study for δ-endotoxin assay has demonstrated that it is a more convenient, rapid and efficient method than the conventional methods.

Mode of action

The exact mechanism of action of *B. thuringiensis* toxins is not well understood (Gill *et al*., 1992; Knowles, 1994) [56, 95]. Following ingestion and solubilisation by intestinal secretions in the insect midgut, the crystal proteins are cleaved by gut proteases. The resulting products are 60-65 kDa activated proteins which bind to specific sites of the brush-border membrane of the columnar cells lining the gut lumen. This triggers a cascade of poorly elucidated events leading to the death of the insect. It is believed that the pore-related increased permeabilisation of the target cells and the resulting cellular ionic and metabolite imbalance constitute the critical steps leading to cell disruption. *Bt* insecticidal toxin studies on the mode of action of *Bt* insecticidal protein have revealed the interacting ligands primarily as amiopeptidase N (APN) and cadherin, which are located at the brush-border membrane in the midgut of susceptible larvae (Morin *et al*., 2003) [121].

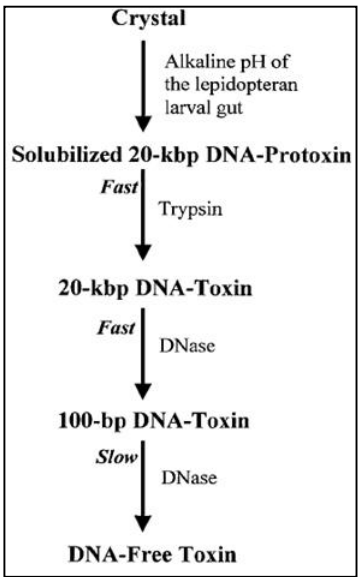

Fig 2.2: Proposed scheme for activation of the crystal protein in the larval gut (Clairmont *et al*., 1998) [36].

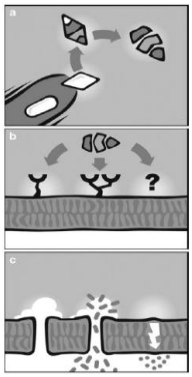

Fig 1.3: Steps of activation of Cry toxins and some mechanisms contributing to its selectivity.

The number of nucleotide bp of DNA per molecule of protein was determined for crystal, solubilized protoxin and toxin-DNA complex (Table 1.1).

Role of DNA in activation of the Cry1Ac crystal protein

The Cry1A insecticidal protein (protoxin) from six subspecies of *Bacillus thuringiensis* as well as the Cry1Aa, Cry1Ab, and Cry1Ac proteins cloned in *Escherichia coli* was found to contain 2.0×10^4 bp DNA. Only the N-terminal toxic moiety of the protoxin was found to interact with the DNA. Analysis of the crystal gave approximately 3 bp of the DNA per molecule of the protoxin, indicating that only a small region of the N terminal toxin moiety interacts with the DNA. It was proposed that the DNA-protoxin complex

is virus like in structure (Fig.1.4), with a central DNA core surrounded by protein interacting with the DNA with the peripheral ends of the C-terminal region extending outward. It is shown that this structure accounts for the unusual proteolysis observed in the generation of toxin in which it appears that peptides are removed by obligatory sequential cleavages starting from the C-terminus of the protoxin. Activation of the protoxin by spruce budworm (*Choristoneura fumiferana*) gut juice is shown to proceed through intermediates consisting of protein-DNA complexes. Larval trypsin initially converts the $2.0x10^4$ bp DNA-protoxin complex, to $2.0x10^4$ DNA toxin complex, which is subsequently converted to a 100 bp DNA-toxin complex by a gut nuclease and ultimately to a DNA free toxin (Clairmont *et al*., 1998) [36].

Table 1.1: Quantification of base pairs DNA/protein ratio.

Bt sample	DNA/molecule protein[a]
	bp
B. thuringiensis kurstaki HD73 crystal	3.5 ± 0.5
B. thuringiensis kurstaki HD73 crystal	3.1 ± 0.4
B. thuringiensis kenya crystal	4.8 ± 1.5
B. thuringiensis sotto crystal	2.0 ± 0.5
Solubilized HD73 protoxin	3.2 ± 1.1
Solubilized HD73 protoxin	4.0 ± 1.4
Cloned *E. coli* Cry1Ac protein	2.3 ± 0.2
HD73 toxin-20-kpb DNA	5.0 ± 1.0
Average:	3.5 ± 1.0

a- The values are given with the 95% confidence interval determined from the standard error in the estimate of the phosphorus content and the standard error in the estimate of the amount of protein from amino acid analysis (Clairmont *et al*., 1998) [36].

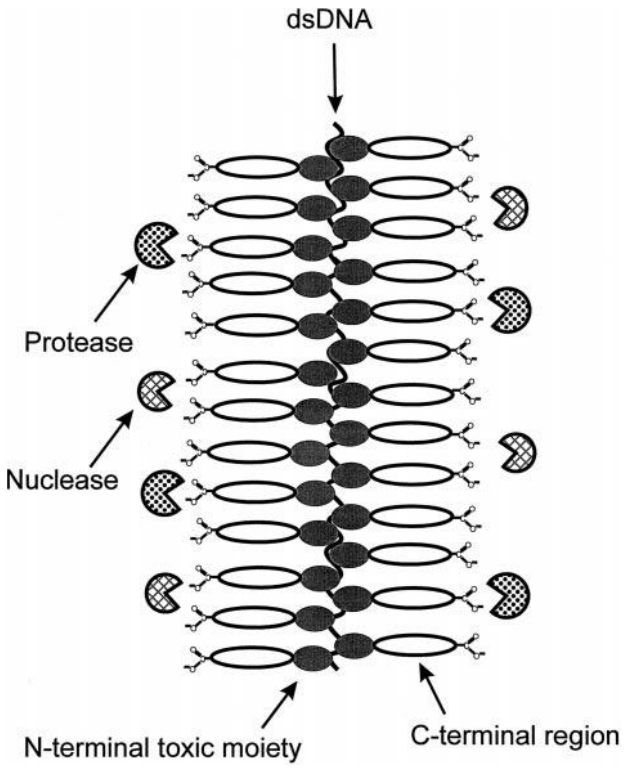

Fig 1.4: Proposed model of the structure of the DNA-protoxin complex.

The protoxin surrounds a central double-stranded DNA strand. Its N-terminal toxic moiety interacts with three nucleotide bp leaving its C-terminal region extending away from the central core. Proteases are only able to attack the peripheral portions of the C-terminal region giving rise to the sequential proteolysis observed in the generation of toxin. The protoxin protects the DNA from attack by nucleases, but once its C-terminal region is removed the DNA becomes exposed and susceptible to nucleases (Clairmont *et al*. 1998) [36].

The proteins are produced as inactive protoxins that are activated in the larval midgut to the insecticidal toxins by solubilization in the high pH (above 10.5) and cleavage by specific proteases (Hofte *et al*., 1989) [77]. The protoxins are insoluble at low pH, with the exception of the CryIII protoxin, which is also soluble at low pH (Koller *et al*., 1992) [96]. The active toxins interact with receptors, which presumably confers specificity, on the epithelial cells of the larval midgut, where the toxins form pores and destroy the cells by colloidal osmotic lysis. Truncated forms of the genes that code for these toxins have been genetically engineered into plants and other bacteria that express the active toxins rather than the inactive protoxins. Because, the active toxins do not require solubilization and proteolytic cleavage, two of the barriers that are involved in specificity are removed. Thus, beneficial insects, as well as organisms at higher trophic levels, could be harmed (Addison *et al*., 1993; Flexner *et al*., 1986; James *et al*., 1993; Johnson *et al*., 1995) [3, 50, 84, 87]. Receptors are also present on the larvae of non-target insects but apparently in lower numbers (Hofte *et al*., 1989; Van Rie *et al*., 1990) [77, 162]. Although, the receptors can be present in higher numbers in some non-susceptible larvae (Garczynski *et al*., 1991; Wolfersberger *et al*., 1990) [55, 171].

Degradation of *Bt*-endotoxin in the environment

Bt-insecticidal proteins may be removed from or inactivated in the environment by (i) consumption by insect larvae, (ii) degradation and eventual mineralization by microorganisms, and (iii) sunlight. When genes that code for the active toxins are expressed by transgenic plants (Adang *et al*., 1987; Barton *et al*., 1987; Fischhoff *et al*., 1987; Vaeck *et al*., 1987; Vaeck, *et al*., 1988; Vaeck *et al*., 1997) [2, 17, 49, 161, 160, 159]. and microorganisms that are indigenous or adapted to natural environments, where in they continue to grow and synthesize the toxins, the toxins may accumulate. Hence, the levels of active toxins in soil could be greater and be present longer than those introduced by periodic spraying of

commercial preparations of *B. thuringiensis* containing protoxins and could exceed consumption, inactivation, and degradation. This could result in sufficiently high concentrations of the toxins to select toxin-resistant target organisms (Addison *et al*., 1993; Alstad *et al*., 1995; Bauer *et al*., 1995; Ferre *et al*., 1991; Ferre *et al*., 1995; Heckel *et al*., 1995; Tabashnik *et al*., 1994) [3, 8, 19, 48, 47], and constitute a hazard to nontarget organisms (Flexner *et al*. 1986; James *et al*. 1993; Johnson *et al*., 1995) [50, 84, 87] especially, if some of the toxins are bound on soil constituents. After commercially usable portions of transgenic plants have been harvested, the rest of the plant biomass will be plowed into soil, where the toxins will bind on clays and humic substances and become resistant to microbial degradation. Thus, beneficial insects, as well as organisms at higher trophic levels, could be harmed (Addison *et al*., 1993; Flexner *et al*., 1986; James *et al*., 1993; Johnson *et al*., 1995) [3, 51, 85, 88].

Differences between bacterial endotoxin and the transgenically produced *Bt*-endotoxin

Cry proteins produced by the bacterium are usually crystalline (called insecticidal crystal proteins-ICPs) and are protoxins with a molecular mass (Mr) of about 130–140 kDa that require cleavage by proteases to produce the biologically active form (toxins) with a Mr of 60–70 kDa (Hofte *et al*., 1989) [77].

Therefore, ICPs must be ingested to have an effect and require alkaline conditions, typically in the range of pH 8-11, in the insect midgut, to be solubilized to a form conducive to activation by midgut proteases (Broderick *et al*., 2006) [23].

cry genes inserted into most *Bt* plants are in a truncated form, and when expressed in plants, truncated active Cry proteins do not form crystals, and they are already solubilized and activated (i.e., no enzymatic cleavage is required) (Gill *et al*., 1992; Aronson *et al*., 2001) [57, 12]. Therefore, most of the specificity that accounts for the safety of Cry proteins in commercial bacterial insecticides (i.e. ICPs) does not apply to these same proteins when expressed in *Bt* crops to make them resistant to specific insects.

Factors affecting toxicity

Certain factors and synergism can impact efficacy and selectivity 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. Risk assessment of genetically engineered plants should put into question the general assumption of a high selectivity and a linear dose–response relationship in the toxicity of *Bt* proteins. Both selectivity and efficacy can be influenced by synergism, which can provoke unexpected and undesired effects in non-target organisms. Synergism between *Bt* toxins and potential extrinsic factors that could impact the spectrum of susceptible organisms. Only non-vertebrates can be seen as potential target organisms for *Bt*-endotoxins. However, Huffmann *et al*., (2004) [81] raise questions beyond receptor-specific activity of *Bt* toxins also being relevant for vertebrates. In addition, Ito *et al*., (2004) [82] 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) [155] 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). Compared to the naturally occurring (non-active) pro-toxin, the *Bt* toxin, as expressed in genetically engineered plants, not only has a different structure but also has, partially, a changed quality in its mode of action (Hilbeck and Schmidt, 2006) [74]. In addition, plant enzymes can help to activate (solubilize) the *Bt* toxin in MON810 (Li *et al*., 2007) [101], so the resistance to native *Bt* toxins acquired in pest insects does not necessarily work on genetically engineered plants (Huang *et al*., 2002; Li *et al*., 2007) [80, 102]. This finding is relevant for the issue of selectivity, since activation (solubilizing) normally requires certain conditions to be met in the gut of insects (de Maagd *et al*., 2001) [39].

Effect of *Bt* endotoxin on non-target organisms

Effect on predatory insect

Dose-response relationship experiment had been conducted on *Chrysoperla carnea (*green lacewing) that showed no adverse effect on them when feed on Cry1Ab toxin (Hilbeck *et al*., 1998) [74]. *Coleomegilla maculate* (Spotted lady betel), also did not show any adverse effect on development and survival in response to Cry1Ab or Cry3Bb proteins (WWW.epa.gov).

Effect on Parasitoid wasp

No adverse effect was observed on the parasitoid wasp *Brachymeria intermedia* when feed on 20 mg/kg body weight Cry1Ab in diet or on the jewel wasp *(Nasonia vitripennis*) (WWW.epa.gov) and also on *Diaeretiella rapae*. (schuler *et al*., 2001) [142]. However, Salama and zaki, (1983) [137] observed reduced emergence and development of parasitoid wasp (*Zele chloropthallamus*) reared on *Bt* fed *S.littoralis.*

Effect on nontarget Lepidoptera

There was a hazard to the monarch butterfly *(Danaus plexippus)* larvae that consumed pollen containing high level of *Bt*-endotoxin. (Hansen-Jesse, 2000; Losey *et al*., 1999) [69, 106]. The potential toxicity to another non toxicity to another non target butterfly, the black swallowtail *(Papilio polyxenes),* was examined in field studies. There was sublethal toxicity observed (Wraight *et al*., 2000) [172].

Effect on soil ecosystem and organisms (Fig.1.5)

Effects of Cry proteins on earthworms:

L. terrestris, *E. fetida,* and *A. caliginosa*, all showed that the Cry1Ab protein had no significant effects on their survival, growth, and reproduction, even though the protein was detected in the gut and feces of the earthworms, indicating that the protein was ingested by the worms (Ahl Goy *et al*., 1995) [106].

Effects on wood lice collembolans and mites

No toxic effects of Cry proteins on woodlice, collembolans, and mites have been reported.

Nematodes

Few studies have investigated the population dynamics of individual nematode species, and some have indicated that

C. elegans showed some sensitivity to the Cry1Ab protein from *Bt* maize, in that growth and reproduction were significantly affected by the presence of the protein in soil (Griffiths *et al*., 2005, 2006; Manachini *et al*., 2003; Manachini *et al*., 2004; Manachini *et al*., 2002) [63, 64, 112, 113, 115].

Protozoans

No toxic effects of the Cry proteins on protozoa have been observed (Donegan *et al*., 1995; Griffiths *et al*., 2006) [64, 42]. However, studies in the greenhouse showed significantly higher numbers of protozoa in soils with *Bt* than with non-*Bt* maize (Griffiths *et al*., 2006) [64].

Microbial community

Microorganisms are the dominant organisms, both in terms of biomass and activity, in soil, and they are involved in numerous important processes, including decomposition of organic matter, nutrient mineralization, regulation of plant pathogens, decomposition of agricultural chemicals, and improvement of soil structure (Gupta and Yeates, 1997) [66]. However, the close interaction between crop cultivation and microbe-mediated soil processes inadvertently leads to contact of soil organisms with Cry proteins released from *Bt* crops. The rhizosphere (the zone of soil directly surrounding and influenced by plant roots) contains the majority of the microbiota in soil (410-fold more than the microbiota in bulk soil) and plant-microbe interactions in the rhizosphere are among the major factors that regulate the health and growth of plants. It is widely acknowledged that root exudates govern which organisms reside in the rhizosphere (Bardgett *et al*., 1999; Lynch, 1994; Wenke *et al*., 2009) [16, 110, 168].

Therefore, any change in the quality and quantity of root exudates could potentially modify the composition (biodiversity) and activity of the soil microbiota and may cause changes in both deleterious and beneficial microorganisms. For example, a decrease in specific microbial populations could lead to a decrease in decomposition processes, alter the level and composition of soil organic matter, and have secondary effects on the survival of plant pathogens. Similarly, loss of particular trophic groups of the mesofauna could cause a loss of specific pathways within nutrient cycling processes, thus affecting important biogeochemical pathways. Different effects, ranging from no effects to minor and significant effects, of *Bt* plants on microbial communities in soil have been reported, but they were mostly the result of differences in geography, temperature, plant variety, and soil type. In general, differences in microbial community structure were transient and not related to the presence of the Cry proteins. Only one study found consistent significant differences between soils with *Bt* and non-*Bt* maize (Castaldini *et al*., 2005) [32].

The rhizosphere bacterial community of *Bt* and non-*Bt* plants were characterized using several techniques, including viable counts, DGGE, CLCP, CLPP, PLFA, ARISA, and T-RFLP. The culturing techniques did not detect any differences in the soil microbiota between soils with *Bt* and non-*Bt* plants, but some molecular techniques indicated that the community structure differed in soils with *Bt* and non-*Bt* plants. Root exudates of *Bt* plants resulted in the development of bacterial communities in soil that differed from those associated with exudates of near-isogenic non-*Bt* plants. However, it was suggested that the exudates of *Bt* plants differ from those of non-*Bt* plants in several ways, not only in the content of the Cry protein (Brusetti *et al*., 2004) [102].

The ecological risks of *Bt* transgenic crops were critically highlighted for potential adverse effects on agroecosystems, in particular, non-target effects on soil microorganisms. No consistent statistically significant differences between rhizosphere soil of *Bt* and non-*Bt* cotton in the numbers of culturable nitrogen-fixing bacteria, bacteria that dissolve organic and inorganic phosphates and potassium-dissolving bacteria during the four sampling stages in the four fields have been found (Hu *et al*., 2009) [79].

Effect on Fungi

Arbuscular mycorrhizal fungi (AMF) are important soil microorganisms providing a range of benefits to the majority of crop plants in the agroecosystem, worthy of monitoring for non-target effects of *Bt* transgenic crops. *Bt* transgenic crops may affect AMF in many ways during their life with regard to the temporal-spatial relevance between the occurrence of *Bt* proteins and fungal symbiotic development of AMF. This may lead to an unwelcome surprise with regard to specific abundance and diversity of AMF, when *Bt* transgenic crops are planted continuously (Clairmont *et al*., 1998; Wenke *et al*., 2008) [36, 168].

Effect on soil biochemical properties

The biochemical properties of soil have often been described as early and sensitive indicators of ecological changes in both natural soil and agroecosystem. Activities of soil enzymes indicate the direction and strength of all kinds of biochemical processes in soil and act as key biological indicators of soil. The significant effect of transgenic *Bt* rice straw was observed on the activities of phosphatase and cellulose. However, the activity of dehydrogenase was seriously inhibited in short time after returning rice straw into flooded soil. Sun *et al*., (2007) [148] suggested that differences between *Bt* and non-*Bt* cotton (Shen *et al*., 2006) [144] activities of soil urease, acid phosphomonoesterase, invertase, and cellulase were stimulated by the addition of *Bt* cotton tissues (GK12 and ZK30), whereas activity of soil arylsulfatase was inhibited. In addition, the activities of urease, phosphatase, dehydrogenase, phenol oxidase, and protease in cotton rhizosphere (*Bt* cotton, Sukang-103, and its non-*Bt* cotton counterpart, Sumian-12) were assayed during the vegetative, reproductive, and senescencing stages of cotton growth and after harvest. There were few significant differences in enzyme activities between *Bt* and non-*Bt* cottons at any of the growth stages and after harvest. Amendment with cotton biomass to soil enhanced soil enzyme activities, but there were no significant difference between *Bt* and non-*Bt* cotton (Shen *et al*., 2006) [144].

Effect on mammals, birds, fish, and aquatic invertebrates

The normal mode of toxic action for the protein is very unlikely to occur in the vertebrate digestive system, and the protein has been used in the direct testing with mammals and birds with no toxic effect reported (www.epa.gov).

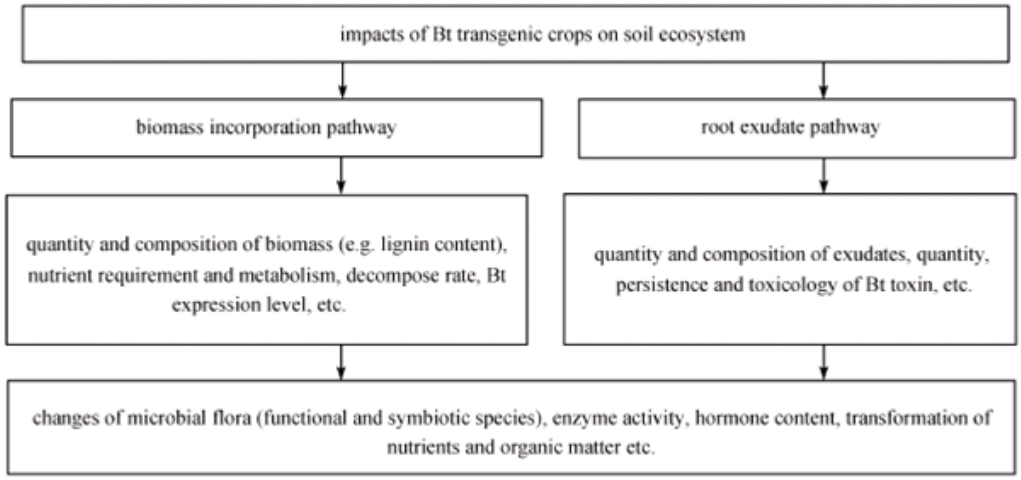

Fig 1.5: Impact mechanism of *Bt* transgenic crop on soil ecosystem.

Soil persistence and dynamics of *Bt* toxins from *Bt* transgenic crops

ICPs (Insecticidal crystal proteins) are degraded by proteases from a variety of sources including those endogenous to the bacterium, those purified from animals and plants, or those found in insects. Proteases in the bacterium function in protein metabolism during sporulation; in some cases they hydrolyse ICPs. Insect proteases are implicated in *Bt* toxin specificity, mode of action and insect adaptation to *Bt* (Oppert, 1999) [127]. Soil persistence and dynamics of *Bt* toxins from transgenic crops (*Bt* transgenic cotton and rice) were mainly investigated in the rhizosphere and soils incubated with *Bt* transgenic crop tissues. The amount of protein in the plant tissue is related to two factors, the event and the promoter. Cry protein expressed at various level in different crops and in different events. Fearing *et al*., (1997) [44] determined that the highest concentration *Bt*-endotoxin per plant occurred at seedling stage and then decreased. However, the largest amount of protein per acre occurred at anthesis, when the plant biomass is greatest.

As the study revealed, there was a significant accumulation (0.2–0.3 μg/g) and then a decreasing process of *Bt* toxin concentration in rhizosphere during entire growth period of *Bt* cotton SGK321 and NuCOTN99B, and finally, there was no detectable *Bt* toxin (Rui *et al*., 2005) [135]. *Bt* protein is incorporated into soil with plant tissue post-harvest, with sloughing of root cells, and potentially through the release of exudates from roots. Saxena *et al*., (1999) [140] reported the presence of protein in plant secretion but did not estimate the concentration of toxin in soil.

Sims *et al*., (1997) [146] calculated that approximately 486 g/acre (1174 g/ha) or 1.6 μg of soil of *Bt* protein would be added to soil from a mature transgenic cotton crop with an assumption of 60 000 plants per acre, if the entire mass of crop is incorporated into the soil. The term DT_{50} and half-life are used to describe the time until the amount of a substance remaining is 50% of the original amount. Half-life applies only to first order dissipation processes. Persistence can also be discussed in terms of detectable residues and bioactivity. Dissipation/persistence of *Bt* protein in soil can also be a function of soil type, environmental conditions, the protein source (purified versus plant produced), and the particularly Cry protein examined. However the lack of reliable accurate and universal analytical method results in differences in results (Clark *et al*., 2005) [37]. Dissipation of *Bt* toxin in soil is generally biphasic (Herman *et al*., 2002) [73]. West *et al*., (1984) [169] characterized the degradation of parasporal *Bt* crystals in soil as a lag phase, followed by a phase of rapid degradation, with the final 10% of the toxin being degraded at much slower rates.

Conclusion

Though Literature literature on interactions of transgenic plants with soil ecosystem it can be concluded that though, lab and field study on such interactions are limited, they have suggest that the transgenic plants and their products mark their footprints on soil ecosystem. In long term this may alter the structure and functioning of soil ecosystem. Transgenic crops may be having an immense potential to render environmental and economic benefit, but reports suggesting negative imprints of transgenic plants on soil ecosystem have created speculations over benefits of transgenic crops. Thus to resolve those doubts, more long term experimental studies are extensively needed.

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