Review Article

BT AS A BIOCIDE AND ITS ROLE IN MANAGEMENT OF TEA PESTS

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ABSTRACT

Bacillus thuringiensis (Berliner) has been under extensive use as a biopesticide for the last 55 years in many parts of the world, contributing 90% of the total commercial biopesticides formulations. It is being utilized as a natural regulating agent for different lepidopteran pests of tea. Recent studies revealed that commercial formulations of Bt were as effective as chemical pesticides. With the advent of genetic engineering, the insecticidal gene of Bt has been successfully isolated and cloned into many commercial crops. This review paper critically discusses the scope of using Bt and Bt transgenics for the management of tea pests.

Keywords: *Bacillus thuringiensis, biopesticides, Bt transgenics, tea insect pests*

INTRODUCTION

Bacillus thuringiensis (Berliner) (*Bt*) is a gram positive endospore forming insecticidal bacterium, producing 45 classes of proteinaceous crystals (Cry) (delta-endotoxins) in the sporangium, specifically toxic to lepidopteran, dipteran, coleopteran insects as well as nematodes (Aronson and Beckman., 1986; Rowe and Margaritis, 1987; Whiteley and Schnepf, 1986; Chandrashekar *et al.*, 2005). On being ingested, the Cry toxins are activated by protease to active toxins of size 65 kDa in the alkaline mid gut, which were then bound by the receptors present in the brush border membrane vesicles resulting in perforation of the membrane and ionic disbalance, which leads to the paralysis of the gut and ultimately to death of the insect (Gill *et al.*, 1992).

Though *Bt* can be isolated from many sources including soil (hence refers as soil bacterium), but its principal ecological niche is insects. In 1901, *Bt* was first discovered in Japan from the silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) (Ishiwata, 1901; Berliner, 1915). Since then, at least 50 serotypes or 63 serovars and probably over 800 isolates were discovered from many insects (Thiery and Frachon, 1997). Out of these, *B.t.* var. *krustaki*, *B.t.* var. *israelensis*, *B.t.* var. *thuringiensis*, *B.t. dendrolimus* and *B.t.* var. *galleriae* (Table 1 next page) (Sivamani *et al.*, 1992) are commercially utilized for controlling 50 species of lepidopteran, coleopteran and dipteran pests for the last 55 years in agriculture, horticulture and public health in different parts of the world.

BIOPESTICIDES AND *BT*

Thrust on biopesticides is increasing due to the introduction of "Organic Agriculture" as well as for high demand of "Organic Tea". Biopesticides, in broad sense, include predators, parasitoids, microbials and botanicals utilized for pest control, which, however, constitute mere 1.4% or $ 380 million of the $ 28 billion global pesticide market. More than 90% of the biopesticides market is governed by the *Bt* products (Rodgers, 1993; Hazarika, 2004). The potency and multiplicity of use of the *Bt* products make them competitive even with chemical pesticides.

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Table 1. *Bacilllus thuringiensis* formulations available in India

Product name	Insecticide potency	Manufacturer /Supplier	Variety of *B.t.*
Agree	NA	Hindustan Ciba Geigy Ltd.	*krustaki*
Bioasp	3200 lu/mg	Bio Tech International	*krustaki*
Biolep, *B.t.k.*-I, *B.t.k.*-II, Lepidocide	3000 lu/mg	Bio Tech International	*krustaki*
B.t.t	1500 lu/mg	Bio Tech International	*thuringiensis*
Biotoxibacillin	3000 lu/mg	Bio Tech International	*thuringiensis*
Dendrobacillin	3000 lu/mg	Bio Tech International	*dendrolimus*
Biobit	32000 lu/mg	Rallis India	*krustaki*
Dipel 8L	17600 lu/mg	Lupin India/Cheminova India Ltd.	*krustaki*
Delfin	53000 lu/mg	Syngenta India/Margo India	*krustaki*
Halt	55000 lu/mg	Wockhardt Life Sciences	Serovar *krustaki* (Indigenous)
Spicturin	1x 10^6 spores/ml	Tuticorin Alkali Chemicals	*galleriae*

(Source: Singh, 2001)

In India, due to alienation of farmer's attitude towards biopesticides from the synthetic pesticides, there is a reduction of the annual pesticide consumption from 61,260 MT in 1995-96 to 46,195 MT in 1999-2000 (Prasad, 2001; Hazarika, 2004). This has resulted in boosting up of the biopesticides market and introduction of many *Bt* formulations in the country (Table 1). Based on available data, the total consumption of pesticide is estimated as 12 kg or l/ha. of tea, which includes insecticides, acaricides, herbicides and fungicides costing Rs. 5000 per ha per annum (Table 2) (Hazarika *et al.*, 1994). Perusal of Table 2 reveals that by reducing the application of chemical insecticides with biopesticides either @ 10% or 25%, the quantity of insecticide on the crop can be reduced to a great extent and so would the cost of production (Hazarika and Puzari, 2001). This reflects the importance of *Bt*, but till date *Bt* has been utilized to a limited extent for tea pest management. This paper reviews the role of *Bt* on tea pest management and discusses advances made on *Bt* transgenic. There are many lepidopteran, coleopteran and dipteran pests of tea against whom *Bt* can be utilized successfully.

Table 2. Saving of pesticide and cost due to application of biopesticides in NE India at different rate of coverage (per hectare basis)

	10%	25%	50%	70%
Quantity	1.2 kg/l	3 kg/l	6 kg/l	9 kg/l
Saving	Rs. 500	Rs. 1250	Rs. 2500	Rs. 3750

(Source: Hazarika and Puzari, 2001)

LEPIDOPTERAN, COLEOPTERAN AND DIPTERAN PEST COMPLEX OF TEA

Tea is a long duration perennial crop grown under monoculture providing favourable condition for variety of pests and diseases. In India, about 14 % of the crop is lost due to these menaces amounting Rs. 5000 million per annum (Borthakur *et al.*, 1993). More than 300 insects and mites pests are associated with the tea plant in India attacking various parts like root, stem, leaf and buds (Hazarika *et al.*, 2001) out of which Lepidoptera and Coleoptera accounts about 31.5% and 18.8% of the tea plant insects, respectively. Lepidopteran, coleopteran and dipteran pests of tea along with their taxonomic identity, time of occurrence and plant parts damaged with severity are shown in Tables 3 and 4. *Bacillus* sp. acted as a natural population regulatory agents against the bunch, *Andraca bipunctata* Wlk. (Lepidoptera : Bombycidae); looper, *Buzura suppressaria* Guen (Lepidoptera : Geometridae); red slug, *Eterusia magnifica* Butl. (Lepidoptera : Zygaenidae) and lymantrid caterpillars, *Euproctis* spp. (Lepidoptera: Lymantriidae) during May to August in NE India (Annon., 1994; Hazarika *et al.*, 1994).

Table 3. Lepidopteran pests of tea along with their taxonomic identity, time of occurrence and plant parts damaged with severity

Sl No	Pests	Taxonomic identity	Time of occurrence	Plant parts damaged				
				Shoot /buds	Young leaves	Matured leaves	Stem	Root
1.	Bunch caterpillar	*Andraca bipunctata* Wlk. (Lepidoptera : Bombycidae)	**Moths:** March-April/ May-June **Caterpillar:** March-April/May -June/July-August/Oct-Nov **Chrysalids:** April-May/June-July /Augst-Sept./Nov.-Dec.	**	***	***		
2.	Looper caterpillar	*Buzura suppressaria* Guen (Lepidoptera : Geometridae)	**Moths:** Feb.-March/ May-June /Augst-Oct **Caterpillar:** March-April/May-June /July-August/Sept.-Oct **Chrysalids:** March-May/July-August/August-Sept./Oct.-Nov.	*	***	**		
3.	Red slug caterpillar	*Eterusia magnifica* Butl. (Lepidoptera : Zygaenidae)	**Moths:** Jan.-March/ March-June/ July-August/Sept.-Oct. **Caterpillar:** Feb.-April/ June-July /August-Sept./Oct-Jan. **Chrysalids:** March-May/ July-August/Sept.-Oct./Nov.-Feb.		*	***	**	
4.	Psychid caterpillar	*Clania crameriiC. sikkima Mahasena theivora* (Lepidoptera : Psychidae)	May-June/Sept.-Dec.			*	**	
5.	Flush worm	*Laspeyresia leucostoma* Mayer (Lepidoptera : Eucosmidae)	Jan.-May	**	**			
6.	Nettle grub	*Parasa pastoralis* Butl. *Thosea* spp. (Lepidoptera : Limacodidae)	April-Sept.		*	**		
7.	Red stem borer	*Zeuzera coffeae* Nietner (Lepidoptera : Cossidae)	**Moths:** March-April/Aug.-Sept.				***	
8.	Bark eating caterpillar	*Indarbela theivora* Hamps. (Lepidoptera: Indarbellidae)	**Moths**: April-May				**	
9.	Cut worm	*Agrotis ypsilon* Hfn. (Lepidoptera : Noctuidae)	Feb.- April				*** (Seedling)	

* Damage caused but no economic loss

** Moderate economic loss

*** Major economic loss

Table 4. Coleopteran and dipetran pests of tea along with their taxonomic identity, time of occurrence and plant parts damaged with severity

Sl No	Pests	Taxonomic identity	Time of occurrence	Plant parts damaged				
				Shoot /buds	Young leaves	Matured leaves	Stem	Root
1.	Cockchafer grubs	*Holotrichia impressa* Burm. *Sophrops plagiatula* (Brenske) (Coleoptera : Scarabaeidae)	**Adult:** March- June					*** (seedling)
2.	Leaf eating cockchafer beetle	*Serica assamensis* Brenske (Coleoptera : Scarabaeidae)	**Adult:** May-June			**		
3.	Large Green weevil	*Astycus chrysochlorus* Wield. (Coleoptera : Curculionidae)	-			**		
4.	Small green weevil	*Astycus lateralis* Fabr. (Coleoptera : Curculionidae)	-		*			
5.	Shot hole borer	*Xyleborous fornicatus* Eichh. (Coleoptera : Scolytidae)	-				**	**
6.	Root borer	*Batocera rufomaculata* L. *Crytognathus indicus* Hope *Melanauster verteegi* Rits. (Coleoptera : Cerambycidae)	**Adult:** April - June					**
7.	Stem borer	*Haplothrix griseatus* Gah. (Coleoptera : Cerambycidae)	**Adult :** May - June **Grub :** Early May				**	
8.	Orange beetle	*Diapromorpha melanopus* Lecord (Coleoptera : Chrysomelidae)	**Adult:** March - October	*	*			
9.	Metallic green beetle	*Chrysolampra flavipes* Jacoby (Coleoptera : Chrysomelidae)	**Adult:** March - October	***				
1.	Tea leaf miner	*Agromyza theae* (Bigot) Meij (Diptera : Agromyzidae)	-		*			

* Damage caused but no economic loss

** Moderate economic loss

*** Major economic loss

In case of bunch, looper, red slug caterpillars and leaf roller, larval stages remain generally active during March-April to October-November, hence *Bt* should be applied during this time of the year only; other than these lepidopterans, no information are available on others. Critical evaluation of *Bt* products against other insects is needed. There are some important points in the biology of these insects, which need consideration in this respect. For example, the red stem borer, bark eating caterpillar and cut worms have typical feeding habit and behaviour. The red stem borer is an internal feeder. *Bt* is a stomach poison and would be difficult to reach this insect. Since the bark eating caterpillars attack the bark of the stem, hence a thorough coverage will be required. Likewise, cutworms mostly cut the seedling at the ground level; they will also be hardly affected by *Bt* formulations.

No researches have been reported against the nine coleopteran pests of tea. *Bacillus popillae* is known to be a pathogen of the scarabaeids, which, however, neither been reported from tea ecosystem nor been introduced for the management of cockchafer beetles. This may act as a potential pathogen for controlling these beetles. Other coleopterans attack the leaves as adults, so chances of being targeted them are less. Leaf miner was found to be susceptible to *Bt* formulations (Kariya, 1977).

MANAGEMENT OF TEA PESTS WITH *BT*

Bt has an established safety residue. The high margin of well established safety records is really encouraging for its use on organically produced food crops by eliminating the problem of pesticide residues. It does not produce tainting of the made tea. They are completely biodegradable and act promptly/rapidly. There is no question of waiting period from the time of application, which is a plus point for tea cultivation.

Kariya (1977) observed that commercial formulations of *Bt* were successfully tested against different species of tea tortricids, *viz.*, tea tortrix,, *Homona magnanima* Diakonoff (Lepidoptera : Tortricidae), smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera : Tortricidae) and tea leaf miner, *Agromyza theae* (Bigot) Meij (Diptera : Agromyzidae). He reported that the 1st instar larvae of tea tortrix were the most susceptible compared to older instars to a *Bt* product, SB-471, which was even superior to methomyl in this respect. The smaller tea tortrix was more prone to *Bt* products like SB-471, Arrow *BT*-601 and Thuricide-A even at lower concentrations compared to the tea tortrix. The mortality increased with time, however, when they were allowed to feed on untreated leaves after treatment, the mortality decreased with time.

While studying the effect of *Bt* on tea leaf miner in the field, two times application of Thuricide-A and single application of SB-471 showed some effectiveness at 8 DAT and at laboratory condition, SB-471, Arrow *BT*-601 and Arrow *BT*-101recorded 100% mortality 4 DAT (Kariya, 1977). It was also found that *Bt* reduced the effectiveness of the hymenopteran parasites at field condition, while it was not observed in the laboratory. This suggests presence of repellency effect of the *Bacillus* or the adjuvants of the product.

Borthakur (1986) was the first from Tocklai Experimental Station, Tea Research Association, Jorhat, Assam to test the pathogenicity of *B.t. thuringiensis* (HB III strain obtained from Central Food Technological Research Institute, Mysore) against looper caterpillar and found that *Bt* induced pathogenicity to all the instars of looper caterpillar and deformity to the adults at sub-lethal doses. Mortality over 90% occurred at a concentration of 800mg/litre of sterile phosphate buffer. He further observed that at a lower concentration of 100 mg/liter, the 3rd instar caterpillars pupated earlier than those of untreated ones, however adults emerged from those of the treated larvae developed abnormalities in forms, behaviour and were not fertile.

Borthakur and Raghunathan (1987) reported that LD_{50} values of *B. thuringiensis* strain HB III isolated from the infected *Heliothis* larvae and mass cultured in liquid medium were 62.4, 135.8 and 177.8 mg/l of spore concentration after 148, 120 and 96 hours of exposure for 3rd, 4th and 5th instar larvae of looper caterpillar, respectively. The LD_{90} for 5th instar was found to be 741.3 mg/l. the larvae feeding the sub-lethal dose, pupated early and became deformed with complete cessation of reproduction. The feeding by larvae ceased within 6-24 hours, afterwards they became inactive.

In a trial for comparative assessment of potency *Bt* formulation with insecticides against looper caterpillar, *Bt* formulation at 1: 50 dilution recorded 100% mortality after 7 days of exposure, similar to that of insecticides with 1: 200 dilution (Anon., 1989-90).

Reddy *et al.* (1990) tested different concentrations of *B.t.* var. *kurustaki* (serotype H3a and 3b) at 10^8/gm, 10^6/gm and 10^4/gm spore load against the bunch caterpillar under both laboratory and field conditions. In laboratory, 100 % mortality of the caterpillars was obtained at all the *Bacillus* treated plants. Likewise, in field, the symptoms of *Bt* infection started only after 24 hours. The per cent reduction of the larvae ranged from 44.4 to 71.42 and 77.7 to 92.85, respectively after 48 and 72 hours.

Further, Borthakur *et al.* (1992) reported that the *Bt* mutant at 1:50, 1:100 dilutions gave 100% and 92% reduction of the leaf roller caterpillar, *Gracillaria theivora* Walsm. (Lepidoptera : Gracilariidae) two weeks after the second round of application. In their experiments, delfin treated caterpillars showed the symptoms of septicemia within 48 hours and eventually died within 96 hours.

B. thuringiensis and its various sub-species, strains and geographic isolates have been studied in details for their efficacy in the field and the laboratory against major lepidopteran insect pests (Borthakur *et al.*, 1992). The results revealed that *Bt* at 1: 2000 dilution recorded 100% mortality of the bunch caterpillar at 24 hours after treatment (HAT), at 1: 8000 and 1: 16000, it caused 80.0% and 77.0% mortality, respectively. In the all the cases, 100.0% mortality was obtained at 144 HAT.

Field trials with an asporogenic crystal mutant of *Bt* against the tea leaf roller revealed that *Bt* at 1:50 dilution gave 100% control and was equitoxic to the standard formulation of the synthetic insecticides like Padan 50SP, Power and Nuvacron after 4 weeks of treatment (Annon., 1990-91). Similar trials with *Bt* crude solution and Delfin at 1: 500 dilution recorded 85.0% and 78.8% mortality, respectively as compared to 100.0% mortality by the chemical insecticides like Fastac 10EC, Fenvel 20EC and Umecron 85EC after 24 HAT. However, the *Bt* produced 100% mortality of the looper caterpillar at 7 DAT (Annon., 1995-96).

Bisen and Hajra (1997) reported that Delfin @ 0.06% concentration, registered more than 60% mortality of the flush worm, *Cydia leucostoma* Meyrick (Lepidoptera : Tortricidae), which was equally effective as that of Fluvalinate and more effective than that of Nethrin and commercial neem formulations.

BT TRANSGENICS

In addition to its use as insecticide, *Bt* gene has been incorporated into the plant system to produce 30 genetically modified species (De Maaged *et al.*, 1999). *Bt* transgenic cotton, corn and potato are already under extensive cultivation in USA, Australia, China, Argentina and South Africa. Recently, *Bt* cotton has also been introduced in India.

Development of clones with productivity and quality attributes is one of the major thrusts in R&D

activities of tea research organizations in India and abroad, which has resulted in release of many improved clones to the industry. However, attempts towards development of *Bt* tea is still at a low key, though Barbora (1993, 1995) emphasized the need of genetic engineering in this sector. Likewise, while reviewing importance of biocontrol of tea pests, Hazarika *et al.* (1994) envisioned that genetically engineered *Bt* tea would contribute immensely not only towards controlling a few lepidopteran and coleopteran pests, but also in reducing the pesticide load, thereby, in eliminating the problems of residues to a great extent. Similarly, Agnihothrudu (1999) pointed out the importance of development of transgenic plants that express *Bt* genes as well as to develop trans-conjugate *Bt* strains that expresses the conventional *Bt* genes with wider host range and construction of fusion protein in which *Bt* with different host spectra are fused together.

In the long run tea insects may also develop resistance against *Bt* as the tobacco budworm, *Heliothis virescens* F. (Lepidopetra : Noctuidae), the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera : Chrysomelidae) Indian meal moth, *Plodia interpunctella* Hubner (Lepidoptera : Anobiidae) and diamondback moth, *Plutella xylostella* L. (Lepidoptera : Plutellidae) have already demonstrated the ability to adapt to *Bt* in the laboratory. It has also been reported that the diamondback moth evolved high levels of resistance in the field as a result of repeated use of *Bt* (McGaughey and Whalon, 1992). However, *Bt* resistance is less stable and can be effectively managed through pesticide rotation.

A few of such examples to produce *Bt* tea have been reviewed here. Sivepalan (1999) have discussed the attempts to transfer the insecticidal crystal protein gene of *B. thuringiensis* into tea plants, using somatic embryogenesis in China. Likewise Wu *et al.* (2002) constructed *Bt* gene expression vector and transferred it in to the tea plant in order to raise new tea cultivars with pest resistance. The *Bt* gene DNA was inserted into the vector PCAMBIA2301. The constructed plasmid with *Bt* gene, intron-GUS gene and NPT gene were transferred into *Escherichia coli* and introduced the same into *Agrobacterium* strains L 134404, EHA 105 and pRil 5834 by triparental cross method. The *Bt* gene was then transferred into leaves and callus of the tea by *Agrobacterium* mediated methods. The transient expression of the GUS gene was successful in both callus and leaves of the tea plant.

CONCLUSION AND FUTURE THRUST

Though *Bt* can be integrated with the other components of IPM, very little work has so far been done in this respect. Field experiments with commercial formulations are also very much limited. A thorough study in this respect on tea crop as well to ascertain the safety of such formulations on the natural enemies and on other non target organisms in and around tea ecosystem is on high demand. Furthermore, isolation and screening of local *Bt* strains from the tea ecosystem may lead to the selection of a novel strain which will perform better compared to the imported ones. Studies on improvement of shelf life of formulations are essential to popularize these. Development of *Bt* transgenic is an important area on which further research is urgently called for. Besides, incorporation of genes responsible for flavour, yield, pest and disease resistance and cold tolerance may be attempted through biotechnology.

North East India is endowed with sericogenous wealth which leads to the growth of sericulture in this region. Sporulating *Bt* is a major threat to the silkworm. Therefore, for their safety, development of non-sporulating mutant strain based formulations of *Bt*, poorly pathogenic to the silk worms but highly pathogenic to the tea pests will be essential. Work on this line must be intimated before popularization.

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