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*Bacillus thuringiensis*as a sustainable approach towards integrated pest management

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Abstract
Bacillus thuringiensis (Bt) has been extensively employed as formulations as well as in genetically modified crop in insect pest management due to its safe environmental and human health records on contrast to chemical pesticides which impedes the efficacy of optimum management of natural enemies to the pest inherently found in abundance and also pose a hazard to the environment. With the incessant revolutionary technology development Bt emerges to be a cornerstone in the management techniques with a high host specificity. Being the most implemented bioagent among the biopesticides with the fact of producing parallel efficacy with some chemical pesticides, the application of Bt cannot be ignored.Here, we review the impact and application of Bt technology and the countermeasures that have been introducedto reduce the evolution of resistant in insect populations.

Keywords: *Bacillus thuringiensis*, natural enemies, biopesticides, environment

Introduction
Bacillus thuringiensis (Bt) is a ubiquitous,rod-shaped, Gram-positive, crystelliferous and spore forming bacteria which are isolated from wide area of the world from a great diversity of ecosystems including soil, water, dead insects, dust from silos, leaves, diverse conifers and mammals, as well as from human tissues with severe necrosis. Bt strains synthesis varieties of insecticidal proteins which are toxic against larvae of lepidopterans, dipterans and coleopterans which are a huge problems in agro ecosystem causing severe reduction in yield (Roh *et al*., 2007) [21]. Thus, Bt-based products are recognized asthe most successful commercial biocontrol agent for insect pests since the genes encodes insecticidal proteins have been successfully employedin the preparation of novel insecticidal formulations and in the production of transgenic crops (Sanchis, 2011) [22]. *B. thuringiensis*has a narrow host spectrum and hence they are harmless to human beings, non-target organisms and mammals. Bt strains synthesize Crystal (Cry) and cytolytic (Cyt) toxins (δ-endotoxins) at the onset of sporulation but during the stationary growth phase as parasporal crystalline inclusions.The spore protein content of about 20 % is represented by Cry⁄Cyt toxins (Aronson, 2002) [2] Once ingested by insects, these crystals are solubilized in the midgut and then proteolytically activated by midgut proteases and bind to specific receptors located in the insect cell membrane leading to cell disruption and insect death (Gonclaves and Pereira, 2012) [11]. Additionally, Bt isolates also synthesize other insecticidal proteins during the vegetative growth phase and have been named as vegetative insecticidal proteins (Vip) (Estruch *et al*., 1996; Warren *et al*., 1998) [10, 24] and the secreted insecticidal protein (Sip). Vip proteins are classified into four families Vip1, Vip2, Vip3 and Vip4 according to the similarity in their amino acid. The binary toxin consisting Vip1 and Vip2 proteins and the Sip toxin (Donovan *et al*., 2006) [9] exhibit insecticidal activity against some coleopterans, whereas Vip3 toxins are toxic against lepidopterans.

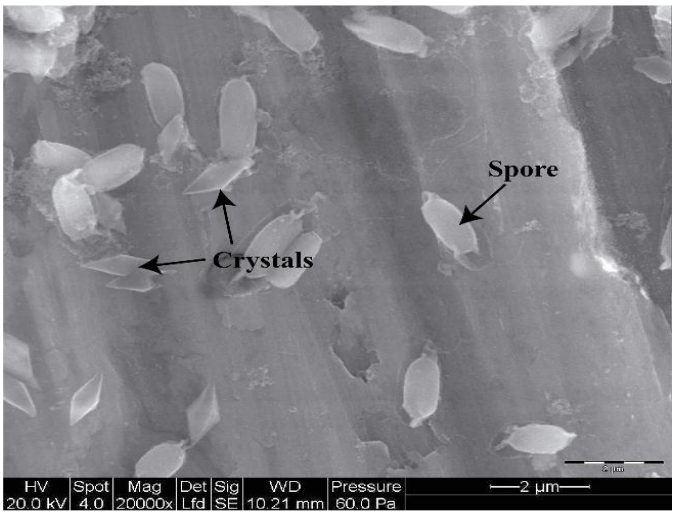

Fig 1: Protein crystals (bipyramidal) mixed with spores from Bt strain H29.3 (Palma *et al.*, 2014) [19]

History of Bt

The insecticidal property of Bt has been known many years before the identification of the bacteria. Some accounts suggest it has been used since ancient Egypt. Berliner (Berliner 1911) [3] first described *Bacillusthuringiensis*from an infected moth *Anagasta kuehniella* (Mediterranean flour moth). He isolated a *Bacillus* species and named it *Bacillusthuringiensis*afterThuringia a province located in Germany where the infected mothwas found. Although, this was the first report under the name *B. thuringiensis*, it was not the first isolation. Japanese biologist, Ishiwata Shigetane, in 1901 discovered a previously unspecified bacterium as the causative agent of a disease afflicting silkworms and he named this particular bacterium as *Bacillus sotto*. Although it has negative impact on silkworm rearing it is considered as the cornerstone of microbial control in insect pest management. The earliest commercial production of Bt began in France in 1938, under the name Sporeine (Lambert and Peferoen, 1996) and Edward Steinhausobtained a culture in 1942 thereby revitalized the activities and interest of Bt and attracted attention to the potential of Bt through his subsequent studies. In 1956, T. Angus (Angus 1956) [1] reported that the insecticidal action of bt was due to the crystalline proteins which are formed during sporulation.

Bt Nomenclature

In the first system, nomenclature for the Cry toxins which are produced during onset of sporulation and stationary growth phase and their corresponding genes were given a roman numeral depending on the insecticidal activity of the crystal protein, namely: CryI for proteins toxic lepidopterans, CryII for proteins with toxicity against both lepidopterans and dipterans, CryIII for proteins toxic for coleopterans; and CryIV for proteins toxic exclusively for dipterans (Höfte and Whiteley, 1989) [13]. However, complications arose in this system, for instance, the activity of new toxins had to be assayed against a growing list of insects before the gene and the toxin could be named, some novel homologous proteins were in fact non-toxic as expected, and others (e.g., Cry1I) exhibited dual toxicity against both dipteran and lepidopteran species (Crickmore *et al.*, 1998) [6]. Hence, inorder to avoid these problems, the *Bacillus thuringiensis* Toxin Nomenclature Committee was established in the year 1993 and a novel system of classification was proposed. In this new system, a novel toxin is given a four-rank name depending on the degree of pairwise amino acid identity to previously named toxins. Furthermore, grouping by this standard does not entail a similar protein structure, host range or even mode of action. In the first and fourth rankArabic numbers are assigned and for the second and third ranksuppercase and lowercase letters are assigned, respectively. In this way, proteins sharing less than 45% pairwise identity are assigned a different primary rank (an Arabic number, e.g., Vip1 and Vip2); two proteins sharing less than 78% pairwise identity are assigned a different secondary rank (a capital letter, e.g., Vip3A and Vip3C); proteins sharing less than 95% pairwise identity are assigned a different tertiary rank (a lowercase letter, e.g., Vip3Aa and Vip3Ab); and, finally, to differentiate between proteins sharing more than 95% pairwise identity, a quaternary rank is assigned an Arabic number e.g., Vip3Aa1 and Vip3Aa2 (Crickmore *et al*., 2014).

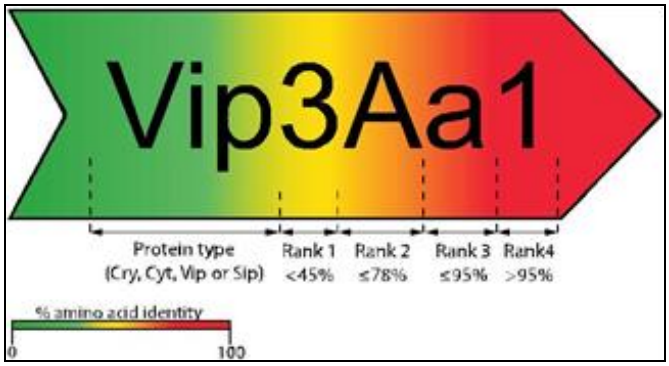

Fig 2: Schematic overview of the current nomenclature system used by the Bt ToxinNomenclature Committee for δ-endotoxins (Cry and Cyt) and secretable (Vip and Sip) toxins. In this example, numbers indicate different Vip proteins changing rank 1 depending of percentage amino acid similarity (for Vip proteins this rank may change to date among Vip1, Vip2, Vip3 and Vip4). The same rule applies for ranks 2, 3 and 4 assigning a different identification digit/letter (Palma *et al*., 2014) [19].

Bt mode of action

In the alkaline pH solution of the midgut following ingestion by the susceptible insect larvae, protoxins are solubilized and proteolytically digested to release the toxic fragments (Guillet and de Barjac, 1979) [12]. Amidst proteolytic activation, removal of peptides from both amino- and carboxyl-terminal ends of the protoxins occurs. Theprotoxins which are of 130 to 140 kDa, the carboxyl terminal proteolytic activation reduce the molecule to half, leading to the formation of an active toxin fragment of 60 to 70 kDa. A generally accepted model for Cry toxin action is that it is amultistage process. First, the activated toxin binds to receptors located on the apical microvillus membrane of epithelial midgut cells (Bravo *et al*., 1992; Hofmann *et al*., 1988) [4]. After the toxin binds the receptor, it is thought that there is a change in the toxin's conformation, allowing toxin insertion into the membrane. This is then followed by toxin oligomerisation, whereby pores are formed by the oligomers leading to osmotic cell lysis.

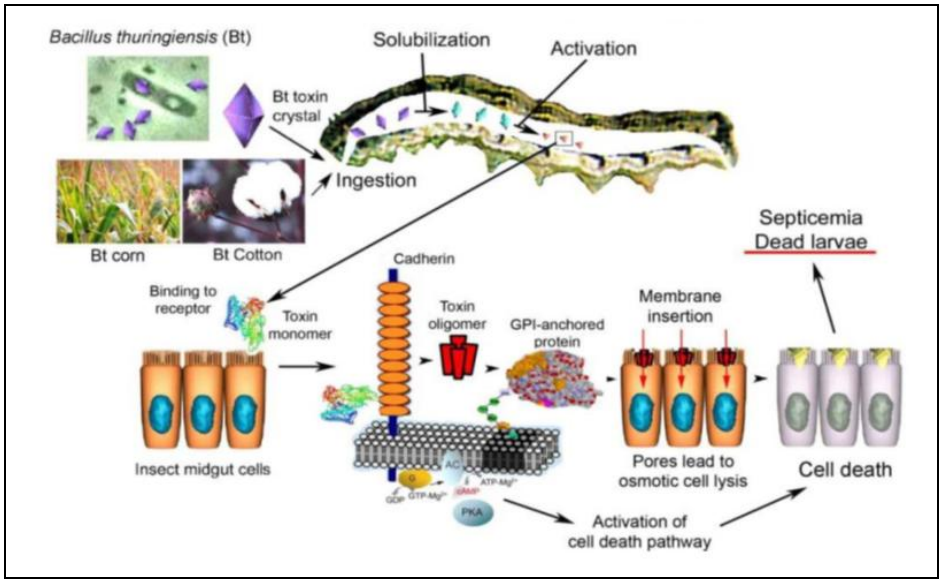

Fig 3: Mechanism of Bt infection (Ibrahim*et al.*, 2010)

Table 1: Bt based products in India

Species/Strains	Target insect	Product
Bacillus thuringiensis var. *kurtaski*	lepidoptera	Biobit, Costar, Thuricide, bactospeine, Javeline, Dipel BL, Delfin WG, Bactin, Bio-Tek, Bio Bit WP, WOCK Biological (Halt-Bt).
Bacillus thuringiensis var.*israeliensis*	Diptera	Aqua Bac XT, vectobac, bactomos, skeetal, teknar
Bacillus thuringiensis var. *tenebrionis*	Coleoptera	Novodor, Trident, M-One
Bacillus thuringiensis var.*galleriae*	lepidoptera	Spicturin
Bacillus thuringiensis var.*sandiego*	Colorado potato beetle	M- Trak, foil
Bacillus thuringiensis var. *aizawai*	lepidoptera	Xentari, certan
Bacillus thuringiensis EG2348	*Lymantria dispar*	Condor
Bacillus thuringiensis EG2371	Lepidopteran larvae	Cutlass
Bacillus thuringiensis EG2424	Coleopteran larvae	Foil

Role and impact of Bt in Agro system:

1. Bt plays a vital role in the management of insect pest with chewing type of mouthparts i.e. the larval stages of insect orders Diptera, Coleoptera and Lepidoptera which results in severe yield reduction due to its voracious feeding habits.
2. It serves as an alternative to chemical pesticides. The efficacy of Bt in controlling the insect pest is comparable with some chemical insecticides. Hence, more inclinations towards employing bt provides a sustainable technique in pest management thus reducing the usage of broad spectrum insecticide. (Purushothaman *et al*., 2013) [20] studied the efficacy of Profenophos, *Bacillus thuringiensis*, Carbaryl and *Beauveria bassiana*and revealed that treatmentsprofenophos @ 1ml/litre at the time of flowering, *Bacillus thuringiensis* @ 1g/litre one at the time of flowering and next one at 15 dayslater, *Beauveria bassiana* @ 20 g/litre at the time of flowering and other one at 15 days interval and carbaryl @ 4 g/litre atthe time of flowering recorded the per cent pod borer incidence of 6.99, 7.40, 7.86 and 8.96, respectively. Significantly higher grain yield was recorded in profenophos (817.18 kg/ha), *Bacillus thuringiensis* (754.89 kg/ha) and *Beauveriabassiana* (748.66 kg/ha) and carbaryl (669.31 kg/ha) treated plots.
3. Since the pollinators are unaffected with the application of biopesticides or when chemical pesticides are avoided, hence crop yield are significantly enhanced.
4. Pollination by honeybees and wild bees significantly increased yield quantity andquality on average up to 62%, while exclusion of pollinators caused an average yield gap of 37% incotton and 59% in sesame (Stein *et al*.,2017) [23].
5. Due to its high host specificity the beneficial insects within the vicinity of its application remains unaffected. Thus, the Natural Enemies which are readily available in the agro system contributes in the management of the insect pest. Lu *et al*., (2012) [18] showed that in the last 13 years GM crops delivered significant environmental benefits by reducing the insecticide usage by 50% and doubling the level of ladybirds, lacewings and spiders. Moreover, the study also stated that the environmental benefits extended to neighboring crops of maize, peanuts and soybeans. Udikeri (2006), UAS, Dharwad, studied the dynamics of cotton aphids and predators in RCH-2Bt and non-Bt cotton hybrids. Laboratory feeding experiments using Bt and non Bt cotton were carried out to study the effect of Bt fed aphids on predator indicated no difference in incubation period, longevity of grubs and adults, fecundity and aphid consumption potential indicating safety of Cry1Ac to predator through intoxicated aphid host. Dong *et al*., (2003) [8] reported only minor effects on some life table parameters in laboratory feeding studies with lacewings and predatory beetles and none with predatory bugs and spiders. There was some evidence of a reduction in numbers of predators and parasitoids which specialized on the Bt controlled bollworms, but also of increases in numbers and diversity of generalist predators such as spiders. A decrease in the parasitoid and predator populations can be associated with decrease in the densities of the pest populations on account of Bt-cotton. Unsprayed Bt cotton sustained 4 times more attack of tarnished bugs, 2.4 times more with boll weevil, 2.8 times more with stink bugs

and *Spodoptera*.

Increased in cotton yield after the introduction of Bt cotton in India

Before the inception of Bt cotton technology in India, most of the insecticides were unsuccessfully used for cotton insect-pest. About 70 % was for bollworm control and the rest for sap sucking insecticides. From 1995 to 2004Insecticide usage for bollworm control was average 6767 M tonnesand from 2005 to 2011 was average 1089 M tonnes. Over all, upto 69% reduction usage of pesticides has been achieved through Bt transgenic cotton (Kranthi, 2012) [16].

Table 2: Thirteen years of adoption and commercial release of Bt Cotton in India, 2002-2014

Year	# of Bt cotton events	# of Bt cotton hybrids	# of seed companies selling Bt cotton	Adopti on of Bt cotton (Mha)	Total cotton area (Mha)	% Bt cotton area	# of Bt cotton farmers (Million)	% of single gene Bt cotton	% of double gene Bt cotton	Cotton producti on (M Bales)	Cotton yield (Kg/ha)	Total insecticides to control bollworms (Metric tons)
2002-03	1	3	1	0.05	7.7	1	0.05	100	-	13.6	302	4470
2003-04	1	3	1	0.1	7.6	1	0.08	100	-	17.9	399	6599
2004-05	1	4	1	0.5	8.9	6	0.3	100	-	24.3	463	6454
2005-06	1	30	3	1.3	8.9	15	1.0	100	-	24.4	467	2923
2006-07	4	62	15	3.8	9.2	42	2.3	96	4	28	521	1874
2007-08	4	131	24	6.2	9.4	66	3.8	92	8	31.5	567	1201
2008-09	5	274	30	7.6	9.4	81	5.0	73	27	29	525	652
2009-10	6	522	35	8.4	10.3	81	5.6	43	57	30.5	503	500
2010-11	6	780	35	9.4	11.0	85	6.2	30	70	31.2	475	249
2011-12	6	884	40	10.6	12.2	88	7.0	18	82	35.3	493	222
2012-13	6	1097	44	10.8	11.6	93	7.2	10	90	33.4	489	-
2013-14	6	1167	45	11.6	12.25	95	7.7	4	96	39	541	-

(Choudhary and Gaur, 2015) [5]

Resistance Management

With the continuous application of Bt cotton the insect pest *Pectinophora gossypiella* started to show resistance. The most globally accepted and effective method in delaying the resistance against bt is Refugia method

Refugia is a method which involves growing of non-bt crops along with the bt crops. This helps in maintaining the resistance to the insect pest at a low frequency as the non bt version crop has no resistance alleles. The strategy is based on the fact that if small defined areas of non-transgenic plants are cultivated in close vicinity of the toxin expressing transgenic plants, they serve as hosts of the target Bt-susceptible insect pests to multiply. These would then serve as reservoirs of the susceptible alleles and when mated with the rare resistant survivors from transgenic plants would result in heterozygous progeny which would express susceptibility, especially if the resistant alleles are recessive in nature. The probability of the susceptible alleles mating with the resistant insects from Bt plants would be high because of the large population of susceptible insects from the non-Bt refuge. Hence having a refuge in close proximity helps in the effectiveness of the refuge. In India the Genetic Engineering Approval Committee (GEAC) has recommended refuge of non-Bt (5 border rows) with Bt-cotton per acre or an area of 20% Bt cotton that can be subjected to insecticide sprays. Recently, in 2009, Pigeonpea has also been approved as Refugia to be cultivated as border rows around Bt cotton (Kranthi, 2012) [16]

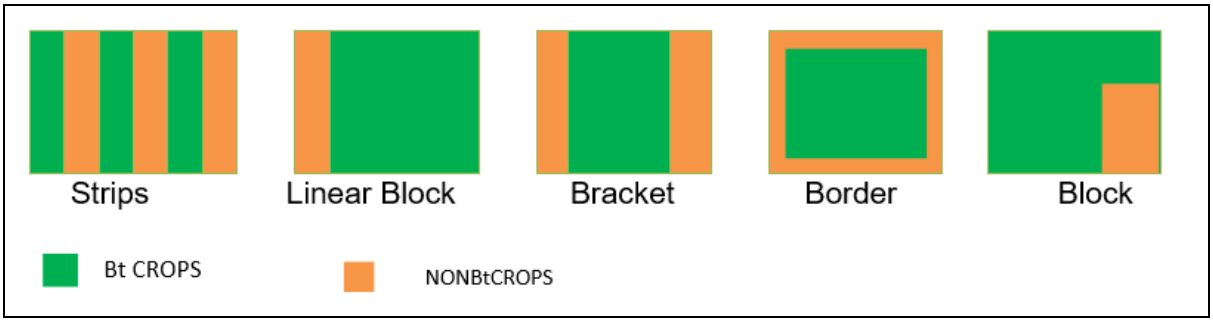

Fig 4: Pattern of Refugia planting

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