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A review on Pest management by using *Bacillus thuringiensis* as biological control: An IPM strategy

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Abstract
Insect population is raising day by day and the crops have become more susceptible to pest attack. Although some pests could be controlled by their natural enemies but some pest are essential to be controlled by pest management. The demand for crops is increasing but the minimum yield is producing due to huge crop damage by multiple insects, pests and other micro-organisms. These pests are the source of production of wide variety of diseases to plants damaging whole crop of the year, not only spoiling quality of crops, causing diseases, but also causing huge economic loss at agricultural level. In this research paper we will describe pest's management strategies that would be responsible for eradicating pests to improve quality of crops with minimum crop damage. Biological control is an effective method for managing pests and it proves to be fruitfull at much extent. *B. thurengiensis* has ability to produce crystal inclusion bodies which are known as Delta endotoxins or insecticidal crystal proteins which have resistance and toxic effects against targeted pests based upon its specifity of mode of action. Various strains of *B. thurengiensis* has been discover yet on the basis of presence of various antigens on its surface and production of its toxicity. It has ability to produce cry (crystal) genes which show toxicity against pests and within a short period of time the insect die due to its toxic effects.

Keywords: biological control, bacillus *thurenginesis* (*B. thurenginesis*) cry genes (endotoxins), CPI (Crystalline Proteinacious Inclusions), VIPs (Vegetative Insecticidal Proteins)

1. Introduction
Integrated pest management, the broad field concept which considers all possible methodologies in which insects or pests are properly managed instead of their complete removal from the crop. Biological control is a process that uses the living organisms to control the pests [1]. The biological method not only reduces the pest concentration but also ensures the less or approximately no damage to non-targeted natural species hence it is environment friendly [2]. Pest management by biological control does not bring harmful effects on human health because it shows resistance to only targeted species of insects. Those species that are responsible for the removal of pest are termed as biological control agents [2].

Bacillus spores were firstly used by Egyptians to save their crops [4]. In 1901, Shigetane Ishiwatari was the first biologist who recognize a bacteria while studying a disease caused by silk worm and he reffered it as *Bacillus sotto* [4]. In 1911, Ernest Berliner during the investigation of infected moth saw a bacteria from the German state of Thurengia and on the name of this state he refferd it as *B. thurengiensis* [4]. In 1901, scientists named Mates performed experiment Against corn Borer and figure out fruitful results from it. The time of 1938 was the golden period for the production of first pest resistant protein refferd as Sporeine. It was developed in France for the first time and the primary purpose of it was to kill the flour moths population from the crops [4]. In 1953, a scientist Christopher Hanny assigned the alternative name of insectisidal proteins of *B. thurengiensis* as 'Parasporal Crystals' Thomas Angus does experiments on killing property of insecticidal proteins in 1953 in coordination with another scientist Flip Fitz James, Haany poses that these parasporal crystals are products of gene translation in 1955 [5].

During 1958, *B. thurengiensis* was commercially introduced in United States [5]. From 1960 to 1990,*B.thurengiensis* took economic importance and piriority of major agricultural industries, the reason of it was the longer time duration for the synthesis of new insecticide, its testation and implementation period and checking of its effectiveness consume much time and cost. Comparatively biologically produced insect killer agents were easy to handle with safety of environment and and did consume much cost [6]. A wide Variety of *B. thurengiensis* based products is spreading around the Globe from 1996 [5]. Different regions of the world start turning their chemical industry into *B. thurengiensis* based industry in which Abbot Laboratories (USA), American Cyanamid (USA), BASF (Germany) are listed [6]. According to latest data almost 50 percent cotton and 40 percent corn produced in US is able to produce *B. thurengnesis* toxins against pests.

a) *Bacillus thuringiensis*
Bacillus thuringiensis is a versatile, facultative anaerobic, spore forming bacteria which posses the ability to give crystal like proteins termed crystal proteins which can kill targeted insects and some worms which are formed in sporulation. The alternative name for cry proteins is *Bacillus thuringiensis* toxins which are insectspecific and act on particular type of insect [3]. *Bacillus thuringiensis* is a bacterium that is unique from all other class of *Bacillus cereus* by its versatile property that is its 25 percent volume of cell is uniquely capable of showing pathogenic effects againt different pest.

B. thurengiensis life cycle posses two distinct stages, vegetative stage and spororulation stage, during vegetative stage the cell distribute its components into two cells of equal size sharing all cellular. While sporulation stage could be a product of many stages in which at a particular time period *B. thurengiensis* a particular structure appears and also insecticidal proteins or spores are the final products formed at the end of sporulation stage ingested by larvae of different orders of insects and developing cell lysis due to the activation of *B. thurengiensis* in insect gut [4].

During the period of sporulation cell continuously shows unorganized pattern of division forming pioneer toxin at third stage and at stage seven it causes breakdown of its cell membrane topiched out its toxic genes or endotoxins [5]. The genes which are responsible for the production of toxicity are located on extacellular DNA of bacterium [7]. The *B. thurengiensis* toxins are also termed as insecticidal proteins due to the killing property of targeted insects causing damage to useful crops and are capable of creating a new way towards the transgenic crops because many genes of interest *B. thurengiensis* are inserted into crops of commercial value like cotton which show resistance in response to targeted pests [3]. It is a bactria that could be available everywhere, it does not exist in soil actually insects or pests are the basic sources that are able to move it into soil, it starts its proliferation or developmental stages in soil when all the necessary machinery required for its growth become available in its habiatat [7]. The toxins produced during sporulation inhabit central position at spore membrane and these inactivated protoxins are converted into activated toxins by the high pH environment or in basic portion of insect digestive system [7].

Biological control by *B. thurengiensis* lowers the use of chemicals to crops which improves the soil conditios for increasing yield [10]. *B. thurengiensis* posses four sub species referred as *B. thurengiensis aizawaio* posses toxic genes that can kill the lepidopterean larvae from the crops, *B. thurengiensis* is raelensis have ability to target toxicity against mosquitos and black fly, *B. thurengiensis kustaki* which posses toxins effective aginst larvae of order lepidoptera and *B. thurengiensis tenebrionis* which is able to reduce the population of Colorado Beetle which harms crops of commercial Importance [6]. Every gene of *B. thurengiensis* has its own specific crystal shape, specific protein size and specific host, for example Cry 1 gene of *B. thurengiensis* have bipyramidal shape, 130 kD protein size and it act specifically on the larvae of order Lepidoptera while Cry3 gene posses flat shape 78 kD protein size and it specifically act on the larvae of order coleopteran [6].

b) Ecology of *Bacillus Thuringiensis*

B. thuringiensis is produced in different environment. Strains of *B. thurengiens* is have been isolated world wild from many different habitats, in which soil, stored-product dust and many leaf surfaces areas included .For the isolation of spore heat treatment is involve in a typical manner *B. thurengiensis* is an insect pathogen [13].

Different kind of *B. thurengiensis* species are isolated from dead or decaying insects, mostly are found from different order for example Coleptera, Diptera and Lepidoptera but most kind of species isolated from soil, leaf surfaces and other habitats. Dead bodies of insects mostly contain large amount of spores and ICPs that may enter into the environment. Some kind of sub-species for example coleopteran-active and lepidopteran-active *B. thurengiensis* sub-species are particularly associated with soil and phylloplane (leaf surfaes). While some kind of subspecies exists in aquatic environment for example diptersn-active *B. thuringiensis* is are generally found in this environment. Spores have the ability to persist and vegetatively grow in favorable and nutrient available environment.

Prevalence

Prevalence of *B. thurengiensis* is detected in 30 out of 39 organic vegetable (76.9%) having mean value of about 2.60 log CFU/g. It is observe that *B. thurengiensis* have heigest frequency in leafy vegatables. Spore of *B. thurengiensis* are sperated from 148 out of 189(78.9) and 13 out of 189 (6.9%) rice sample [13].

Table 1: Prevalence of *B. thurengiensis* in organic vegetables.

Isolates	No. of samples containing *B. thuringiensis* (%)	Mean ± SD [a,b]
Leafy vegetables (n = 26)	23 (88.5)	3.09 ± 1.47
Flower head brassicaceae (n = 6)	4 (66.7)	2.02 ± 0.23
Fruiting vegetables (n = 4)	3 (75.0)	1.89 ± 1.66
Root and tuber vegetables (n = 3)	0 (0.0)	-
Total (n = 39)	30 (76.9)	2.60 ± 1.59

STD[ab]: Standard deviation
Unit: log CFU/g

Moreover, it is assumed that *B. thurengiensis* is more readily isolated from stored product or insect cadavers than from soil. *B. t*hurengiensis have ability to multiply in blood of insect to stimulate their circulatory system. It also produce antibiotic compounds that perform fungal activity [11].

2. Genetics of *Bacillus thurenginesis*

Genetically, It is assumed that *Bacillus thuringiensis* and *Bacillus cereus* are from single sub- specie. *B. thurengiens*is higly specific against insects because of production of crystal (*cry)* protein (endo-toxin) used as biological method in worldwide. *B. thurengiensis* is not only produce crystal but also produce cytolytic (cyt) protein. Toxin is produced by combination of both two crystal and cytolytic protein that cause disruption of cell and heamolytic (destruction of red blood cell) against mammalian cell line [14].

In 1980, it was established that, in *B. thurengiensis* most of genes are coding for ICPSs that is present on large transmissible plasmid which are immediately exchange by conjugation between strains. Most *B. thuringiensis* toxin genes appear to reside on plasmid [11]. According to these Initial studeies ICPs gene can be produce hindered in numbers. ICPs can be propagate and form identical copies to construct *B. thurengiensis* strains [12]. Cry is the type of gene that encode for ICPs, it is specific for specific order like Lepidoptera (cryI),Diptera and lepidoptera (cryII), coleopteran (cryIII), Diptera(cry IV), coleoptera (cry V) [12]. ICPs gene provide sequence for the production of *B. thurengiensis* strains consist of specific gene by hyberdization and PCR analysis is also done to know about already present nucleotides sequences [14].

Other than cry protein many pesticidal protein are produced from *B. thuregiensis* strains during the vegetative growth. these VIPs are secreted from the cell and never having ability to produce toxicity i.e Crystal protein. These are non crystal-forming proteins term VIPs is misnomer in the sence that *B. thurengiensis* cry protein produce during vegetative growth as well as during sporulation or stationary phase. Location of *B. thurengiensis* genome has not been describe, In spite of the fact that it is not surprising to find that residing on large plasmid that encode cry gene [11].

2.1 Genes coding protein

A 100-kDa protein that is encoded by *vip 1A* appearently prepared by special process from its N-terminus to yield 80-kDa protein upon secretion.80-kDa protein is toxic to western corn rootworm larvae when conjugate with Vip2A protein.

88-kDa protein is encoded by a gene i.e. vip3A, which is produced not by a special process during vegetative growth. This protein is reported to produce toxicity in wide variety of lepidopteran insect pests, included Agrotis Ipsilon, spodopterafrugiperda. Vip 3A cause gut paralysis and cause breaking of epithelial layer of midgut when a susceptible insect fed at lethal concentrations [11].

2.2 *B. thurengiensis* genome

Genome size of *B. thurenginesis* strain is about 2.4 to 5.7 million bp. Physical map for two *B. thurengiensis* and *B. cereus* have been suggested that all of these chromosomes have same organization. It is recongnize the protein that comprise proposal crystal particularly encoded by large plasmid.

B. thurengiensis species have transposable element (jumping gene or sequence of gene that jump from one location genome to another. Transposable element of *B. thurengiensis*is regarded to their structural association with crygene. Role of transposable element in *B. thurengiensis* is amplification of cry gene in bacterial cell, but this hypothesis is not clearly tested. Second role is transfer of plasmid by conduction process involving the formation of covalently bonding between self conjugative plasmid and chromosomal DNA plasmid [11].

2.3 Cry gene Expression

Cry gene show their expression during the stationary phase, product of cry gene particularly accumulated in mother cell compartment for the formation of crystal inclusion of about 20 to 30% dry weight of sporulation. In *B. thurengiensis* high level of crystal protein sysnthesis and coordianation with stationary phase is controlled by variable mechanism occurring at the transcriptional, post-transcriptional and post-translation levels [11].

cry1Aa, cry2Aa, cry3Aa and *cry4Aa* gene

High level of toxic production in *B. thurengiensis* is due to stability of mRNA. Half-life of *cry* mRNA, about 10 min, is at least fivefold greater than the half-life of an average bacterial mRNA. In post transcriptional mechanism *cry1Aa* gene (stem loop structure) act as a positive retro regulator. Cry1Aa transcriptional terminator increase the cry mRNA stability by processing it from exonucleolytic degradation from 3'endmRNA stability has not been tested by deleting them from a *cry* gene and measuring stability of message [10].

The three dimensional sturucture of three activated forms of Bt toxins i. ecry1Aa, cry2 and cry3 have been solved by X-ray crystallography and relatively each consist of three domains. N-termila domain I consis of seven alpha-helics (six aliphatic helicals around a central core helix).Domain II is so- called beta-prism, with three folds symmetry consisting of three beta sheets having a having a Greek key conformation. C-terminal domain III consist of two antiparallel beta sheets in a jelly roll formation. Domain I is involved in membrane insertion and pore formation. Domains II and III and both involve in receptor recognition and binding [14].

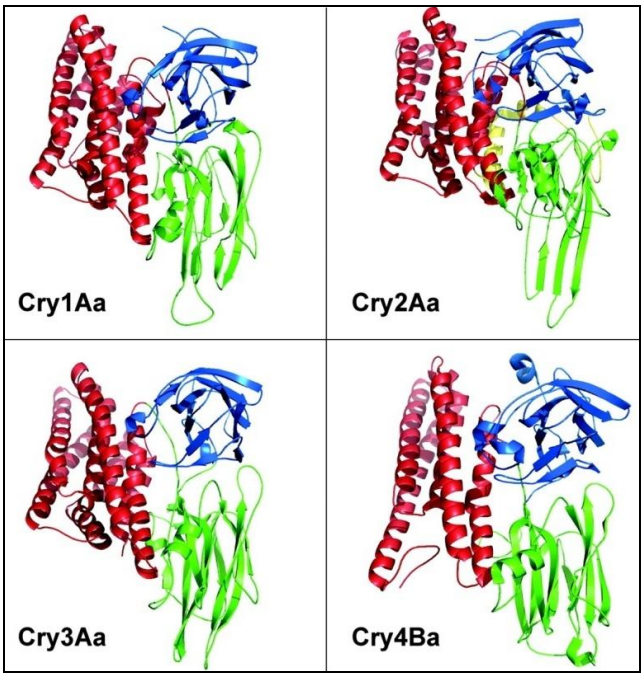

Fig 1: Three dimensional structucture of cry1Aa, cry2Aa, and cry3Aa and cry4Aa [10]

2.4 Beta-exotoxin

Beta-exotoxin is toxin and associated with *B. thurengiensis* sub-species and those product which is formed from the *B. thurengiensis* sub-species contain toxin. Heat stable nucleotide (Adenin, Glucose, allaric acid) inhibit RNA polymerase enzymes by acting competitively with ATP. In life RNA synthesis is a vital process, beta-exotoxin exerts its toxicity almost in numerous insects specis order Coleoptera, Diptera and Lepidoptera. In some countries beta–exotoxin is used for the control of houseflies but regulatory agencies currently inhibit the use of beta exotoxin for any purpose.

3. Mechanism of action of endotoxins:

Crystal included in *B. thurengiensis* i.e. endotoxins kill the targeted insect when larvae of that insect ingest it and incorporate them into its digestive system. These endotoxins then start binding to their particular receptors there specially in the mid gut receptors due to the availability of alkaline conditions over there, alkalline conditions reinforce this receptor specific binding, as binding proceeds holes are created in cell membrane of gut epithilia and eventually this

causes the imbalance in movement of ions across the pores across gut epithila of midgut producing toxicity [4]. Now it was noticeable that what factor is responsible for the production of toxicity or activation of cry genes in midgut, it was basically the presence of Specific receptors at brush borders of epithilial Cells, host cell proteases enzymes for catalytic activity and the availability of alkaline medium over there which converts Inactive protoxin Into active form of toxin to cause death of the insect [4]. Now it is clear that's why these toxins are harmless to mammals because they lack specific receptors in gut and acidic medium exist there [4].

There exixts three basic steps ivolved in pathogenicity listed below:

In first step, Ingestion of Insecticidal Proteins by Scusceptible larvae, dissolution of insecticidal endotoxins by specillized enzymes (proteases) of host body [8]. In second step binding to specific receptors at plasma memberane of gut epithilia of insect targeted binding to specific receptors at plasma memberane of gut epithilia [8] In last third step, entry of endotoxin into pest epithelia and formation of holes there to allow to make membrane permeable for multiple ions which also allow the movement of water into the cell causing the cell to burst and die instantly [8]. Another hypothesis for the action of that endotoxins was the sequential stimulation of G proteins in pest [8]. As endotoxin get attached to Cadherin protein on cell memebrane of gut epithillia it initiates a cycle that stimulate G proteins which in turn stimulates Adenyle Cyclase, its activation build up high level of cyclic AMP which initiate formation of a protein known as protein Kinase which ultimately leads to pathogenesis in host cell [7].

Till now there exist 82 various serotypes and 235 toxic genes of *B. thuringiensis* [7]. Beside the production of Crystal proteins or Crystal genes another type of proteins are also derived from the *B. thurengiensis* which are known as VIPs or vegetative insecticidal proteins [7]. The important feature of crystal genes is their expression during vegetative stage of their lifecycle [7].

The specificity of insecticidal proteins of *B. thurengiensis* is of great importantce, every endotoxin show specificity in its structure and kill a particular type of pest, for example lepidoptearan (butterflies and moths) could be mostly eradicated from the crops by the sub species of *B. thurenginesi kurstaki* while *israelensis specifically* remove the diptearans and *morrisoni* strains are responsible for the causage of death in coleopterans specifically, similarly *B. thurengiensis* strains that posses CRY-5 and CRY-6 genes are specifically able to cause death of nematodes [4].

B. thurenginesis genes are classified by taking into account the toxins they produce, while toxins are studied by their amino acids sequence and protein configuration and toxicity production, cry genes could also be classified on the basis of specific receptor they bind of insect midgut while CYT genes show relationship with the plama membrane lipids showing their own specificity and classification criteria for studying purpose and invention of new genes of particular specificity [4]

There exist a wide variety of cry toxins out of which some are listed here (Cry1Aa, Cry2Aa, Cry3Aa, Cry3Bb, Cry4Aa and Cry4Ba).

Although there are numerous endotoxins but posses some common structural feature that is the presence of two distinct regions, C terminus side is capable of producing crystal inclusions or endotoxins while N terminus is toxin itself that posses three distinct domains therefore these are refferd as 3D toxins as well [4] The subspecies of *B. thurengenesis* that is *B. thurenginesis kurstaki HD-1, B. thurengiensis aizawai, B. thurengiensis kursatakiSA-12, B. thurengiensis kurstaki SA-11* specifically produce toxins againt the order butterflies and moths while subspecies *B. thurengiensis tenebrionis* produce toxicicity against order Coleoptera and *B. thurenginesis isralienesis* are capable of producing toxins aginst order Diptera [4]. Crystal proteins which have been discovered yet constitute Cry 1 with nine sub-species, Cry2 with three sub-species, Cry3 with also three sub-Specis and Cry 4 with four sub-species [6]. Beside this these genes Cry5B, Cry6A, Cry14A, and Cry21A are pathogenic against nematodes.

4. Conclusion

As *B. thurengiensis* specificity in its mode of action bt has no harmful effects on non targeted species of insects. It is environmental friendly and causes no damage to human health. Products which are produced comercially by *B. thurengiensis a*re easy and safe to use and have biopesticidal properties making a successful pest management to save crops for gaining commercial importance. *B. thurengiensis* products could be used at agricultural and horticultural level to control pests. *B. thurengiensis* could also be used in aquatic environment to safe drinking water reservoir from mosquitoes and black flies. various novel strains of *B. thurengiensis* could be produce by modifying its genome toxic and resistant to wide variety of arthropods.

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