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# Toxins and Targets of Insecticidal Genes of Entomopathogenic Bacteria: A Review

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**Insects are susceptible to a variety of diseases caused by microbes which are exploited for their biological control through inundative applications. Control of insects by microbs is intensively investigated to develop environmental friendly pest management strategies in agriculture. Among these the most successfully utilized for insect management is *Bacillus thuringiensis* (*Bt*), which is used extensively against lepidopteran pests. Insecticidal toxins used in agriculture are predominantly from Gram-positive bacteria and derived mostly from *Bacillus thuringiensis* producing the toxins a) neurotoxins b) digestive toxins and c) cytotoxins, categorized based on their target tissues. Members of the Enterobacteriaceae, *Photorhabdus*, *Xenorhabdus*, *Serratia,* and *Yersinia* spp. produce insecticidal toxins with oral toxicity. The haemolymph are the novel targets of bacterial toxins while as most toxins are known for their toxicity to gut as well as nervous tissues in lepidopteran larvae. Therefore, this review describes the virulence factors associated with both Gram-negative and Gram-positive bacteria as well as their mode of action and their target organ in insect pests.**

**Keywords:** Bacteria, Insect, Management, Microbial, Bacteria.

In modern agricultural system the research on microbial pathogens of insects is increasing considerably to find out environmental friendly alternatives to hazardous chemical pesticides. In this regard, a Science Congress held in Europe entitle "Pesticide use and risk reduction in European farming systems", aimed to reduce the dependence on pesticides in modern agriculture through the implementation of general principles of Integrated Pest Management (IOBC, 2015). The microbials, broadly speaking biopesticides are the biological agents that are usually applied at appropriate formulation and application in a manner similar to synthetic chemical pesticides to achieve desirable pest management (OMICS, 2015; Matthews *et al*., 2014) in an environmentally friendly way. Whileas, the concept of EcoPesticides is developing, means biological based pesticide and encapsulation technology, designed and aimed to extend the potency and performance of the insect controlling properties of "green" material, especially the naturally occurring bacteria and fungi against to insect pests (Lux, 2015). Currently, the microbial pesticide market is $2 billion and is estimated to double by 2017 and will expectedly reach $5 billion by 2020 (ALBUQUERQUE, 2015). However, the market was assumed to increase to $3.3 billion in 2014 against the pesticide market of $51.1 billion (BCC, 2014) and biopesticides sales in crop protection market is expected to grow by 15% annually until 2020 (Lux, 2015).

The pathogen that cause most diseases in insects and successfully used for insect pest control is bacterium, *Bacillus thuringiensis* (*Bt*). Each one of *Bt* strains produces different mix of toxins and specifically kills one or a few related species of insects as *Bt* sub. *kurstaki* and *aizawai* for lepidopteran larvae and *Bt* sub. *tenebrionis* for coleopteran larvae. *Bt* subspecies *israelensis* is specific to mosquitoes (Diptera). Research suggests that, in response to bacterial and fungal infections in insects, an innate immune pattern

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recognition receptors (PRRs) initiate highly complex intracellular signaling cascades, which induce a variety of immune functions that restrain the spread of microbes in the host population (Stokes *et al*., 2015).In the current year the biopesticide *Isaria fumosorosea* strain Apopka 97 (*Paecilomyces fumosoroseus*) have been approved for use (Bird, 2015) as a component in IPM.

The discovery that *Bt* spore associated toxins are extremely virulent and persist in the environment with high potency (Koch *et al*., 2015) prompted the development of bacterial spray formulations and also the transgenic (Genetically Modified Plants) pants express the bacterial toxins against the insect pests (Di and Tumer, 2015; Nhan *et al*., 2015; Kamthan *et al*., 2015; Katriraee, 2015). The advancement in the characterization of bacterial pathogens, whole genome characterization and comparison has prompted the discovery of novel pest management tools. Insecticidal molecules expressed and secreted by various entomopathogenic bacteria have been targeted for the genetic manipulation to enhance toxicity (Sellami *et al*., 2015; Lin *et al*., 2015). Recently other insect pathogenic bacteria with mode of action similar to Bt have been hailed as agriculturally relevant. Currently insect pathogenic bacteria of diverse taxonomic groups and phylogenetic origin have been shown to have striking similarities in the virulence factors which are often encoded on plasmids and bacteriophages and can easily be spread through horizontal gene transfer. For example, *Photorhabdus luminescens* and *Heterorhabditis bacteriophora* have been shown to produce virulence factors (Gerdes *et al*., 2015; Castaneda *et al*., 2015) similar to that of Bt. *B. thuringiensis* produce crystal protein. When an insect ingests these proteins they are activated by proteolytic cleavage. The N-terminus is cleaved in all of the proteins and C-terminal extension is cleaved. Once activated the endotoxin binds to the gut epithelium and causes cell lysis by the formation of cation-selective channels leads to insect death.

**Entomopathogenic bacteria**

One of the best modern agricultural defenses against plant eating insects is *Bacillus thuringiensis* (Ibrahim and Shawer, 2014). In recent times, it has become a source of agriculture innovations, providing a new solution to the age of old problems. Biotechnology is often equated with genetic engineering and the support or opposition to genetically engineered crops is often distilled down to being for or against 'science' (Vogel, 2014). Plant genes are being cloned, genetic regulatory signals deciphered and genes transferred from the entirely unrelated organisms to confer new agriculturally useful traits on crop plants (Josine *et al*., 2011). *Bt* protein toxins are highly selective to their target insect and are completely biodegradable. Therefore, *Bt* is a viable alternative for the control of insect pests in agriculture and disease spreading vectors in public health. Transgenic crops based on insecticidal crystal proteins of *Bt* are now an international industry with revenues of several billion dollars per year (James, 2011). Classification of *Bt* strains have been accomplished by H serotyping, the immunological reaction to the bacterial flagellar antigen. Specific flagellin amino acid sequences have been correlated to specific *Bt* H serotypes and at least 69 H serotypes and 82 serological varieties (serovars) of *Bt* have been characterized from around the world (Lecadet *et al*., 1999). After few decades of research on *Bacillus thuringiensis* (*Bt*), new novel bacterial species are being discovered and developed into new products especially derived from *Brevibacillus laterosporus*, *Chromobacterium subtsugae* and *Yersinia entomophaga* (Ruiu *et al*., 2013). It is reported that the entomopathogenic bacteria of diverse taxonomic groups and phylogenetic origin have striking similarities in the virulence factors they produce (Castagnola and Stock, 2014) on infestation.

**Insecticidal toxins produced by entomopathogenic bacteria**

Insecticidal toxins used in agriculture are predominantly from Gram-positive bacteria and derived mostly from *Bacillus thuringiensis*. Foliar sprays containing *B. thuringiensis* represent an organic alternative to synthetic foliar sprays. Entomopathogenic Gram-negative bacteria produce toxins which are categorized into three types based on their target tissues. These are a) neurotoxins b) digestive toxins and c) cytotoxins. According to Ricardo *et al*. (2015) reported that toxification involves the binding of Cry toxins produced by *Bacillus thuringiensis* to specific cellular receptors like CADP (cadherin-like protein),

a GPI (glycosylphosphatidyl-inositol)-anchored APN (aminopeptidase-N), a GPI-anchored ALP (alkaline phosphatase) and a 270-kDa glycoconjugate. Members in the Enterobacteriaceae such as *Photorhabdus*, *Xenorhabdus*, *Serratia*, and *Yersinia* spp. produce insecticidal toxins with oral toxicity similar to that of *Bt* toxins, but have not yet been fully utilized. This section describes the virulence factors associated with both Gram-negative and Gram-positive bacteria as well as their mode of action.

**Toxin genes produced by gram positive bacteria**

**Bacillaceae**

Bacillus comprised of three species viz., *B. thuringiensis, Bacillus cereus, Baccillus anthracis* which are the most widely studied taxa in terms of insecticidal toxins (Tahany *et al.*, 2015). Due to their unique pathogenicity properties and the diverse modes of actions of their insecticidal toxins, that support their distinctiveness, therefore, giving each separate species names (Priest *et al*., 2004). Bacillus *thuringiensis* upon sporulation forms crystals of proteinaceous endotoxins, called *Cry* proteins or crystal proteins, which are encoded by *cry* genes (Hofte and Whiteley, 1989). The *Cry* toxins, are toxic to may insect of order Lepidoptera, Diptera (Gough, 2002), Coleoptera (Kreig *et al.*, 1984) and Hymenoptera (Rose *et al*., 1999) and also against nematodes (Hui *et al*., 2013). The Cry protoxins are first solubilized by the alkaline pH (6-8) of the insect (Lepidopteran larva) gut, and then proteolytically activated by proteases. The toxins bind to specific receptors on the columnar cells of the larval midgut epithelium causing pore formation and gut cell death. Cry toxins are commonly classified as gut poisons, as they compromise the epithelial-hemocoel layer and ultimately lead to starvation and septicemia and finally the larval death (Schnepf *et al*., 1998). Crystal toxins after they bind, form pores, and damage midgut epithelial goblet cells in mixed midgut cell cultures (Loeb *et al.*, 2001) and *in vivo*. The Cry toxins from *B. thuringiensis* are almost exclusively considered as digestive toxins, however, they have homology to neurotoxins and attacks diverse tissues of lepidopteran larvae as the toxin meet suitable alkaline conditions in gut of larvae. They have been shown to kill larval neurons of the cerebral ganglia of central nervous system *in vitro* (Cerstiaens *et al*., 2001), invade liposomes causing morphological deformities to lipid bilayers (Haider and Ellar, 1989), initiate apoptosis in ovary and its derived cell (Zhang *et al*., 2006) and bind ATP-binding cassette (ABC) transporter (Tanaka *et al*., 2013). The mutated ABC trasporters are correlated with resistance to *Bt* toxins in silkworm, *Bombyx mori* (Atsumi *et al*., 2012), tobacco budworm, *Heliothis virescens* (Gahan *et al*., 2011), diamond back moth *Plutella xylostella* and cabbage looper *Trichoplusia ni* (Baxter, 2011). Recently it is reported that recombinant fusants have more efficient and potential toxicity values, compared with insecticidal *Bt* and the mosquitocidal *Bt* strains alone against *S. littoralis* and *C. pipiens* larvae, respectively (Tahany *et*., 2015).

*Bacillus cereus* has been mostly known for its role in human digestive food poisoning; and the endospore of *B. cereus* is not insecticidal unlike to *Bt*. Both *B. thuringiensis* and *B. cereus* produce non-proteinous insecticidal exotoxins; in addition, a small proteinous exotoxin is also produce by *B. cereus* (Perchat *et al*., 2005). Although, *B. cereus* grow and proliferate in the insect gut, and is mostly regarded as an opportunistic pathogen with the production of virulence factors that are most effective when titers are high. The *Bacillus* species known to produce insecticidal toxins are *Bacillus circulans* (Firmicutes: Bacillaceae) and *sphaeriscus* (Firmicutes: Bacillaceae) (=*Lysinibacillus sphaeriscus*). Virulence factors produced by the former species have been shown to affect most dipteran insects and other invertebrates such as nematodes and mollusks (Zwick *et al*., 2012). During the vegetative growth of the *L. sphaeriscus* the toxin produced is Sphaericolysin (Berry, 2011). Sphaericolysin toxin has heamoceolic toxicity toward *Blattela germanica* and *S.litura* (Nishiwaki *et al*., 2007).

**Clostridiaceae**

*Clostridium* species: These are anaerobic and spore-forming bacteria (Vaishnavi, 2015), produces binary proteinous toxins that are proteolytically activated by serine proteases (Barth *et al*., 2004). For example, *Clostridium bifermentans* serovar *malaysia*, produces larvicidal toxin active against mosquitoes (Nicolas *et al*., 1993). *Clostridium perfringens* (Firmicutes: Clostridiaceae) has an Iota toxin which binds actin by ADP-ribosylation and has a C-domain structure

like *Bt*, ultimately targets mammals (Tsuge *et al*., 2003). The *Clostridium difficile* produce the exotoxin known as cytotoxin B which cause reorganization of cytoskeletons, similar to *Mcf* and makes the caterpillars floppy and dull in appearance. The *Clostridium difficile* has a three domain structure, consisting of receptor binding, translocation, and one catalytic domain (Just *et al*., 2005). The actin binding C2 toxin of *C. botulinum*, has four domains of activation for pore formation and receptor recognition. The C2 toxins ribosylate at arginine involves dysfunctioning the actin by inducing actin polymerization (Aktories *et al*., 2012). The translated product of the *plu0822* gene, referred to as *Photox* toxin, stop actin polymerization by targeting an arginine amino acid with ADP-ribotransferase activity (Visschedyk *et al*., 2010).

**Toxin genes produced by gram negative bacteria *Photorhabdus***

*Photorhabdus* species: Like *Bacillus*, three species of *Photorhabdus* have been identified namely, *P. luminescens*, *P. temperate* and *P. asymbiotica* (Fischer *et al*., 1990). All photorhabdus species have a strong mutualistic association with *Heterorhabditis* nematodes which are parasitic to insects. However, the species *P. asymbiotica* has also been found associated with skin injuries in human (Gerrard *et al*., 2006) and is considered as an emerging human pathogen model system (Garrard *et al*., 2004). *Photorhabdus* spp. are facultative anaerobes and cannot live freely in the soil environment unlike to *B. thuringiensis*. Almost all the *Photorhabdus* species are vectored by the nematodes and together with it form an insecticidal complex that kills the insect in general and use the carcass for various life processes like reproduction and nutrition. Once the bacteria are delivered by the nematodes in the insect hemocoel, it first invades insect immune system and then produces toxins which break its epithelial tissues and finally kills the insect. The *Photorhabdus* genome contains a multitude of lump like pathogenicity islands with an abundance of toxin genes (Duchaud *et al.*, 2003). The major virulence factors produced by *Photorhabdus* consist of *mcf1* and *mcf2* (=makes caterpillar floppy) genes, the *Tc* (toxin complex) genes, *Pir* (Photorhabdus insect related) operon, and a multitude of other virulence factors associated with *Photorhabdus* virulence cassettes (PVC) (Rodou *et al*., 2010). The *Mcf* toxins are responsible for both rearrange actin cytoskeletons and induce apoptosis in both insect hemocytes and epithelial tissue, leading to tissue damage to the extent that there is a complete loss of turgor pressure throughout the infected insect (Daborn *et al*., 2002). The *Tc* toxin factors, similar to *Bt* Cry toxins, are orally ingested toxic compounds that have been known to be insecticidal to the insect taxa including coleopteran, lepidoptera, dipteral and hemiptera. Experiments has shown that *P. luminescens* is highly insecticidal and pathogenic when injected into hosts such as African cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae) and *P. xylostella* (Lepidoptera: Pluttidae). As the *Tc* are orally toxic compounds; therefore, are active in the lumen side (inside the lumen) of the insects' midgut epithelium and not in the basal side of this tissue, which is common rout for a hemocoel pathogen (Waterfield *et al*., 2005a). *Pir* proteins are other group of *Photorhabdus* toxins, known to have hemolymph (Waterfield *et al*., 2005b) and oral (Blackburn *et al*., 2006) toxicity. The *Pir* proteins have similarity in certain aspects to neurotoxin, leptinotarsin (Blackburn *et al*., 2006) and are binary (Rodou *et al*., 2010) in structure. The binding and destructive effects to insect neural tissue are a major factor in toxicity when *Photorhabdua* are injected into susceptible host. There are yet many more toxins to be characterized from the *Photorhabdus* genome which are responsible for hemolymph-based insect toxicity. For example, the *Txp40* protein has been identified in 59 different strains of both *Photorhabdus* and *Xenorhabdus* species, cause injectable toxicity to many lepidopteran pests, like greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), corn earworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), and Australian sheep blowfly, *Lucilia cuprina* (Diptera: Calliphoridae) (Brown *et al*., 2006). *Escherichia coli* when expresses *txp40* gene, it has been shown to be insecticidal to *P. xylostella* (Park *et al*., 2012). The midgut and the body cell lines of dipteral and lepidopteral insects are damaged by *Txp40* protein in *vitro* (Brown *et al*., 2006) while as, hemolymph (Waterfield *et al*., 2005b) and oral (Blackburn *et al*., 2006) toxicity were exhibited by *Pir* toxin proteins and

*Photorhabdus* toxins. The *Xenorhabdua* species of bacteria in this genus are also non-free living; though, they are symbiotically associated with nematodes of genus *Steinernema* (Brown *et al.*, 2004). Like *Heterorhabditis*, *Steinernema* nematodes are play a key role in vectoring the *Photorhabdus* and *Xenorhabdus* from one insect host to another, thereby dispersing the bacteria and finally mange the pest population. *Xenorhabdus* also produce a large number of insecticidal toxins and one example of toxin is from *Xenorhabdus nematophila* (Proteobacteria: Enterobacteriaceae) called *A24tox*, which kills *G. mellonella* and *H. armigera*. However, this toxin has a hypothetical homology in *Photorhabdus*, but without a significant match outside of this group (Sicard *et al.*, 2003).

The xenocin operon consists of two genes, *xciA* and *ximB*, when it is expressed; these proteinous molecules get secreted through flagellar type II secretion pathway. Xenocin *xciA* gene has RNAse activity and cytotoxicity; once these proteins are co-expressed it has an antimicrobial effect killing competing microbes in insect larvae (Sing *et al.*, 2013). *Xenorhabdus* bacteria also produce another insecticidal protein called *HIP57*

**Table 1.** Paralytic effects of ingested *Bt* on various lepidopteran families and species (Castagnola and Stock, 2014)

| Family | Species | Bt component | Response |
|---|---|---|---|
| Noctuidae | *Spodoptera* spp. | Not specified | No paralysis |
| | *H. virescens* | Bt var. *kurstaki* | Midgut paralysis |
| | *T. ni* | Not specified | Type I paralysis |
| Saturniidae | *Philosamia ricini* | Bt var. *sotto* crystals | Whole body paralysis (type I) |
| Crambidae | *Ostrinia nubilalis* | Bt var. *thuringiensis* crystalline paraspores | Gut paralysis |
| Pyralidae | *Phlegathontius* | Thuricide (International Minerals and | Abnormally quiescent, cessation of feeding |
| | *G. mellonella* | Spores and crystals derived from Thuricide | No paralysis, Type III most susceptible |
| | *Ephestia cautella* | Not specified | Type II paralysis |
| Sphingidae | *quinqueaculatus* | Chemical Corp., Libertyville, IL, USA) | No paralytic effect |
| Erebidae | *L. dispar* | Not specified | Type II paralysis |
| Plutellidae | *P. xylostella* | Bt biological products | Decreased movement with subsequent paralysis |
| | *P. xylostella* | Bt var. *kurstaki*-HD1 | Reduction of movements |
| Papilionidae | *Papilio demoleus* | Bti Berliner spore | Fairly rapid paralysis followed by alkalinity |
| Gelechiidae | *Pectinophora gossypella* | Delta-endotoxin endotoxin | Evidence of gut paralysis gut muscles surrounding the disorganized epithelium relaxed |
| Bombycidae | *B. mori* | *Bacillus sotto* | Paralysis within four hours |
| | *B. mori* | Bt var. *sotto* | Paralysis |
| | *B. mori* | Not specified | Type I paralysis |
| | *Quinquemaculata* | Bt | General paralysis |
| | *P. quinquemaculata* | Not specified | Type I paralysis |
| | *Protoparce sexta* | Bt crystals | General paralysis |
| | *Antheraea pernyi* | Bt crystals | General paralysis |
| Pieridae | *Colias eurytheme* | Bt var. *thuringiensis* | No paralysis |
| | *Pieris rapae* | Not specified | Type II paralysis |
| Hesperiidae | *Urbanus acawoios* | Bt var. *kurstaki* wettable powder | no general paralysis |
| Tortricidae | *Urbanus acawoios* | Bt Dipel foliar spray | Interruption of feeding due to gut paralysis |
| | *fumiferana* | Bt Dipel foliar spray | resulted in reducing rate of development |

that is similar to chaperonins like *GroEL* produce by *E. coli*. *GroEL* chaperonins help to combat problems such as aggregation when nascent proteins have hydrophobic residues exposed before reaching a fully folded native state (Ellis, 2005). The *HIP57* have injectable toxicity to *G.mellonella,* and the insecticidal property of it is a novel function for the *GroEL* proteins.

**Serratia**

*Serratia* species: These bacteria often possess fungicidal properties, but are facultatively associate with insect (Lamelas *et al.,* 2011) and nematodes (Abebe *et al.,* 2011). Genome studies have found several insecticidal genes in the *Serratia* genome and few species are responsible for causing amber disease in grass grubs, *Costelytra zealandica* (Coleoptera: Scarabaeidae) (Jackson *et al.,* 2001). However, contrastingly *Serratia marcescens* (Proteobacteria: Enterobacteriaceae) infects other host such as poorly reared *H. virescens* (Sikorowski *et al.,* 2001). Whileas, pADAP plasmid from *Serratia entomophila* contains the genes *sepA, sepB* and *sepC,* which are similar to the *Tc* genes described in *P. luminescens* and the *xpt* genes observed from *X. nematophila.* There is no need for the entire pADAP plasmid to be associated with the *sep* genes to cause death. However, when only *sep* genes are expressed without the entire plasmid, the scarab beetles do not cease feeding (Hurst *et al.,* 2000) which is one symptom of amber disease. Actually the virulence factor of pADAP that stops feeding in amber disease is the antifeeding prophage (Afp) (Hurst *et al.,* 2007). Therefore, it is concluded that both *sep* genes and Afp are needed for full virulence of *Serratia* in grass grubs that leads to its death.

**Yersinia**

*Yersinia* species: *Yersinia pestis*, the causative agent of bubonic disease is associated with fleas (UNC, 2015; CDCP, 2014) humans and rodent intermediates. Whileas, the two other species *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* often cause diarrheal disease and fever with inflammation in human beings. *Yersinia entomophaga* (Proteobacteria: Enterobacteriaceae) and *Yersinia frederiksenii* (Dodd *et al.,* 2006), cause disease in grass grubs and plague in humans (Gonzalez *et al.,* 2015).

**Table 2.** Susceptibility of *Helicoverpa virescens* neonate larvae to the combinations of the insecticidal proteins Vip3A and Cry1 crystals protoxins (Lemes *et al.,* 2014)

| S. No | Crystal and vegetative insecticidal protein combination | Respective concentrations[a] | Per cent larval mortality | | Fisher's test[d] (T-test) | Chi ($ü^2$) square Test (P)[e] |
|---|---|---|---|---|---|---|
| | | | Observed frequency[b] | Expected frequency[c] | | |
| 1 | Cry1Aa | 3.50 | 44 | 50 | No significance | No significance |
| 2 | Cry1Ac | 0.04 | 52 | 50 | do | do |
| 3 | Cry1Ca | 3.10 | 42 | 50 | do | do |
| 4 | Vip3Aa | 1.65 | 52 | 50 | do | do |
| 5 | Vip3Ae | 0.95 | 50 | 50 | do | do |
| 6 | Vip3Af | 0.87 | 50 | 50 | do | do |
| 7 | Vip3Aa+Cry1Aa | 1.65+3.50 | 69 | 73 | 0.4113 | 0.2017 (0.6534)* |
| 8 | Vip3Aa+Cry1Ca | 1.65+0.04 | 67 | 77 | 0.1823 | 1.2882 (0.2554)* |
| 9 | Vip3Ae+Cry1Ca | 1.65+3.10 | 33 | 72 | 0.00009** | 15.101(0.0001)*** |
| 10 | Vip3Ae+Cry1Aa | 0.946+3.50 | 73 | 72 | 0.5907** | 0.0000 (1.0000)* |
| 11 | Vip3Ae+Cry1Ac | 0.946+0.04 | 63 | 76 | 0.1354** | 1.7455 90.1864)* |
| 12 | Vip3Ae+Cry1Ca | 0.946+3.10 | 31 | 17 | 0.0001** | 15.048 (0.0001)* |
| 13 | Vip3Af+Cry1Aa | 0.874+3.50 | 50 | 72 | 0.0177** | 5.3211 (0.0211)* |
| 13 | Vip3Af+Cry1Ac | 0.874=0.04 | 50 | 76 | 0.0149** | 5.7501 (0.0165)* |
| 14 | Vip3Af+Cry1Ca | 0.874-3.10 | 37 | 71 | 0.0009** | 10.741 (0.001)** |

Concentrations of proteins were chosen such as to equal their respective LC50 values. Values are expressed as mg/cm$^2$.
[b] Each value represents the mean from three replicates of 16 larvae per replicate (n = 48).
[c]Expected mortality considering simple independent action.
[d]Asterisks indicate significant differences at P, 0.05, and two asterisks at P,0.001.
[e]Chi-square and P values.

**Targets of insecticidal genes and toxins produced by bacteria**

This section summarizes the current knowledge of hemolymph based toxicity caused by various entomopathogenic bacteria in relation to the neurobiology of insect pests.

**Hemolymp as a Novel Target**

The genomic organization of *P. luminescens* and *asymbiotica* consists of *Pir* operon with promoter region, Pir toxin i.e, *PirA* and *PirB* (Waterfield *et al*., 2005a), but it is not known how *PirA* and *Pir B* are differentially expressed when targeting an insect host. *Pir* toxin is a binary protein that may have an interesting mode of action based upon its homology profile, while *PirB* is homologous endotoxins consisting of a pore-forming domain unit and leptinotarsin. Leptinotarsin has been obtained from the infected Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and has homology to juvenile hormone esterase which is a regulatory protein present mainly in insect immature. However, *Pir* toxin does not disturb insect metamorphosis (Ffrench and Waterfield, 2005) but it (Leptinotarsin) is a neurotoxin that stimulates and activates the release of acetylcholine at the presynaptic nerve terminal (McClure *et al.,* 1980). That is why, the mode of action of Pir toxin and its potential relationship to the insect nervous system is yet to be elucidated. *Serratia* and *Photorhabdus* have phage related loci, and in *Serratia* these loci are pADAP, which causes decreased feeding in infected insects. Both *Serratia* and *Photorhabdus* phage related loci confer hemolymph based injectable toxicity on *Galleria* larvae, with hemolymph-circulating phagocytes (Yang, 2006) as a virulence factor. There are also the cycle inhibiting factors (*cif*) produced by *P. luminescens*, but it is not known how *Cif* interacts with an insect host, when the *cif* gene is incorporated into *Spodoptera* derived Sf9 cells. The cells once infected undergo apoptosis and thereby, the cell cycle arrests (Chavez *et al*., 2014).

**Hemolymph based toxicity and ecofriendly insect pest control**

The insecticides lack pest specificity therefore, promotes development of pest resistance (Davies *et al.,* 2012) in ecosystem, often leads to a problem referred to as the pesticide treadmill (Knight, 1989). One of the best examples is *Bt* applied as foliar sprays which are organic have high specificity and negligible environmental impact compared to their synthetic counterparts (Castagnola and Fuentes, 2012). The toxins produced by mites, spiders and other venomous organisms have been known to have neurotoxic effects on insects (Windley *et al.,* 2012) without deteriorating the environment. Some of the genes encoding these toxins can be expressed in transgenic plants, thereby contribute to decrease of target insect population (Khan *et al.,* 2006).

As mentioned, many neurotoxins have been discovered from invertebrates. For example, leptinotarsin, isolated and identified from the Colorado potato beetle, has been shown to disrupt the release of acetylcholine at the presynaptic nerve terminal of rat synaptosomes (Yoshino *et al*., 1980). Although peptides of leptinotarsin have shown homology to both juvenile hormone esterase (JHE) of insects and Cry toxins of *Bt*, there is no evidence that leptinotarsin has JHE activity. Because of the way proteinous neurotoxins interact and disrupt neural tissues function, they can be used to study the physiological consequences of the nervous system dysfunction (Khan *et al*., 2006). Understanding their mode of action and interactions with the insect nervous system, Bt can be a powerful tool with application in insect pest management.

**Insect Paralysis by *B.thuringensis***

Whether a particular *Bt* protein bind to insect gut receptor or not, it is necessary to be determined (OMICS, 2015) at first. Once the *Bt* Cry toxins are accepted by sensory receptors only then these are ingested by larvae of lepidopteran, coleopteran and dipteran insect and causes a number of toxic effects *viz*. paralysis, cessation of feeding and reduced movements. Paralysis of midgut is a predominant characteristic caused by *Bt*, and is a preliminary way to discriminate among different insecticidal *Bacillus* species and strains (Heimpel and Anguset 1958). Paralysis induced by *Bt* can be categorized by insect type. Type I insects are characterized by the symptom of whole body paralysis; larvae become inactive and fall off their host plant. The increase of pH in the insect hemolymph is the main cause for this type. Unlike Type I insects, type II insects involves paralysis symptoms limited to gut movement; however, increased pH once again plays a key role in toxicity

intensification. Paralysis is thought to be caused by the breakdown of the epithelial integument, characterized by inhibiting insect physiological function and movement (Heimpel and Anguset, 1959). Gut paralysis in Type II insects involves the cessation of feeding and frass production. Second generation Bt crops (*B.thuringiensis* genes) used for the management of crop pests by combined action of more than one genes exhibited synergism and antagonism between Vip3A and Cry 1 Proteins in response to the damage done by *Heliothis virescens, Diatraea saccharalis* and *Spodoptera frugiperda* (Lemes *et al.,* 2014)

Knoweldege about the potential role of the insect nervous system, brain, or neuromuscular junction in insect gut or whole body paralysis is limited; however in general the peristalsis in insects is controlled by the stomatogastric nervous system (Hartenstein, 1997). The frontal ganglion especially the frontal connective controls the peristaltic movements, thereby enabling the foregut to empty food into the midgut. So, it is clear that if digestion has ceased then this aspect of the nervous system may have been targeted. However, larva regurgitation is a common physiological defense response during ingestion of plant defense molecules. In depth we can say that toxins of insect pathogens which have both oral and injectable toxicity could selectively silence specific insect nervous tissues involved in digestion and midgut muscle movement. Understanding this nature of toxicity of microbial toxins, we may judge the function of frontal connective tissues (nervous tissues) in insect behavior, especially the tissues involved in food consumption (Rodriguez *et al.,* 2008). One Cry toxin of *Bt,* Cry1C, targets the tissues of nervous system (Cerstiaens *et al.,* 2001) and digestive system especially the gut epithelium (Aronson and Shai, 2001) of various lepidopteran species.

**Role of other *Bt* strains in Neurotoxicity of insects**

Neurotoxic symptoms have been observed when *Bt* var. i*sraelensis (Bt)* are injected into Cabbage semiloopers. *Trichoplusi ni*. When injected into hemolymph at higher does *Bti* stops heart activity. Moreover, symptoms like loss of motor activity, paralysis and flaccidity were observed. The proteins conferring neurotoxicity in lepidopteran insects were components of the crystal endotoxin from *Bti* (Cheung *et al.,* 1985). It is assumed that the presynaptic nerve terminal function were blocked; whereas, the postsynaptic membranes and axons in the ventral nerve cord remained unaffected. The symptoms like $6^{th}$ abdominal ganglion transmitter release, calcium uptake and complete blockage of transmitters were observed. However, in rat muscle degeneration were observed when treated with crystal protein of *Bti*. In short, the mode of action of crystal toxins involve $Na^+/K^+$ ATPase damage and $K^+$ levels decrease; whileas, the $Na^+$ levels increase within muscles cells with increasing $Ca^{2+}$ influx (Cahan *et al.,* 1985).

## CONCLUSION

Insecticidal toxins are important options for the biological control of insect pests. Their use in the genetic engineering of plants could provide a new generation of resistant crops. The Bt whether incorporated into a foliar spray or toxins expressed in transgenic plants, is regarded as the premier entomopathogens used in pest management. Once the *Bt* Cry toxins are accepted by sensory receptors only then these are ingested by larvae of insect pests and cause a number of toxic effects *viz*. paralysis, cessation of feeding and reduced movements. Paralysis of midgut is a predominant characteristic caused by *Bt,* and is a preliminary way to discriminate among different insecticidal *Bacillus* species and strains. Recently, similar toxins to that of Bt have been identified throughout bacterial kingdom. The use of Bt for insect control help to gain further knowledge of the origin of entomopathogens and their associated virulence factors. As it would be interesting to investigate if the combination of virulence factors of the two different entomopathogenic bacteria such as *Photorhabdus* and *Bacillus* delay resistance. Combining toxins with different modes of actions, may delay the onset of resistance by forcing insects to develop two separate mechanisms of resistance. Combination of toxins could result in a more lethal insecticide that would result in a better control tactic for a problematic insect pest. Furthermore the transfer and delivery mechanism of *Photorhabdus* into the haemocoel suggests to existence of virulence factors with novel tissues as targets in the lepidopteran pests that can be further investigated.

The neurotoxic effects of Bti were investigated in American Cockroach, *Periplaneta americana* (Blattodea: Blattidae). The presynaptic nerves terminal function was suspected to be blocked were as the postsynaptic membranes and axons in the ventral nerve cord remained unaffected. The sixth abdominal ganglion transmitter release calcium uptake and complete blockage of transmitters were observed. The mode of action were related to $Na^+/K^+$- ATPase damage upon incubation and $K^+$ level decrease while $Na^+$ level increase within muscle cells with increasing $Ca^{2+}$ influx. In general the entomopathogens have history of horizontal gene transfer shuffling the toxin containing plasmids resulting in Pathogenicity Island between each other. The acquisition of insecticidal genes may be a strategy that may develop to onset virulence once bacteria were ingested; thereby broadening the availability of possible sources and helps in insect pest management.

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