11

Biological Control of Mosquito Larvae by *Bacillus thuringiensis* subsp. *israelensis*

Mario Ramírez-Lepe[1] and Montserrat Ramírez-Suero[2]
[1]*Instituto Tecnológico de Veracruz, UNIDA, Veracruz,*
[2]*Université de Haufe-Alsace, LVBE EA-3991, Colmar,*
[1]*Mexico*
[2]*France*

1. Introduction

Chemical insecticides provide many benefits to food production and human health and has proven very effective at increasing agriculture and forestry productivities. However, they also pose some hazards as contamination of water and food sources, poisoning of non-target fauna and flora, concentration in the food chain and selection of insect pest populations resistent to the chemical insecticides (Wojciech & Korsten 2002). It is well documented that chemical pesticides reduced natural-enemy populations and chemical applications can disrupt biological control and may cause outbreaks of secundary pests previously suppressed by natural enemies (Bartlett, 1964) and pest species develop pesticide resistance but natural enemies not (Johnson & Tabashnick, 1999).

The use of synthetic organic pesticides has had serious economic, social and environmental ramifications. Economically, the rapidy increasing cost for development and production of petrochemically derived insecticides, together with the declining effectiveness due to widespread insect resistance. As a result the chemical pesticide industry continues to develop new more expensive compounds and increasing pesticide prices. Socially and ecologically they have caused death and disease in human and damaged the environment. It is estimated that only a minute fraction of the insecticides applied is required for suppression of the target pest. The remainder, more than 99.9%, enters the environment through soil, water and food cycles (Metcalf, 1986).

Alternative methods of insect management offer adequate levels of pest control and pose fewer hazards. One such alternative is the use of microbial insecticides, that contain microorganisms or their by-products. Microbial insecticides are especially valuable because their toxicity to non-target animals and humans is extremely low. Compared to other commonly used insecticides, they are safe for both, the pesticide user and consumers of treated crops. Microbial insecticides also are known as biological pathogens, and biological control agents. Chemical insecticides are far more commonly used in the world than microbial control, however some microbial control agents, at least in part, can be used to replace some hazardous chemical pest control agents. A number of biological control agents formulated with bacteria, fungi, virus, pheromones, and plant extracts have been in use mainly for the control of insects responsible for the destruction of forests and agriculture crops (McDonald & Linde, 2002).

The microbial insecticides most widely used in the world are preparations of *Bacillus thuringiensis* (*Bt*). The insecticidal activity of *Bt* is due to the proteic parasporal inclusions that are produced during sporulation. Insecticides based on the proteinaceous δ-endotoxin of *Bt* constitute part of a more ecologically rational pest control strategy. *Bt* strains have been isolated world wide from many habitats, including soil, insects, stored-product dust, and deciduous and coniferous leaves, all of which have a limited host range, however together span a wide range of insects orders which include: Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Phthiraptera, Orthoptera, Acari, and Mallophaga and other organisms such as nematodes, mites, and protozoa (Federici, 1999). *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) or serotype H-14, exhibit acute toxicity towards dipteran insects such as larval mosquitoes and black flies (de Barjac 1978) and is currently used in mosquito control programs world wide (Priest, 1992). The World Health Organization's (WHO) Onchocerciasis Control Program (OCP) in West Africa using *Bti* toxins has been one of the success stories of international co-operation in the control of infectious diseases program (Webb 1992; Drobniewski 1993). Due to the importance of *Bti* to control several tropical diseases such malaria and dengue, our purpose in this chapter is to provide an overview for non-*Bt* specialists of the basic knowledge of *Bti.*

2. *Bacillus thuringiensis* strains

2.1 Origin of some strains of *B. thuringiensis*

A large number of strain of *Bt* have been isolated from which to date. *Bt* as currently recognized is actually a complex of subspecies. They have grouped in 79 serotypes (Zeigler, 1999). The first *Bt* strain was isolated from diseased larvae of the silkworm, *Bombi mori*, in Japan by Ishiwata (1901). Iwabushi (1908) describe the bacillus as *Bacillus sotto.* Aoki & Chigasaki (1915) and Mitani & Wataral (1916) purified a highly toxic substance from sporulated *Bt* cultures. It was not officially described, however, until it was reisolated by Berliner in 1915 from diseased larvae of the Mediterranean flour moth, *Anagasta kuehniella*, in Thuringia, Germany, hence the derivation of species name thuringiensis (Federici, 1999). A *Bt* strain was isolated again by Mattes (1927) and described briefly the inclusion rhomboidal body. The activity of the *Bt* strains against lepidopteran larvae was described by Metalnikov (1930) and by Husz (1931). The association of the inclusion bodies of *B. thuringiensis* with toxicity against insects was established by Steinhaus. In 1951 published a paper which described the morphology of *Bt* and its possible use in the biological control against the alfalfa caterpillar. In France, a product named "Sporeine" was developed and used against *Ephestia kuhniella* (Lepidoptera) (Jacobs, 1950). Hannay, (1953) described a parasporal body in bipyramidal shape produced by the bacterium during sporulation and suggested that the crystal was involved in the toxic activity. The protein nature of the crystals was determined by Hanna and Fitz-James (1955).

2.2 Features of *B. thuringiensis* strains

Bt is a facultative anaerobic, gram-positive bacterium that forms characteristic protein inclusions adjacent to the endospore (Fig.1). The crystalline inclusion are composed of proteins known as ICPs crystal proteins. Cry proteins, or δ-endotoxins is the basis for commercial insecticidal formulations of *Bt.* Insecticides containing *Bt* in pest control programs is now considered as a viable strategy, which has proven to be both safe and reliable over the last 45 years (Chungjatupornchai et al. 1988).

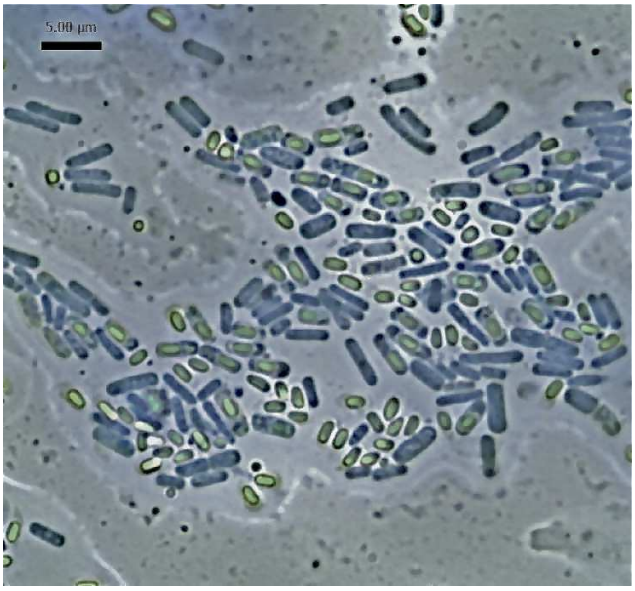


Fig. 1. *Bacillus thuringiensis* subsp. *israelensis.*

2.3 Advantages and disadvantages of *Bt*

According to Federici (1999) the main reasons for the success of *Bt* include (i) the high efficacy of its insecticidal proteins (ii) the existence of a diversity of proteins that are effective against a range of important pests (iii) its relatively safety to nontarget insect predators and parasites (iv) its easy to mass production at a relatively low cost, and (v) its adaptability to conventional formulations and application technology.

Advantages and disadvantages of *Bt* with chemical insecticides were summarized by Rowe and Margaritis (1987).

Advantages of *Bt:*

- High specificity, i.e. no mammalian or nontarget effects; use permitted up to date of harvest.
- No broad insect resistance observed or expected to develop.
- Adaptable to many types of formulations; potential to incorporate feeding stimulants or baits to increase the attractiveness of the formulations to the insects and thereby increase their efficacy.
- Probability of producing more potent formulations and reducing production costs through improved-fermentation technology.
- High probability that strain selection and/or genetic engineering will lead to better control of pest insects by newly found or created strains of Bt having novel host spectra or increased activity

Disadvantages of *Bt:*

- Narrow host spectrum
- Lack of patent protection on new strains
- Proper timing of application require due to slower effect than chemical insecticides

- Activity dependent of ingestion, and feeding activity depends on environmental conditions
- Relatively higher cost compared to chemical insecticides

2.4 Clasification of strains of *Bacillus thuringiensis*

Bt is a crystalliferous spore-forming bacterium close genetic relationship with *B. cereus, B. anthracis* and *B. mycoides* (Höffe & Whiteley, 1989). The classification of *Bt* is difficult because DNA sequencing studies of conserved gene regions of theses species have suggested that they belong to a single specie. *Bt* strains are distinguished from *B. cereus, B. anthracis* and *B. Mycoides* by the ability to produce parasporal crystalline inclusions during sporulation (de Barjac, 1978). Crystal formation is the criterion for distinguishing between *B. cereus* and *Bt*, otherwise they could be considered as the same specie. Research based on a comparative study of 16s rRNA sequences, *Bt* and *B. cereus* var. *mycoides* differed from each other and from ***B.*** *anthracis* and *cereus* by less than nine nucleotides (Ash and Collins 1992). Chen & Tsen (2002) amplified 16S rDNA and *gyrB* gene by PCR and they found that the discrimination between *B. cereus* and *Bt* strains, when a large number of Bacillus strains were tested was difficult. They proposed, to distinguish *Bt* from *B. cereus*, a single feature, such as the presence of a parasporal crystal protein or cry gene is reliable.

Several attemps were made to classify *Bt* strains. de Barjac and Frachon (1990) didn´t find correlation between biochemical reactions and 27 H serotypes using 1,600 *B. thuringiensis* isolates. They demonstrated that the current biochemical tests have no value as the sole criteria for differentiating *Bt* strains. Another approach ot these authors for classification was the use of susceptibility to certain bacterial viruses called phages. There are 14 bacteriophages that have been used for *Bt* but phage typing is inconsistent with serotyping and does not permit classification. They found frequent cross-reactions.

One widely clasification system for *Bt* strains is based in the determination of the H-flagellar antigen technique described by Barjac & Bonnefoi (1962). This technique needs very motile bacterial cultures to prepare flagellar suspensions. These suspensions are titrated against antisera directed against *B. thuringiensis* strains of each serotype. Presently *B. thuringiensis* strains are classified within 79 serotypes. Table 1 shows the classification of *Bt* strains by serotypes.

Bt strains that had discovered previously to 1977 were pathogens towards lepidopteran larvae. However, in 1975 was discovered *B. thuringiensis* serovar *israelensis* toxic to mosquito larvae (Goldberg & Margarit 1977) and in 1983 a strain from *Bt* serovar *morrisoni* was found to be pathogenic to Coleoptera larvae (Krieg et al. 1983). According to these findings, serological clasification, although still in use as a basic method to clasify *Bt* strains could not be related with pathogenicity. Subsequently studies showed that, within a serotype, different activity, spectra can be found. For example, some strains of *B. thuringiensis* serotype *morrisoni* in their parasporal inclusión bodies contain different proteins and have activity against Diptera, Coleoptera or Lepidoptera.

With the knowledge of sequence of the genes that encode the proteins cry it was proposed a classification based on the cry toxin genes. Höfte and Whiteley (1989) proposed a nomenclature clasification scheme for *Bt* crystal proteins based in their structural aminoacid sequence, deduced from the DNA and host range. They named *cry* (crystal protein) genes and their related proteins, "Cry proteins". They clasified 42 *Bt* crystal protein genes into 14 distinct genes grouped into 4 major clases. The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera-and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Dipter-specific).

Serotype	Serovar	Serotype	Serovar
1	thuringiensis	28a, 28c	jeghatensan
2	finitimus	29	amagiensis
3a, 3b, 3c	kurstaki	31	toguchini
3a, 3c	alesti	32	cameroun
3a, 3d	sumiyoshiensis	33	leesis
3a, 3d, 3e	fukuokaensis	34	konkukian
4a, 4b	soto/dendrolimus	35	seoulensis
4a, 4c	kenyae	36	malayensis
5a, 5b	galleriae	37	andralousiensis
5a,5c	canadensis	38	owaldocruzi
6	entomocidus/subtoxicus	39	brasilensis
7	aizawai/pacificus	40	huazhongensis
8a.8b	morrisoni	41	sooncheon
8a, 8c	ostriniae	42	jinghongiensis
8b, 8d	nigeriensis	43	guiyangiensis
9	tolworthi	44	higo
10a, 10b	darmstadiensis	45	roskildensis
10a, 10c	londrina	46	chanpaisis
11a, 11b	toumanoffi	47	wratislaviensis
11a, 11c	kyushuensis	48	balearica
12	thompsoni	49	muju
13	pakistani	50	navarrensis
14	israelensis	51	xiaguangiensis
15	dakota	52	kim
16	indiana	53	asturiensis
17	tohokuensis	54	poloniensis
18a, 18b	kumamtoensis	55	palmanyolensis
18a, 18c	yosoo	56	rongseni
19	tochigiensis	57	pirenaica
20a, 20b	yunnanensis	58	argentinensis
20a, 20c	pondicheriensis	59	iberica
21	colmeri	60	pingluonsis
22	shanongiensis	61	sylvestriensis
23	japonensis	62	zhaodongensis
24a, 24b	neolonensis	64	azorensis
24a, 24c	novosibirsk	65	pulsiensis
25	coreanensis	66	graciosensis
26	silo	67	vazensis
27	mexicanensis	none	wuhanensis
28a, 28b	monterrey		

Table 1. Classification of *B.thuringiensis* strains by serotype (modified after Zeigler, 1999).

The *cryI* genes can be distinguished from the other *cry* genes simply by sequence homology (>50% aminoacid identy) and encode 130 to 140 kDa proteins which accumulate in

bypiramidal crystalline inclusions during the sporulation of *Bt*. *cryII* genes encode 65-kDa proteins which form cuboidal inclusions in strains of several species. *cryIII* genes produces romboidal crystals containing one major protein, a 72kDa protein. cryIV class genes (*cryIVA*, cryIVB, cryIVC, and cryIVD) as well as *cytA* were all isolated from the same 72-Mda plasmid present in *Bt* subsp. *israelensis.*

3. *Bacillus thuringiensis* subsp. *israelensis* as an important part of mosquito control

3.1 Properties of *Bti*

In 1975-76 under a World Health Organization sponsored project, a new *Bt* strain was discovered in the Negev desert in Israel by Goldberg and Margalit (1977). The strain was isolated from *Culex* sp. dead larvae mosquito. Later was identified as *Bt israelensis*, serotype H14 according to its fagellar antigenicity by de Barjac (1978).

Bti has all the features taxonomic, morphological, growth, sporulation, of isolation, cultivation of other varieties of *B. thuringiensis* (Fig.1). The larvicidal activity of *Bt israelensis* on mosquito transmitted diseases was the most important feature of the strain. The insecticidal properties of this bacteria are due primarily to insecticidal proteins produced during sporulation. The key proteins are Cyt1A(27.3 kDa), Cry4A (128 kDa), Cry4B (134 kDa) and Cry11A (72 kDa) and in three different inclusion types assembled into a spherical parasporal body held together by lamellar envelope (Ibarra & Federici, 1986). The inclusions are realtively small (0.1 to 0.5μm) and there are usually two to four inclusions per cell which vary in shape from cuboidal to bipyramidal, ovoid or anamorph (Charles & de Barjac 1982; Mikola *et al.* 1982; Yamamoto *et al.* 1983)

3.2 Mosquitoes, important vectors of tropical diseases

Mosquitoes are important vectors of several tropical diseases that suck blood from human and animals. They are vectors of multiple of diseases of man through transmision of pathogenic viruses, bacteria, protozoa and nematodes (Priest, 1992). From the medical point of view, mosquitoes are among the most important insects due their capacity to transmit human diseases such as malaria and dengue.

Vector	Disease
Anopheles	Malaria, lymphatic filariasis
Culex	Lymphatic filariasis, Japanese encephalitis, other viral diseases
Aedes	Yellow fever, dengue, dengue hemorrhagic fever, other viral diseases, lymphatic filariasis
Mansonia	Lymphatic filariasis

Table 2. Some diseases transmitted by mosquito (Rawlins, 1989; Walsh 1986).

There are about 3000 species of mosquito, of which about 100 are vectors of human diseases. Some of the more important of these diseases are listed in Table 2. It is estimated two billion people worldwide living in areas where these are endemic (World Health Organization, 1999). Thus, there is an urgent need for new agents and strategies to control these diseases.

3.3 Susceptibility of mosquito species to *B. thuringiensis* serotype *israelensis*

Bti is highly pathogenic against *Culicidae* (mosquitoes) and *Simuliidae* (blackflies), and has some virulence against certain others Diptera, especially Chironomidae (midges). Mosquito have four distinct stages in their life cycle: egg, larva, pupa and adult. Depending on the specie a female lays between 30 and 300 eggs at a time on the surface of the water, singly (*Anopheles*), in floating rafts (*Culex*) or just above the water line or on wet mud (*Aedes*). Once hatched the larvae grow in four different stages (instars). The first instar measures 1.5 mm in length, the fourth instar about 8-10 mm. The fully grown larvae then changes into a comma shaped pupa. When mature, the pupal skin splits at one end and a fully developed adult emerges. The entire period from egg to adult takes about 7-13 days under good conditions (Wada, 1989). First instar is more suceptible to *Bti* than fourth instar (Mulla *et al.* 1990). Pupa does not feed and therefore is not affected by *Bti*. For almost all species tested, increasing age of the larvae resulted in reduced susceptibility in mosquito (Chen et al. 1984: Mulla *et al.* 1985). *Bti* was found to be specific toxic to larvae of 109 mosquito species (Table 3).

Mosquito genus	Species
Aedes	40
Anopheles	27
Culex	19
Culiseta	5
Mansonia	5
Psorophora	3
Armigeres	3
Toxorhynchites	2
Limatus	2
Trichophospon	1
Uranotaenia	1
Tripteroides	1
	Total 109

Table 3. Larvicidal activity of *Bti* on mosquito species (Glare & O´Callaghan 1998).

Among mosquitoes, different preparations of *Bti* have shown different levels of toxicity to host species. Others factors influencing the susceptibility of mosquito larva to *Bti*. For example, the effect of a given dosage of toxin could produce different results depending on weather the lethal dose is administered all at once or in same doses over a long period (Aly et al. 1988).

Bti has an LC50 in the range of 10–13 ng/ml against the fourth instar of many mosquito species (Federici *et al.* 2003). Generally, *Culex* and *Aedes* are highly susceptible while *Anopheles* are less suceptible, but can be killed with *Bti* (Balaraman *et al*. 1983). Much higher concentrations of *Bti* are required to induce mortality in anopheline larvae than in *Aedes aegypti* larvae (Goldberg & Margalit 1977; Nugud & White 1982)

The exception is *A. franciscanus* that is as susceptible as other genera (García *et al.* 1980, Sun *et al.* 1980). Furhtermore, even within one genus, some species are more suceptible than others (Chui et al. 1993). Sun et al. (1980) suggested that a difference in feeding behavior might account for differences in susceptibilities. Filtering rates vary between genera and species. For example Aly (1988) showed that in the absence of *Bti*, larvae cleared the suspensions with constant relative filtration rates of 632 (*Ae. aegypti*), 515 (*Cx. quinquefasciatus*) or 83.9

μL/Larvae/h (*An. albimanus*). Another factor to be considered for the suceptibility to *Bti* is the behavior of the larvae. *Anopheles* larvae filter-feed on food particles present at the surface of water or a few centimeters below it, where as *Culex* and *Aedes* larvae not only feed faster but are capable of filter-feeding at much deeper water dephts (Aly et al. 1988).
Mosquito species which are not filter feeders do not seem suceptible. For example, against *Culicoides occidentalis* (Colwell 1982) and *Coquillettidia perturbans*, *Bti* larvicides had no effect (Walker et al 1985). Solubilization of the protein reduced the toxicity dramatically (50- to -100 fold) this is attributed to reduced level of toxin ingestion by larvae owing to their filter feeding behaviour (Chungjatupornchai et al. 1988).

3.4 Susceptibility of mosquito species to purified *Bt* proteic crystals

Several efforts have been made to purify the proteins Cry of *Bti* and other *Bt* subps. with the purpose of studying the chemistry of Cry proteins, the synergism between them, and the effect of each crystal on larvae of different mosquito species. It is somewhat difficult to separate the *Bt* spores and crystals because they are of similar size and surface characteristics. For that reason several methods have been used to purify *Bt* crystals proteins. Using NaBr gradients (Chang et al. 1992), sucrose gradients (Debro et al. 1986), renografin gradients (Aronson et al. 1991), and in a separatory funnels (Delafield et al. 1968). Bioassays of purified Cry proteins have been allowed to know that not only *Bti* Cry proteins have activity on mosquito larva. Other purified Cry proteins of other *Bt* strains have also activity on mosquito larvae. Table 4 shows the mosquito toxicity of purified crystals of some *Bt* strains.

Name	Source Strain	Mosquito toxicity
Cry4Aa1	*B.t. israelensis* 4Q2-72	*Aedes agypti, Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)
Cry4Ba1	*B.t. israelensis* 4Q2-72	*Aedes agypti* (Diptera: Cuclidae)
Cry10Aa1	*B.t. israelensis* ONR60A	*Aedes agypti,* (Diptera: Cuclidae)
Cry11Aa1	*B.t. israelensis* HD-567	*Aedes agypti, Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)
Cry11Ba1	*B.t. jegathensan* 367	*Aedes agypti, Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)
Cry11Bb1	*B.t. medellin*	*Aedes agypti, Anopheles albimanus*, *Culex quinquefasciatus* (Diptera: Cuclidae)
Cry16Aa1	*Clostridium bifermentans malasya* CH18	*Aedes agypti, Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)
Cry19Aa1	*B.t. jegethesan*	*Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)
Cry20Aa1	*B.t. fukuokaensis*	*Aedes agypti,* (Diptera: Cuclidae)
Cry21Aa1	*B.t. higo*	*Culex pipiens molestus* (Diptera: Cuclidae)
Cyt1Aa1	*B.t. israelensis* IPS82	*Aedes agypti, Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)
Cyt1Ab1	*B.t. medellin* 163-131	*Aedes agypti, Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)
Cyt2Aa1	*B.t. kyushuensis*	*Aedes agypti, Anopheles stephensi*, *Culex pipiens* (Diptera: Cuclidae)

Table 4. Mosquitocidal activity of Cry and Cyt proteins (Modified after "Zeigler, 1999").

3.5 Resistance

One major problem with insects control via chemical insecticides is the evolution in insects of resistance to those insecticides. The use of *Bti* on biological control of mosquitoes has no resulted in the development of resistence in host populations. Laboratory attemps to induce resistance by continual exposure to *Bti* have generally failed to detect resistence (Lee & Chong 1985; Georghiou & Wirth 1997).

The lack of resistance development to *Bti* could be due to its complex mode of action, involving synergistic interactions between up to four proteins (Becker & Maragrit 1993). Use of a single protein from *Bti* for mosquito control resulted in resistance after only a few generations in the laboratory (Becker and Margalit 1993). Georghiou & Wirth (1997) also showed that resistance could be raised in only a few generations when single *Bti* toxin was used (i.e. Cry 4Aa, 4Ba, 10Aa or 11Aa), and was progressively more difficult to raise in mosquitoes with combinations of two, and three toxins. When all four *Bti* toxins were used, resistance incidence was remarkably low. On the other hand Wirth et al. (2005) have shown that the lack of resistance in *Bti* is due to the presence of the Cyt1Aa protein in the crystal. For example, *Culex quinquefascitus* populations resistant to CryIVA, Cry4B and Cry11A have been obtained in the laboratory but not mosquito larvae resistant to Cry and Cyt1Aa proteins (Georghiou & Wirth 1997)

3.6 Synergism

Bti produces four crystal proteins Cry (4Aa, 4Ba, 10Aa, and 11Aa) and two Cyt (1Aa and 2Ba) (Guerchicoff *et al.* 1997). No single crystal component is as toxic as the intact crystal complex (Chan *et al.* 1993; Wu *et al.* 1994; Chilcott & Ellar, 1998). One possible explanation for this is that two or more proteins act synergisticall, yielding a higher activity than would be expected on the basis of the specific toxicity of the individual protein (Finney, 1971). For example, the toxicity against mosquito larvae of Cyt1Aa is lower compared to each of the four Cry proteins (Crickmore et al. 1995). However, cytA can potentiate the activity of the toxins and synergistic interactions that seems to account for the high toxicity of the *Bti* strains (Delecluse et al. 1993).

Tabashnick (1992) proposed a method to measure synergistic effect. Using the proposed method discuss the data reported by two authors: in bioassays with *A. aegypti* larvae, Wu and Chang (1985) found that mixtures of the 27- and 65-kDa proteins from *B. thuringiensis* subsp. *israelensis* were more toxic than expected on the basis of their individual toxicities, however, Chilcott and Ellar (1988) concluded from their own data that no synergism between these two proteins occurred. With this new interpretation of Tabashnick method, both studies support the same conclusions: positive synergism between the 27-kDa protein (CytA) and either of the CryIV proteins (65 and 130 kDa) and no such synergism between CryIV proteins (65 and 130 kDa). Other studies have been carried out with the aim of increasing the synergistic activity of *Bti.* Ramirez-Suero et al. (2011) evaluated the synergistic effect of *S. griseus* and *Bt aizawai* chitinases with *Bt israelensis* spore-toxin complex against *Aedes aegypti* larvae. The synergistic factor values according to Tabashnik (1992) method were 2 and 1.4, respectively.

3.7 Effect of *Bti* on no-target organisms

Bti has no direct effect on aquatic organisms other than mosquitoes, blackflies and chironomids. Other aquatic organisms, such as shrimps, mites and oysters are generally unaffected (Glare & O'Callaghan, 1998). This large safety margin of preparations of *Bti* for

non-target organisms indicate their suitability for mosquito control programs in areas where protection of the natural ecosystem is important (Sinegre et al. 1980)
Several authors have reviewed the non target effects of *Bti* (Becker & Margalit 1993; Lacey & Mulla 1990). Field applications have often been monitored for effects on non-target organisms but no significant non-target effects have been reported (Ali, 1981; Jackson et al. 1994; Hershey et al. 1995)

4. Production of *Bti* by fermentation

4.1 Culture medium for *Bti* production

Commercial production of *Bti* is performed using culture media based on complex nutrients sources. The main purpose of the fermentation is to obtain high quantities of *Bti* crystals. The *Bti* parasporal crystal can account for up to 25% of the sporulated cell dry weight. To optimize the cry production it is necessary to have a suitable culture medium because the toxicity obtained at the end of the fermentation depend on the culture medium and operating conditions. The culture media that have been reported in the literature for high growth and sporulation can be used for any variety of *Bt*. Not always a high cell growth ensures an elevated Cry protein production or an increased insecticidal activity. Various culture mediums have been used for high growth and sporulation of *Bt* in the laboratory: 2XSG medium (Leighton & Doi, 1971), PA medium (Thorne, 1968), G-Tris medium (Aronson et al. 1971), CDGS medium (Nakata, 1964). Other media with inexpensive substrates have been reported by Pearson & Ward (1988), Smith (1982), Foda et al. (1985), Dulmage et al. (1970), Salama et al. (1983), Goldberg et al. (1980).

4.2 Factors affecting Cry production

There are several factors that influence the production of crystals: (1) Carbon source. Glucose is the most appropiate carbon source either for high *Bt* growth and sporulation (Smith, 1982). When glucose has been exhausted in the fermentation, the abscence of this can trigger sporulation. The use of one or other carbon source affects the biological activity and the morphology of the crystals (Dulmage, 1970). (2) Nitrogen source. An appropiate source of aminoacids provides high growth rates and high sporulation of *Bt* strains. Its absence delays sporulation and low yield in Cry proteins (Goldberg et al. 1980) (3) Carbon:Nitrogen ratio. Higher C:N rates glucose do not deplete at the end of fermentation and biomass yield decrease. Several authors have recomended a carbon nitrogen ratio of 7.5:1. (Salama et al. 1983; Foda et al. 1985) (4) Oxygen. High aeration rates are important for high spore and toxin formation. As k_La, increase biomass and Cry protein formation are increased (Rowe & Margaritis, 1987)(5) pH. Optimum pH for *Bt* growth is 6.8-7.2. If pH rises to 9.0 Cry protein can be dissolved (6) Temperature. Optimum temperature of *Bt* is 28-32°C. Higher temperatures favours plasmid losses or *Bt* mutants (Rowe & Margaritis, 1987).

5. Molecular biology of *Bti*

5.1 *Bti cry* and *cytA* genes

All *Bt* strains contain extrachromosomal DNA. *Bt* strains are well known for its numerous plasmids ranging in size from 1.5 MDa to 130 MDa. Plasmids have been found in each variety examined and the plasmid profiles appear to be strain specific. However these plasmid profiles depend on the media type and growth rate of the strain and can be readily

gained or lost (Federici, 1999). The mere presence of plasmids in *Bt* does not prove that they are involved in crystal formation, many non-crystalliferous bacteria also contain plasmids The *cry* genes are located on large plasmids although some Cry genes have been reported on the chromosome (Baume & Malvar, 1995). As mentioned earlier, *Bti* produces four different Cry proteins: CryIVA, CryIVB, Cry11A, and the cytolytic CytA protein (Hoffe and Whiteley, 1989). The Cry proteins are codified by *cryIVA*, c*ryIVB*, *cry11A*, and *cytA* genes, respectively. These genes responsible for the toxicity of *Bti* have been sequenced by various researchers (Table 5).

Gene name	GenBank Accesion No.	Coding Region	Reference
cryIVAa1	Y00423	1-3540	Ward &Ellar, 1987
cry4Aa2	D00248, E01676	393-3935	Sen *et al*. 1988
cry4Ba1	X07423, X05692	157-3564	Chungjatupornchai *et al*. 1988
cry4Ba2	X07082	151-3558	Tungpradubkul *et al*. 1988
cry4Ba3	M20242	526-3930	Yamamoto *et al.* 1988
cry4Ba4	D00247, E01905	461-3865	Sen *et al*. 1988
cry11Aa1	M31737	41-1969	Donovan *et al.* 1989
cry11Aa2	M22860	1-235	Adams *et al.* 1989
cyt1Aa1	X03182	140-886	Waalwijk *et al.* 1985
cyt1Aa2	X04338	509-1255	Ward & Ellar, 1986
cyt1Aa3	Y00135	36-782	Earp a& Ellar, 1987
cyt1Aa4	M35968	67-813	Galjart *et al*. 1987

Table 5. *cry* and *cytA* genes DNA sequences of *Bti.*

In *Bti* the elements responsible of the toxicity against mosquito larvae are located in a large plasmid of 72 MDa (125 kb) and contribute to the formation of a complex parasporal body (Aronson 1993). Figure 2 shows the partial map of the *Bti* 125 kb plasmid.

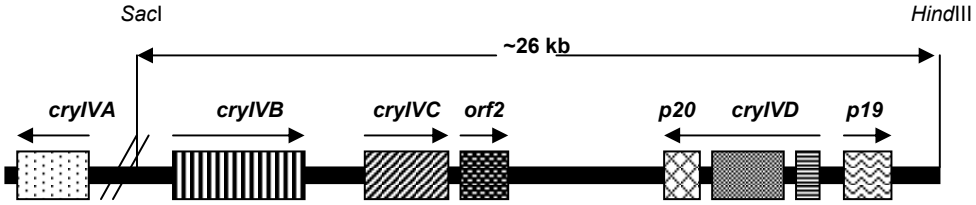


Fig. 2. Map of 26kb DNA fragment carrying the DNA genes responsibles of the toxicity of *Bti* (Modified after Ben-Dov *et al.* 1996).

5.2 *Bti* operon of *cryIVD* gene

The DNA sequence indicated that *cryIVD* gene is the second gene of an operon which includes three genes. A gene that encodes a 19 kDa polypeptide, *cryIVD* gene and a gene that encodes a 20 kDa polypeptide (Dervyn et al. 1995). Transcription of *cryIVD* gene in *Bti* is induced 9 h after the beginning of the sporulation. DNA sequence analysis and potential promoters are recognized by the RNA polimerase associated with the σ^{35} and σ^{28} , specific sigma subunit of the RNA polymerase genes related with control of sporulation of *B.*

thuringiensis. *cryIVA* and *cryIVB* promotors are activated in the mid-sporulation phase (Ben-Dov et al. 1996), *cryIVA* is regulated by the σ^{35} RNA polymerase gene and *cryIVB* is under control of σ^{35} (Yoshisue et al. 1994). *cytA* gene is transcribed by two promotors, pBtI and pBtII, regulated by the RNA polymerase σ^{35} and σ^{28}, respectively (Brown & Whiteley, 1988, 1990). These results have demostrated that *cryIVD* transcription is subjected to σ^{35} regulation.

5.3 Identication of *Bacillus thuringiensis* pesticidal crystal genes by PCR

The polymerase chain reaction (PCR) is a molecular tool widely used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The identification of *Bt* toxin genes by PCR can partially predict the insecticidal activity of a given strain. Several studies have reported that the type of *cry* and *cyt* genes present in a *Bt* strain correlates to some extent with its insecticidal activity (Porcar & Juarez-Perez). Thus, the identification of the gene content in a *Bt* strain can be used to predict its insecticidal potential. The PCR-based identication of *B. thuringiensis* cry genes was first developed by Carozzi et al. (1991), who introduced this technique as a tool to predict insecticidal activity. They found correspondence with the toxicity predicted on the basis of the amplification product profiles. Carozzi et al. proposed PCR as an accurate, fast methodology for the identification of novel strains and the prediction of insecticidal activity of new isolates, and they also forecast the possible use of PCR for the discovery of previously unknown cry genes. highly conserved regions and recognizing entire cry gene subfamilies are often used in a preliminary screening prior to performing a second PCR with specific primers. The primers used to amplify *cry4A, cry4B, cry11A*, and *cytA* genes have been designed by various researchers and are shown in Table 6.

Direct	Sequences (5´⇒3)	Reverse Sequences (5´⇒3)	Amplifies/Product (bp)
EE-4A(d)	GGGTATGGCACTC AACCCCACTT*	Un4(r) GCGTGACATACCC ATTTCCAGGTCC*	cry4A/1529
Dip2A(d)	GGTGCTTCCTATTC TTTGGC**	Dip2B TGACCAGGTCCCT TGATTAC**	*cry4A*/1290
EE-4B(d)	GAGAACACACCTA ATCAACCAACT*	Un4(r) GCGTGACATACCC ATTTCCAGGTCC*	*cry4B*/1951
EE-11A(d)	CCGAACCTACTAT TGCGCCA*	EE-11A(r) CTCCCTGCTAGGA TTCCGTC*	*cry11A*/445
gral cyt1(d)	AACCCCTCAATCA ACAGCAAGG***	gral cyt(r) GGTACACAATACA TAACGCCACC***	*cyt1*/522-525

Table 6. PCR primers pairs and the cry or cyt genes they amplify of *Bti*. Sources: Ben-Dov *et al.* (1997)*, Carozzi et al. (1991)**, Bravo *et al*. (1998)***

5.4 Expression of *Bti* genes in other strains

Expression of *Bti* genes either individually or in combination in crystal-negative *Bt* or other strains have being carried on by several researchers. The genes encoding these proteins have been expressed in *Caulobacter* (Thanabalu et al. 1992), cyanobacteria (Manasherob et al. 2002; Murphy & Stevens 1992), *Escherichia coli* (McLean & Whiteley 1987; Tanapongpipat et al. 2003), *Bacillus subtilis* (Ward et al. 1986), and *Bt* (Crickmore et al. 1990). However, *Bt* toxins have been expressed as active or inactive toxins, especially when expressed in *E. coli* (Ogunjimi et al. 2002).

Quintano et al. (2005) reported the expression of *cry11A* from *Bti* in *S. cerevisiae*. The *cry11A* gene was expressed as fusion proteins with gluthathione *S*-transferase under the control of the *S. cerevisiae HXK1* promoter. The protein was purified by affinity chromatography using gluthathione *S*-transferase–Sepharose beads. Insecticidal activity against third-instar *Aedes aegypti* larvae of the recombinant *S. cerevisiae* cell extracts (LC_{50} = 4.10μg protein/mL) and purified GST-cry11A fusion protein (LC_{50} = 4.10μg protein/mL) was detected in cells grown in ethanol.

Servant et al. (1999) constructed a recombinant *B. sphaericus* strain containing the *cry11A* gene from *Bti*. They found an LC_{50} for the cry11A protein of 1.175 μg/mL against fourth-instar *A. aegypti* larvae. Poncet *et al.* (1997) constructed a recombinant *B. sphaericus* strain with *cry11A* and *p20* genes integrated into the chromosome. In this case, the LC_{50} value reported against third- instar *A. aegypti* larvae was 0.023 μg/mL. Xu *et al.* (2001) studied the expression of cry11A and *cry11A+p20* gene cluster in recombinant *E. coli* and *Pseudomonas putida*. They found that both recombinant bacteria contained higher levels of Cry11A protein when the adjacent *p20* gene was present on the same DNA fragment. Yamagiwa et al. (2004) reported that the solubilized cry11A protein, obtained from a nonrecombinant *Bti*, was less toxic against *Culex pipiens* larvae than the crystal itself (LC50 of 0.267 and 0.008 μg/mL, respectively). These authors obtained 2 GST fusion proteins of 36 and 32 kDa from cry11A. The LC50 against *C. pipiens* larvae obtained using both proteins were 0.818 μg/mL. In other study with the purpose to preserve the toxicity of sunlight-sensitive Cry proteins, Manasherob et al. (2002) constructed a transgenic cyanobacterium *Anabaena* PCC 7120 to express the genes *cry4Aa*, *cry11Aa* and an accesory protein (*p20*) under control of two tandem strong promotors. Cyanobacterium *Anabaena* can multiply in breeding sites of mosquito larvae and serve as their food source. Higher toxicity against *Aedes aegypti* larvae was obtained in this study.

6. Mode of action of *Bti* Cry proteins against mosquito larvae

6.1 Pore-forming-toxins

Bt Cry and Cyt toxins belong to a class of bacterial toxins known as pore-forming toxins (PFT) that are secreted as water-soluble proteins undergoing conformational changes in order to insert into, or to translocate across, cell membranes of their host (Bravo et al. 2007). In most cases, PFT are activated by host proteases after receptor binding inducing the formation of an oligomeric structure that is insertion competent. Finally membrane insertion is triggered, in most cases, by a decrease in pH that induces a molten globule state of the protein (Parker and Feil, 2005). Cry and Cyt proteins are PFT proteins. Both proteins are solubilized in the gut of suceptible diperans insects and proteolytic activated by midgut proteases. For the Cry 11Aa protoxin, proteolytic activation involves amino-terminal processing and intramolecular cleavage leading to two fragments of 36 and 32 kDa that remain associated and retain insect toxicity.

6.2 Mechanism of action of *Bti* toxins

An acepted model for Cry toxin action against mosquito larvae is that it is a multistage process. (i) Ingestion of Cry protein by the larvae (ii) Solubilization of the crystals in the alkaline midgut (iii) Proteolytic activation of the insecticidal solubilised protein (iv) Toxin binds to receptors located on the apical microvellus membrana of ephitelial midgut cell walls (v) Alter the toxin binds the receptor, it is though that there is a change in the toxin conformation allowing toxin insertion into the membrane (vi) Electrophysiological and biochemical evidence suggest that the toxins generate pores in the cell membrane, thus disturbing the osmotic balance, consecuently the cells swell and lyse (vii) The gut becomes paralyzed and the insect stops feeding. Most mosquito larvae die whitin few hours of ingestion, generally cease feeding within 1 hour, show reduced activity by two hours and die six hours after ingestion (Chicott et al. 1990; Marrone & Macintosh, 1993).

Several authors have studied the mechanisms of action of the δ-endotoxin of *Bti* on mosquito larvae. Thomas and Ellar (1983) found that δ-endotoxin active against mosquito larvae was inactivated by prior incubation with lipids extracted from ephitelial midgut *Aedes albopictus* cells. They reported that toxin binds to membrane lipids (phosphatidyl choline, sphingomyelin and phosphatidyl ethanolamine). According to their results, they proposed a mechanism in which the interaction of toxin with membrane lipids causes a detergent-like rearrangement of the lipids and as a consequence cytolisis. Others authors have corroborated these results: Cyt protein, unlike Cry toxins, do not recognize specific binding sites and do not bind to protein receptors, directly interact with membrane lipids inserting into the membrane and forming pores (Knowles *et al.* 1989; Promdonkoy & Ellar, 2000) or destroying the membrane by a detergent-like interaction (Butko, 2003).

The high efficacy of *Bti* is because of the production of multiple toxins with different modes of action. Perez *et al.* (2005) reported that Cyt1Aa protein functions as a receptor for the Cry11Aa toxin and suggest that this interaction explains the synergism between the Cyt1A and Cry11A proteins. Further, the Cyt proteins in *Bti* synergize the toxic effect of Cry11A and Cry4 toxins and, even more, suppresses the resistance to these Cry toxins (Wirth *et al.* 1997).

7. Formulation of *Bti* toxins

7.1 Potency in *Bti* formulations

Formulation is a preparation of an insecticide for a particular application method. Formulation plays an important rol in determining final virulence. The vast majority of the formulations of *Bt* have been developed to control agricultural and forest pests, mainly *Lepidoptera*. However, the feeding habits of *Lepidoptera* are different to feeding habits of *Diptera*. Mosquito larvae feed by filtering water and concentrate organic particles. Product formulations based on *Bti* should consider the mosquito larvae habits and the environmental conditions, promote that Cry proteins retain their toxic activity and promote that the larva have access to them. Products based on strains of *Bti* are given a potency based on bioassays on third or fourth instar mosquito larvae. Bioassays are conducted using 6 to 7 dilutions of the toxin by duplicate in 100 mL cups containing 20 third instar *Aedes aegypti* larvae. Duplicate cups with 20 mosquito larvae in 100 mL of deionized water without test material serves as a control (McLaughlin *et al.* 2004). Concentration-mortality data are obtained, transformed to a log-probit scale, and potency is obtained by comparing the estimated LC_{50} of a test substance with that of a standard with a known potency (de Barjac

1985). The international standard recognized for *Bti* is IPS82. LC_{50} and LC_{90} are the dose require to kill 50 and 90 percent of the mosquito larvae of a tested population after 24h tested duration. Each sample is bioassayed at least 3 times on various days and the results are average values. LC_{50} and LC_{90} are measured in micrograms or milligrams of material per liter, or parts per million (ppm).

For potency calculations, it is used the international recognized standard for mosquito assay, IPS82 (15 000 ITU/mg) provided by Institute Pasteur, Paris, France. Standard vials are kept at -18°C.

Product potency is calculated by the Abbott (1925) formula:

$$\text{Potency (A)} = \text{Potency (std)}\ LC_{50}\ (\text{std})/LC_{50}\ (\text{A})$$

where (a) is the product and (std) is the standard.

The size of the particle could be a factor that influences the potency of the toxin. A product with small particles is more homogenously distributed in the water than a product with larger particles and small particles which sink slower. Changes in LC_{50} are not necesary regarded to reflect changes in amount of toxin, but could be a function of particle size/distribution (Skovmand et al. 1997). Change of particle size also change LC_{50} . Decreasing particle increased LC_{50} and thus decrease the calculated potency. The slope measured between LC_{50} and LC_{90} values should have high value. Higher value will require a smaller quantity to kill a greater number of larvae. The slope of the dosage-mortality curve is in function of the heterogeneity of the product effect. If product availability is in function of particle sizes, particles with broad ranges of particle size distribution will also have low slopes.

7.2 *Bti* formulations

A variety of *Bti* formulations have been studied for mosquito control under laboratory and field conditions. *Bti* fluid formulations are not stable in heat and high humidity and cannot be stored for months under tropical conditions (McGuirre et al. 1996) . In many cases, and especially in areas exposed to the sun, the residual effect is very short (Leong et al. 1980) and the product has to be reapplied. Photoinactivation seems to be one of the major environment factors affecting the stability of *Bti* delta-endotoxin (Morris, 1983). Yu-Tien et al. (1993) reported that *Bti* completely lost its toxicity to mosquito larvae when exposed to irradiation at 253nm. Poszgay et al. (1987) showed that exposure of *B. thuringiensis* toxin to 40 h of ultraviolet light irradiation resulted in lost activity. Cry proteins inactivation by the solar radiation is the result of the destruction of the tryphtophan (Pusztai et al. 1991). Research and development efforts are focusing on formulations to avoid the ultraviolet light effect. Ramirez-Lepe et al. (2003) encapsulated *Bti* spore-toxin within aluminum/ carboxymethylcellulose using green malachite, congo red or ponceau red as photoprotective agents against ultraviolet light in lab conditions. The encapsulated form of the *Bti* spore-toxin complex with photoprotectors avoided the limitation in controlling mosquito larvae caused by ultraviolet light. Yu-Tien et al. (1993) achieved photoprotection of the spore-toxin complex by addition of melanin.

Other *Bti* formulations have been developed. For example, Ramirez-Suero et al. (2005) evaluated maltodextrin, nixtamalized corn flour and corn starch for entraping active materials in *Bti* spore-toxin complexes dried by aspersion. Dried products had water activity values below 0.7 suggesting that the formulations are long shelf-life because keep

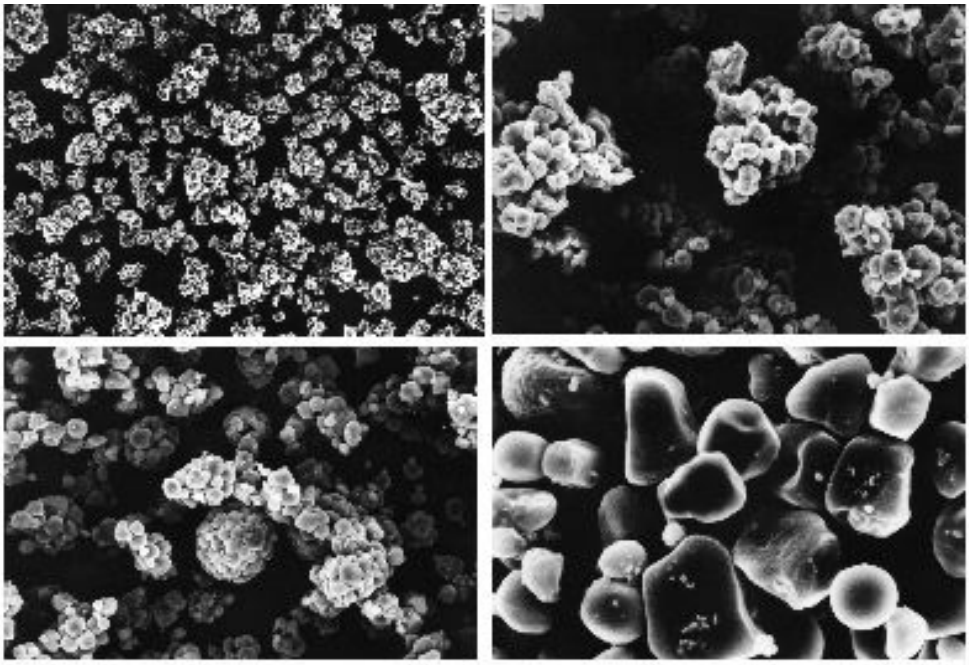


Fig. 3. Scanning electron micrograph of spray/dried formulation of *Bti* spore/toxin complex with Grits a) 379X and b)1500X and Nixtamalized corn flour c) 370X and d) 1500X.

the products without microorganisms for longer periods and increases the larval feeding and as a consequence have higher activity against mosquito larvae. Fig. 3 shows the scanning electron microscope of spray/dried formulations with grits and nixtamalized corn flour. It shows smoth spherical grits and corn particles entrapping the spore/toxin ingredient. Manasherob et al. (1996) encapsulated *Bti* in the protozoan *Tetrahymena pyriformis* and the activity against *A. stephensi* was enhanced 8 times when exposed to protozoan cells filled with *Bti* compared to exposed to the same concentration of *Bti* alone. Elcin (1995) encapsulated *Bti* in alginate microcapsules and increase its stability and its effect against *Culex* sp larvae. Another alternative to protect *Bti* crystals from ultraviolet light is obtaining mutants that protectrs *Bti* crystal. Hoti & Balaraman (1993) obtained a mutant of *Bti* that produced a brown pigment during sporulation, the pigment was identified as melanin. Other *Bti* formulations have been developed. Culigel superabsorbent polymer controlled-release system for the slow release of *Bti* to mosquito larvae (Levy et al. 1990). Combination of chemical and biological agents susch as insect growth regulator s-methoprene + *Bti* (Bassi et al. 1989). Sprayed-dried *Bti* powder as a fizzy tablet (Skovmand & Eriksen 1993). Floating bait formulations designed to improve the effect of bacterial toxins, especially against *Anopheles* spp (Aly et al. 1987).

Highly concentrated liquid formulations are available for control of floodwater mosquitoes while formulations which float for as long possible have been developed for use in fast-flowing or turbulent waters. Formulations which settle and persist at the bottom are required for buttom feeders. Granules which float on the surface are the most effective against surface feeders such as *Anopheles* spp. (Mulla et al. 2004).

Briquettes for mosquitoes with continual successive generations like *Culex* spp (Becker & Margalit 1993; Kase & Branton 1986). Briquetts or pellets, in particular seem to be useful for overcoming lack of persistence, which is one of main limitations of *Bti.* Granules using plant, such corn (maiz) grits or clay carriers are particular useful in aerial application to breeding sites with dense foliage as salt marshes or rice fields. Sustained release formulations such as floating briquettes or semi submersible pellets are designed to provide long-lasting larvicidal activity in containers or small ponds. Ingestion of the toxin depends on the rate of feeding, the rate at which the toxin falls to the bottom of the pool and becomes inaccesible, and competition to ingestion from other suspended organic materials. In turbid and polluted waters the rate of application needs to be at least two-fold greater than in clear water (Mulla et al. 1985). The feeding habits of mosquito larvae influence formulation design. *Anopheles* larvae are surface feeders and ingest particulate material from water surface such as yeast or flour and filter feed poorly. This has led to the development of formulations that present the toxin at, or just below, the water surface and such prepartions are particulary effective against certain *Anopheles* larvae (Aly et al. 1987)

8. Field application

One of the major drawbacks in the use of *Bti* is its rapid inactivation (24-48 h) in the environment (Mulla et al. 1993). Thus larvae populations of stagnant water mosquitoes recover within 5-7 days following treatment. Therefore the use of *Bti* is limited by the low efficacies of current preparations under field conditions (Tyanum & Mulla 1999). Since there is a little persistence of the toxin further applications are necessary to effect continuous control. Formulation and applications techniques can extend the persistence of activity for over one month in some situations, but activity remains sensitive to factors like UV degradation. In areas exposed to the sun, the residual effect is very short (Leong et al. 1980) and the product has to be reapplied. Other factors that affect the toxicity of *Bti* are particle sedimentation (Rushed & Mulla, 1989), protein adsorption onto silt particles, organic matter, elevated temperatures (Ohana et al. 1987), consumption by other organisms to which the toxin is not lethal (Blaustein and Margalit, 1991), dissolved tannins (Lord & Undeen, 1990) and inactivation by sunlight. Other factors that have been observed are that *Bti* does not recycle, under simulated field conditions, is unable to germinate and multiply in mud at the botton of pools although it did remain viable viable for up to 22 days, and higher water depth where applied decrease its activity (Ohana et al. 1987)

Early reports showed that a primary powder formulations of *Bti* had virtually no residual effect against mosquito larvae beyond application (Aguilar-Meza et al. 2010), although the delta-endotoxin remained chemically stable in neutral and acid waters (Sinegre et al 1980). Extend persistence with *Bti* is possible through use of improved formulations. Gunasekaran *et al.* (2002) tested a floating sustained release formulation of *Bti* in polluted water habitats against *Culex quinquefasciatus* larvae. Briquetts may result in more prolonged control than liquid formulations as these products have greater persistence through slow release (Kase & Branton 1986).

On the other hand, Aguilar-Meza et al. (2010) tested the residual insecticidal activity after field exposure of an aluminum-carboxymethylcellulose microencapsulated formulation of *Bti* spore-toxin complex with malachite green as photoprotective agent. The formulation improved the activity against *Aedes aegypti* larvae for 30 days and was comparable to that of the chemical insecticide temephos.

9. Conclusions

Bti is a bacterium that has been applied with success in biological control programs against mosquitoes and flies larvae all over the world. The study in each of its facets addressed in this review will open new perspectives to improve their effectiveness in biological control.

10. Acknowledgments

We thank Dr. Vladimir Sanchez-López of the University of Papaloapan, Tuxtepec, Oaxaca and Tiburcio Laez Aponte of the INECOL, Xalapa, Veracruz for taking optical an electronic microscope microphotographs, respectively.

11. References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, Vol. 18, pp. 265-267.

Adams, L.F.; Visick, J.E. & Whiteley H.R. (1989). A 20-kilodalton protein is required for efficient production of the *Bacillus thuringiensis* subsp. *israelensis* 27-kilodalton crystal protein in *Escherichia coli*. *Journal of Bacteriology*, Vol 171, pp. 521-530

Aguilar-Meza, O.; Ramırez-Suero, M.; Bernal, J.S. & Ramırez-Lepe, M. (2010). Field evaluation against *Aedes aegypti* larvae of aluminum-carboxymethylcellulose-encapsulated formulation of *Bacillus thuringiensis* serovar *israelensis*. *Journal of Economic Entomology*, Vol.103, pp. 570–576

Aronson, A.I. Angelo, N. & Holt, S.C. (1971). Organic Nutrients Required For Growth and Sporulation of *Bacillus Cereus*. *Journal of Bacteriology*, Vol.106, p.p.1016-1025.

Ali, A. (1981). *Bacillus thuringiensis* serovar. *israelensis* (ABG-6108) against chironomid midges and some nontarget invertebrates. *Journal of Invertebrate Pathology* Vol 38, pp. 264–272.

Aly, C.; Mulla, M.S. & Schnetter, W. (1987). Floating formulations increase activity of *Bacillus thuringiensis* var. *israelensis* against *Anopheles* larvae. *Journal of the American Mosquito Control Association* Vol 3, pp. 583–588.

Aly, C.; Mulla, M.S.; Xu, B.Z. & Schnetter, W. (1988). Rate of ingestion by mosquito larvae (Diptera:*Culicidae*) as a factor in the effectiveness of a bacterial stomach toxin. *Journal of Medical Entomology,* Vol 25, pp.191–196.

Aoki, K & Chigasaki, Y. (1915) Uber des toxin von sog. Sotto-bacillen Mitt. Med. Fak. Kais. Univ. Tokyo. Vol. 14, pp. 59-80

Aronson, A.I.; Han, E.S; McGaughey, W & Johnson, D. (1991). The solubility of inclusion proteins from *Bacillus thuringiensis* is dependent upon protoxin composition and is a factor in toxicity to insects. *Applied and Environmental Microbiology*, Vol 57, pp.981-986

Ash, C. & Collins, M.D. (1992). Comparative analysis of 23s ribosomal RNA gene sequences of *Bacillus anthracis* and emetic *Bacillus cereus* determined by PCR-direct sequencing. *FEMS Microbiology Letters,* Vol 73, pp. 75-80

Bassi, D.G.; Weathersbee, A.A.; Meisch, M.V. & Inman, A. (1989). Efficacy of Duplex and Vectobac against *Psorophora columbiae* and *Anopheles quadrimaculatus* larvae in Arkansas ricefields. *Journal of the American Mosquito Control Association,* Vol 5, pp. 264-266.

Bauer, L.S. (1995). A threat of the insecticidal crystal protein of *Bacillus thuringiensis*. *Florida Entomologist*, Vol 78, pp. 414-443

Baum, J. A. & T. Malvar, T. (1995). Regulation of insecticidal crystal protein production in *Bacillus thuringiensis*. *Molecular Microbiology,* Vol 18, pp. 1-12.

Bartlet, B.R. (1964). Integration of Chemical and Biological Control. In: *Biological Control of insect pests and weeds*, P. DeBach, (Ed.), 489-511, Chapman & Hall, London.

Becker, N. (2000). Bacterial control of vector-mosquitoes and black flies. In *Entomopathogenic Bacteria: From Laboratory to Field Application*, (Ed.) J.F. Charles, A. Delécluse and C. Nielsen-LaRoux), pp. 383-398. Dordrecht, The Netherlands: Kluwer.

Becker, N. & Margalit, J. (1993). Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies. In *Bacillus thuringiensis, an EnvironmentalBiopesticide: Theory and Practice,* P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs (Ed.)), pp. 147-170. John Wiley & Sons, New York.

Ben-Dov, E.; Zaritsky, A.; Dahan, E.; Barak, Z.; Sinai, R.; Manasherob, R.; Khamraev, A.; Troitskaya, E.; Dubitsky, A.; Berezina, N. & Margalith, Y. (1997). Extended screening by PCR for seven *cry*-group genes from field-collected strains of *Bacillus thuringiensis*. *Applied and Environmental Microbiology*, Vol 63, pp. 4883-4890

Bravo, A.; Sarabia, S.; Lopez, L.; Oniveros, H.; Abarca, C.; Ortiz, A.; Ortiz, M.; Lina, L.; Villalobos F.J.; Peña, G.; Nuñez-Valdez, M.E.; Soberón, M. & Quintero. R. (1998). Characterization of *cry* genes in a Mexican *Bacillus thuringiensis* strain collection. *Applied and Environmental Microbiology,* Vol 64, pp. 4965-4972

Bravo, A.; Gill, S.S. & Soberon, M. (2007). Mode of Action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon,* Vol.49, No.4 (March 2007). pp.423-435

Brown, K. L. & Whiteley, H.R. (1988). Isolation of a *Bacillus thuringiensis* RNA polymerase capable of transcribing crystal protein genes. *Proceedings of the National Academy of Sciences,* Vol 85, pp. 4166–4170.

Brown, K. L., & H. R. Whiteley. (1990). Isolation of the second *Bacillus thuringiensis* RNA polymerase that transcribes from a crystal protein gene promoter. *Journal of Bacteriology,* Vol 172, pp. 6682–6688.

Butko, P. (2003). Cytolytic toxin Cyt11A and its Mechanism of Membrane Damage: Data and Hypotesis. *Applied and Environmental Microbiology*. Vol69, No.5, (May 2003) pp 2415-2422

Carozzi, N. B.; Kramer, V.C.; Warren, G.W.; Evola, S. & Koziel, M.G. (1991). Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product profiles. *Applied and Environmental Microbiology,* Vol 57, pp. 3057-3061

Crickmore, N.; Zeigler, D.R.; Feitelson, J.; Schnepf, E.; Vanrie, J.; Lereclus, D.; Baum, J. & Dean, D.H. (1998). Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews,* Vol 62, pp. 807-813

Crickmore, N.; Nicholls, C.; Earp, D.J.; Hodgman, T.C.; & Ellar, D.J. (1990). The construction of *Bacillus thuringiensis* strains expressing novel entomocidal ™-endotoxin combinations. *Journal of Biochemistry,* Vol.270, pp. 133–136.

Chang, C.; Dai, S.M Frutos, R.; Federici, B.A. & Gill, S.S. (1992). Properties of a 72-kilodalton mosquitocidal protein from *Bacillus thuringiensis* subsp. morrisoni PG-14 expressed in *Bacillus thuringiensis* subsp. *kurstaki* by using the shuttle vector pHT3101. *Applied and Environmental Microbiology,* Vol 58, pp. 507-512

Charles, J. F. & deBarjac, H. (1982). Sporulation et crystal- logenese de *Bacillus thuringiensis* var *israelensis* en microscopie electronique.*Annals of Microbiology* (Inst.Pasteur)133A:425-442.

Chen, M.L.& Tsen H.Y. (2002). Discrimination of Bacillus cereus and Bacillus thuringiensis with 16S rRNA and gyrB gene based PCR primers and sequencing of their annealing sites. *Journal of Applied Microbiology*. Vol.92, pp. 912–919

Chilcott, C. N. & Ellar, D.J. (1988). Comparative toxicity of *Bacillus thuringiensis* var. *israelensis* crystal proteins *in vivo* and *in vitro. Journal of General Microbiology,* Vol.134, pp.2552-2558

Chilcott, C.N.; Knowles, B.H.; Ellar, D.J. & Drobniewski, F.A. (1990). Mechanism of Action of *Bacillus thuringiensis israelensis* Parasporal Body. In *Bacterial Control of Mosquitoes and Blackfles,* eds de Barjac, H. & Sutherland, D.J. pp. 45-65. New Brunswick : Rutgers University Press.

Chungjatupornchai, W.; Höfte, H.; Seurinck, J.; Angsuthanasombat, C. & Vaeck, M. (1988). Common features of *Bacillus thuringiensis* toxins specific for *Diptera* and *Lepidoptera*. *European Journal of Biochemistry*, Vol.173, pp.9-16

de Barjac, H. & Bonnefoi, A. (1973). Mise au point sur la classification des *Bacillus thuringiensis. Entomophaga.* Vol 18, pp. 5-17

de Barjac, H. 1978. A new subspecies of *Bacillus thuringiensis* very toxic for mosquitoes; *Bacillus thuringiensis* serotype H-14. *Compte Rendu Académie Sciences Paris Series D*. Vol. 286 pp.797-800.

de Barjac, H. & Frachon, E. (1990). Classification of *Bacillus thuringiensis* Strains Entomophaga. Vol. 35, pp. 233-240.

de Barjac, H. & Larget-Thiery, I. (1985). Characteristics of IPS82 standard for bioassay of *B. thuringiensis* H-14 preparations. WHO Rep/VBC/84.892.

Dervyn, E.; Poncet, S.; Klier, A. & Rapoport, G. (1995). Transcriptional regulation of the *cryIVD* gene operon from *Bacillus thuringiensis* subsp. *israelensis*. *Journal of Bacteriology,* Vol.177, pp.2283-2291

Donovan, W. P.; Dankocsik, C. & Gilbert, M.P. (1988). Molecular characterization of a gene encoding a 72-kilodalton mosquito-toxic crystal protein from *Bacillus thuringiensis* subsp. *israelensis*. *Journal of Bacteriology,* Vol.170, pp.4732-4738

Dulmage, H.T. (1970). Production of the spore- endotoxin complex by variants of *Bacillus thuringiensis* in two fermentation media. *Journal of Invertebrate Pathology*, Vol.16, pp.385-389.

Earp, D.J. & Ellar, D.J. (1987). *Bacillus thuringiensis* var. *morrisoni* strain PG14: nucleotide sequence of a gene encoding a 27 kDa crystal protein. *Nucleic Acids Research*, Vol 15, pp. 3619-3624

Dervyn, E.; Poncet, S.; Klier, A,; Rapoport, A.; Yoshisue, H.; Nishimoto, T.; Sakai, H. & Komano, T. (1994). Identification of a promoter for the crystal protein-encoding gene *cryIVB* from *Bacillus thuringiensis* subsp. *israelensis*. *Gene* Vol.137, pp.247–251

Debro, L.; Fitz-James, P.C. &Aronson, A. (1986). Two different parasporal inclusions are produced by *Bacillus thuringiensis subsp. finitimus. Journal of Bacteriology,* Vol.58, pp. 507-512

Delafield, F.P.; Somerville, H.J. & Rittenberg S.C. (1968). Immunological homology between crystal and spore protein of *Bacillus thuringiensis. Journal of Bacteriology,* Vol.96 pp.713-720

Dunkle, R.L., & B.S. Shasha. 1989. Response of starch encapsulated *Bacillus thuringiensis* containing ultraviolet screens to sunlight. Environ. Entomol. 18: 1035-1041.

Drobniewski, F.A. (1993) Onchocerciasis and the Onchocerciasis Control Programme. *Microbiology Europe* Vol.1, pp. 26-28,

Elcin, Y.M.; Cokmus, C. & Sacilik, C.S. (1995). Aluminum carboximethylcellulose encapsulation of *Bacillus sphaericus* 2362 for control of *Culex* spp. (Diptera: *Culicidae*) larvae. *Journal of Economic Entomology* Vol.88 pp. 830-834

Federici, B.A. (1999). *Bacillus thuringiensis* in Biological Control. . In: *Handbook of Biological Control.* T. Fisher (Ed.)Academic Press (Ed.) 575-593, ISBN 10: 0-12-257305-6

Federici, B.A.; Park, H.W.; Bideshi, D.K.; Wirth M.C. & Johnson, J.J. (2003). Review. Recombinant Bacteria for Mosquito Control. *The Journal of Experimental Biology*, Vol.206, pp.3877-3885

Finney, D.J. (1971). Probit Analysis. Cambridge Univ. Press, Cambridge, United Kingdom.

Foda M.S.; Salama, H.S. & Selim, M. (1985). Factors afecting growth physiology of *Bacillus thuringiensis. Applied Microbiology and Biotechnology*, Vol.22, pp.50-52.

Galjart, N. J.; Sivasubramanian, N. & Federici, B.A. (1987). Plasmid location, cloning and sequence analysis of the gene encoding a 23-kilodalton cytolytic protein from *Bacillus thuringiensis* subsp. *morrisoni* (PG-14). Current Microbiology, Vol.16, pp. 171-177

Glare T.R. & O'Callaghan M. (1998). Environmental and Health Impacts of *Bacillus thuringiensis israelensis. Report for the Ministry of Health.* Biocontrol & Biodiversity, Grasslands Division, Ag. Research. Lincoln, New Zealand.

Goldberg, L. J. & Margalit, J. (1977). A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univitattus, Aedes aegypti* and *Culex univitattus*. *Mosquto News,* Vol.37, pp. 355–358

Georghiou, G.P. & Wirth, M.C. (1997). Influence of Exposure to Single versus Multiple Toxins of *Bacillus thuringiensis* subsp. *israelensis* on Development of Resistance in the Mosquito *Culex quinquefasciatus* (Diptera: *Culicidae*). *Applied and Environmental Microbiology,* Vol.63, No. 3, pp.1095-1101

Goldberg, I.; Sneh, B.; Battae, E. & Klein, D. (1980). Opt i mi zat i on o f a medium for a high product i on o f spore-crystal preparat i on o f *Bacillus thuringiensis* effective against Egyptian cotton leaf worm *Spodoptera littorallis. Biotechnology Letters* Vol.2, pp. 419-426.

Hannay, L.C. (1953). Cristalline inclusion in aerobic spore forming bacteria. Nature. Vol. 172, pp. 1004.

Hannay, C.L. & Fitz-James P.C. (1955). The protein crystals of *Bacillus thuringiensis Berl. Canadian Journal of Microbiology.* Vol 1, pp. 694-710.

Hershey, A. E., Shannon, L., Axler, R., Ernst, C. & Mickelson, P. (1995). Effects of methoprene and *Bti* (*Bacillus thuringiensis* var. *israelensis*) on non-target insects. *Hydrobiologia,* Vol. 308, pp.219-227

Höfte, H. & Whiteley, H.R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiology Reviews* Vol.53 pp. 242-255.

Hoti, S.L, & Balaraman, K. (1993). Formation of melanin-pigment by a mutant of *Bacillus thuringiensis* H-14. *Journal of General Microbiology* Vol.139, pp. 2365-2369.

Husz, B. (1931). Experiments during 1931 on the use of *Bacillus thuringiensis Berl*. in controlling the corn borer. *Science Reports International of Corn Borer Investigation*, Vol. 4, pp. 22-23.

Ibarra, J. E. & Federici, B. A. (1986). Isolation of a relatively nontoxic 65-kilodalton protein inclusion from the parasporal body of *Bacillus thuringiensis*subsp. *israelensis*. *Journal of Bacteriology,* Vol.165, pp.527-531

Ignofo, C.M., Shasha, B.S. & Shapiro, M (1991). Sunlight ultraviolet protection of the *Heliothis* nuclear polyhedrosis virus through starch encapsulation technology. *Journal of Invertebrate Pathology,* Vol 57: 134-136.

Ishiwata, S. (1901). On a kind of severe flacherie (sotto disease). *Dainihon Sanshi Kaiho* Vol.114, pp.1-5

Iwabushi, H. (1908) Popular silkworm Pathology A.A. Meibundo (Ed.) Tokyo, pp. 428.

Kase, L.E. & Branton, L. (1986). Floating particle for improved control of aquatic insects. U.S. Pat. 4631857

Knowles, B. H.; Blatt, M.R.; Tester, M.; Horsnell, J.M.; Carroll, J.; Menestrina G. & Ellar, D.J. (1989). A cytolytic delta-endotoxin from *Bacillus thuringiensis* var. *israelensis* forms cation-selective channels in planar lipid bilayers. *FEBS Letters*, Vol.244, pp.259–262

Krieg V.A.; Huger A.M.; Langenbruch G.A. & Schnetter W. (1983). *Bacillus thuringiensis* var. *tenebrionis*: Ein neuer, gegenüber lair ven von coleopteren wirksamer pathotyp. *Z. Äng. Ent.* Vol.96, pp. 500-508

Jacobs, S.E. (1950).Bacteriological control of the flour moth *Ephestia kuhniella*. *Proceedings of the Society of Apply Bacteriology,* Vol.13, pp. 83-91.

Jackson, J. K.; Sweeney, B. W.; Bott, T. L.; Newbold, J. D. & Kaplan, L. A. (1994). Transport of *Bacillus thuringiensis* var. *israelensis* and its effect on drift and benthic densities of nontarget macroinvertebrates in the Susquehanna River, northern Pennsylvania. *Canadian Journal of Fisheries and Aquatic Sciences* Vol.51, pp.295-314

Johnson, M.W. & Tabashnik B.E. (1999). Enhanced Biolgical Control Through Pesticide Selectivity. In: *Handbook of Biological Control.* T. Fisher (Ed.)Academic Press (Ed.) 297-317, ISBN 10: 0-12-257305-6

Lacey L.A.; Urbina, M.J. & Heitzzman, C.M.(1989). Sustained release formulations of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) for control of container-breeding *Culex quinquefasciatus*. *Mosquito News* Vol.44, pp.26-32.

Lacey, L. A. & Mulla, M. S. (1990). Safety of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* to nontarget organisms in the aquatic environment. In *Safety of Microbial Insecticides.* Laird-M, Lacey-LA and Davidson-EWSO (Ed.), pp. 259 pp. CRC Press Inc.; Boca Raton; USA.

Leighton, T.J. & Doi, R.H.(1971). The Stability of Messenger Kibonucleic Acid during Sporulation in *Bacillus subtilis*. *The Journal of Biological Chemistry*. Vol.246, pp.3189-3195.

Leong, K.L.; Cano, R.J. & Kubinski, A.M (1980). Factors affecting *Bacillus thuringiensis* total field persistence. *Environmental Entomology*, Vol.9 pp.593-599.

Levy, R.; Nichols, M.A. & Miller, T. W. (1990). Culigel superabsorbent polymer controlled-release system: application to mosquito larvicidal bacilli. *Proceedings and Abstracts, Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia* , 107

Lord, J.C. & Undeen A.H. (1990). Inhibition of the *Bacillus thuringiensis* var. *israelensis* toxin by dissolved tannins. Environmental Entomology, Vol.19 pp.1547-1551

Manasherob, R., Ben-Dov, E., Margalit, J., Zaritsky, A. & Barak, Z. (1996). Raising activity of *Bacillus thuringiensis* var. *israelensis* against *Anopheles stephensi* larvae by encapsulation in *Tetrahymena pyriformis* (Hymenostomatidia: Tetrahymenidae). *Journal of the American Mosquito Control Association,* Vol.12, pp. 627-631

Manasherob, R.; Ben-Dov, E.; Xiaoqiang, W.; Boussiba, S., & Zaritsky, A. (2002). Protection from UV-B damage of mosquito larvicidal toxins from *Bacillus thuringiensis* subsp. *israelensis* expressed in *Anabaena* PCC 7120. *Current Microbiology,* Vol 45, pp. 217–220.

Marrone, P.G. & MacIntosh, S.C. (1993). Resistance to *Bacillus thuringiensis* and Resistance Management. *In: Bacillus thuringiensis*, an Environmental Biopesticide: Theory and

Practice, Entwistle, P. F., Cory, J. S., Bailey, M. J., and Higgs, S., Eds., John Wiley and Sons, Chichester, UK, pp.221-235.

McDonald B.A. & Linde C. (2002). Pathogen Population Genetics, Evolutionary Potential, and Durable Resistance. *Annual Review of Phytopathology*, Vol. 40, pp. 349-379

McGuirre, M.R.; Shasha, B.S.; Eastman, C.E. & Sagedchi H.O. (1996). Starch and fluor-based sprayable formulations: Effect on rainfastness and solar stability of *Bacillus thuringiensis*. *Journal of Economic Entomology*, Vol.89, pp. 863-869

McLaughlin, R.E.; Dulmage, H.T.; Alls, R.; Couch, T.L.; Dame, D.A.; Hall, I.M.; Rose, R.I. &.Versol, P.L. (1984). U.S. standard bioassay for the potency assessment of *Bacillus thuringiensis* var. *israelensis* serotype H-14 against mosquito larvae. *Bulletin of the Entomological Society of America*, Vol.30, pp. 26-29

McLean, K.M. & Whiteley, H.R. (1987). Expression in *Escherichia coli* of a cloned crystal protein gene of *Bacillus thuringiensis* subsp. *israelensis*. *Journal of Bacteriology,* Vol.169, pp. 1017–1023

Mattes, O. (1927) Parasitare kronkheiten der mehlmotten larven und versuche uber ihre Verwendborkeit als biologische Bekampfungsmittel. *Sisher Ges. Beforder. Ges Naturw. Marburg*. Vol.62., pp. 381-417

Metalnikov, S.; Hengula, B. & Strail, D.M. (1930). Experiments on the application of bacteria against the corn borer. *Science Reports International Corn Borer Investigation,* Vol3, pp. 148-151.

Mikkola, A. R.; Carlberg, G.A.; Vaara, T. & Gyllenberg, H.G. (1982). Comparison of inclusions in different *Bacillus thuringiensis* strains. An electron microscope study. FEMS Microbiology Letters, Vol.13, pp.401-408

Mitani, K & Watari, J. (1916). A new method to isolate the toxin of *Bacillus sotto* Ishiwata by passing through a bacerial filter and a preliminary report on the toxic action of this toxin to the silkworm larvae. *Archi Gensanshu Serzojo Hokoku*. Vol 3, pp. 33-42.

Metcalf, R.L. (1986). The Ecology of Insecticides and the Chemical Control of Insects. *In Ecological Theory and Integrated Pest Managment in Practice*, M. Kogan (Ed.) 251 297 John Wiley & Sons, New York.

Morris, O.N. (1983). Protection of *Bacillus thuringiensis* from inactivation from sunlight. *Canadian Entomology*, Vol 115, pp.1215-1227.

Mulla, M. S. & Darwazeh, H.A. (1985). Efficacy of formulations of *Bacillus thuringiensis* H-14 against mosquito larvae. *Bulletin fo the Society of Vector Ecology*. Vol.10, pp.14–19

Mulla, M. S.; Darwazeh, H.A.; Ede, L.; Kennedy, B. & Dulmage H.T. (1985). Efficacy and field evaluation of *Bacillus thuringiensis* (H-14) and *B. sphaericus* against floodwater mosquitoes in California. *Journal of American Mosquito Control Association* Vol1, pp.310–315.

Mulla, M. S.; Darwazeh, H.A. & Zgomba, M. (1990). Effect of some environmental factors on the efficacy of *Bacillus sphaericus* 2362 and *Bacillus thuringiensis* (H-14) against mosquitoes. *Bulletin of the Society of Vector Ecology,* Vol.15, pp.166–175.

Mulla, M. S.; Chaney, J.D. & Rodchareon, J. (1993). Elevated dosages of *Bacillus thuringiensis* var. *israelensis* fail to extend control of *Culex* larvae. *Bulletin of the Society of Vector Ecology*, Vol.18, pp.125–132.

Mulla, M. S.; Thavara, U.; Tawatsin, A. & Chompoosri, J. (2004). Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. *Journal of the American Mosquito Control Association* Vol.20, pp.64–73

Nakata, H.M. (1964). Organic Nutrients Required For Growth And Sporulation Of *Bacillus Cereus*, *Journal of Bacteriology,* Vol.88, pp.1522-1524;

Ohana, B.; Margalit, J. & Barak, Z. (1987). Fate of *Bacillus thuringiensis* subsp *israelensis* under simulated field conditions. *Applied and Environmental Microbiology,* Vol.53, pp.828-831.

Ogunjimi, A.A.; Chandler, J.M.; Gbenle, G.O.; Olukoya, D.K., & Akinrimisi, E.O. (2002). Heterologous expresión of *cry2* gene from a local strain of *Bacillus thuringiensis* isolated in Nigeria. *Biotechnology Applied Biochemistry*, Vol.36, pp.241–246

Pearson, D. & Ward, O.P. (1988). Effect of culture conditions on growth and sporulation of *Bacillus thuringiensis* susp. *israelensis* and development media for production of the protein crystal endotoxin. *Biotechnology Letters*, Vol.10, pp.451-456.

Perez, C.; Fernandez, L.E.; Sun, J.; Folch, J.L.; Gill, S.S. & Soberon M. (2005*). Bacillus thuringiensis* subsp. *israelensis* Cyt1Aa synergizes Cry11Aa Toxin by Functioning as a Membrane-Bound Receptor. *Proceedings of the National Academy of Sciences*. Vol 102, No. 51. pp. 18303-18308

Poncet, S.; Bernard, C.; Dervyn, E.; Cayley, J.; Klier, A.; & Raport, G. (1997). Improvement of *Bacillus sphaericus* toxicity against dipteran larvae by integration, via homologous recombination, of the *cry11A* toxin gene from *Bacillus thuringiensis* subsp. *israelensis*. *Applied and Environmental Microbiology,* Vol.63, pp 4413–4420

Porcar, M. & Juarez-Perez, V. (2003). PCR-based identification of *Bacillus thuringiensis* pesticidal crystal genes. *FEMS Microbiology Reviews*. Vol. 26, pp. 419-432.

Pozsgay, M.; Fast. P.; Kaplan, H. & Carey, P.R. (1987). The effect of sunlight on the protein crystals from *Bacillus thuringiensis* var. *kurstaki* HD1 and NRD12: A raman spectroscopy study. *Journal of Invertebrate Pathology,* Vol.50 pp.620-622

Priest, F.G. (1992). A review. Biological control of mosquitoes and other biting flies by *Bacillus sphaericus* and *Bacillus thuringiensis*. *Journal of Applied Bacteriology,* Vol.72, pp.357-369.

Promdonkoy, B. & Ellar, D.J. (2000). Membrane pore architecture of a cytolytic toxin from *Bacillus thuringiensis. Journal of Biochemistry.*Vol. 350, pp.275–282.

Pusztai, M.; Fast, P.; Gringorten, L., Kaplan, H.; Lessard, T. & and Carey, P.R. (1991). The mechanism of sunlight-mediated inactivation of *Bacillus thuringiensis* crystals. *Journal of Biochemistry*, Vol.273, pp.43-47

Quintana-Castro, R.; Ramírez-Suero, M.; Moreno-Sanz, F. & Ramírez-Lepe, M. (2005). Expression of the *cry11A* gene of *Bacillus thuringiensis* ssp. *israelensis* in *Saccharomyces cerevisiae*,.*Canadian Journal of Microbiology, Vol*.51, pp.165-170

Ramırez-Lepe, M.; Aguilar, O.; Ramırez-Suero, M. & Escudero, B. (2003). Protection of the spore-toxin complex of *Bacillus thuringiensis* serovar *israelensis* from ultraviolet irradiation with aluminum-CMC encapsulation and photoprotectors. *Southwestern. Entomology,* Vol.28, pp.137-143.

Ramírez-Suero, M.; Robles-Olvera, V. & Ramírez-Lepe, M. (2005). Spray-dried *Bacillus thuringiensis* serovar *israelensis* formulations for control of *Aedes aegypti* larvae. *Journal of Economic Entomology*, Vol.98, pp.1494-1498

Ramırez-Suero, M.; Valerio-Alfaro, G.; Bernal, J.S. & Ramirez-Lepe M. (2011). Synergisitic effect of chitinases and *Bacillus thuringiensis israelensis* spore-toxin complex against *Aedes aegypti* larvae. *The Canadian Entomologist* Vol,143: pp. 157–164.

Rawlins, S.C. (1989) Biological control of insect pests affecting man and animals in the tropics. *CRC Critical Reviews in Microbiology* Vol.16, pp.235–252

Rowe, G.E. & Margaritis, A.M. (1987). Bioprocess Developments in the Production of Bioinsecticides by *Bacillus thuringiensis. CRC Critical Reviews in Biotechnology.* Vol.6, No.4, pp. 87-127.

Rushed, S.S. & Mulla, M.S. (1989). Factors influencing ingestion of particulate materials by mosquito larvae (Diptera: Culicidae). *Journal of Medical Entomology,* Vol.26, pp. 210-216

Salama, H.S.; Foda, M.S. & Dulmage H.T. (1983). Novel Media for Production of δ-endotoxins form *Bacillus thuringiensis*. *Journal of Invertebrate Pathology,* Vol.41, pp.8-19.

Sen, K.; Honda, G.; Koyama, N.; Nishida, M.; Neki, A.; Sakai, H.; Himeno, M. & Komano, T. (1988). Cloning and nucleotide sequences of the two 130 kDa insecticidal protein genes of *Bacillus thuringiensis* var. *israelensis*. *Agriculture Biology Chemistry*, Vol.52, pp.873-878

Servant, P.; Rosso, M.L.; Hamon, S.; Poncet, S.; Delécluse, A. & Rapport, G. (1999). Production of cry11A and cry11B toxins in *Bacillus sphaericus* confers toxicity towards *Aedes aegypti* and resistant *Culex* populations. *Applied and Environmental Microbiology*, Vol.65, pp.3021–3026.

Sinegre, G.; Gaven, B.; Jullien, J. L. & Crespo, O. (1980). Effectiveness of the serotype H-14 of *Bacillus thuringiensis* against the principal species of man-biting mosquitoes on the Mediterranean coast of France. *Parassitologia* Vol.22, pp.223-231.

Skovmand, O. & Eriksen, A. G. (1993). Field trials of a fizzy tablet with *Bacillus thuringiensis* subsp. *israelensis* in forest spring ponds in Denmark. *Bulletin of the Society for Vector Ecology, Vol.* 18, pp.160-163.

Skovmand, O.; Hoeg, D.; Pedersen, H.S., & Rasmussen T. (1997). Parameters Influencing Potency of *Bacillus thuringiensis* var. *israelensis* Products. *Journal of Economic Entomology* Vol 90, No.2. pp. 361-369.

Smith, A.R. (1982). Effect of strain and medium variation on mosquit production by *Bacillus thuringiensis* var. *israelensis*. *Canadian Journal of Microbiology,* Vol.28, pp.1090-1092.

Tabashnik B.E. (1992). Evaluation of Synergism among *Bacillus thuringiensis* Toxins. *Applied and Environmental Microbiology,* Vol.58, pp.3343-3346

Tanapongpipat, S.; Luxananil, P.; Boonhiang, P.; Chewawiwat, N.; Audtho, M. & Panyim, S. (2003). A plasmid encoding a combination of mosquito-larvicidal genes from *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* confers toxicity against broad range of mosquito larvae when expressed in Gram-negative bacteria. FEMS Microbiology Letters, Vol.228, pp.259–263

Thanabalu, T.; Hindley, J.; Brenner, S.; Oei, C. & Berry, C. (1992). Expression of the mosquitocidal toxins of *Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis* by recombinant *Caulobacter crescentus*, a vehicle for biological control of aquatic insect larvae. *Applied and Environmental Microbiology,* Vol.58, pp. 905– 910

Thomas, W.E. & Ellar, D.J. (1983) *Bacillus thuringiensis* var. *israelensis* crystal delta-endotoxin : effects on insect and mammalian cells *in vitro* and *in vivo. Journal of Cell Science* Vol.60, pp.181-197.

Thorne, C.B. (1968). Transducing Bacteriophage for *Bacillus cereus*. *Journal Of Virology,* Vol.2, pp. 657-662.

Tungpradubkul, S.; C. Settasatien, & Panyim, S. (1988). The complete nucleotide sequence of a 130 kDa mosquito-larvicidal delta-endotoxin gene of *Bacillus thuringiensis* var. *israelensis*. *Nucleic Acids Research* Vol.16, pp.1637-1638.

Tyanyun, S. & Mulla, M.S. (1999). Field evaluation of new water dispersible granular formulations of *Bacillus thuringiensis* subp. *israelensis* and *Bacillus sphaericus* against Culex mosquito in microcosms. *Journal of the American Mosquito Control Association* Vol 15 pp. 356-361.

Waalwijk, C.; A.; Dullemans, M.; VanWorkum, M.E.S. & Visser, B. (1985). Molecular cloning and the nucleotide sequence of the Mr28,000 crystal protein gene of *Bacillus thuringiensis* subsp. *israelensis*. *Nucleic Acids Research*, Vol.13, pp. 8207-8217

Walsh, J. (1986) River blindness, a gamble pays off. *Science*, Vol.232, pp.922–925.

Ward, E.S.; Ridley, A.R.; Ellar, D.J. & Todd, J.A. (1986). *Bacillus thuringiensis* var. *israelensis* endotoxin. Cloning and expression of the toxin in sporogenic and asporogenic strains of *Bacillus subtilis*. *Journal of Molecular Biology*, Vol.191, pp. 13–22

Ward, E.S., & Ellar, D.J. (1986). *Bacillus thuringiensis* var. *israelensis* delta-endotoxin: nucleotide sequence and characterization of the transcripts in *Bacillus thuringiensis* and *Escherichia coli*. *Journal of Molecular Biology*, Vol.191, pp.1-11

Ward, E. S., & Ellar, D.J. (1987). Nucleotide sequence of a *Bacillus thuringiensis* var. *israelensis* gene encoding a 130 kDa delta-endotoxin. *Nucleic Acids Research*, Vol 15, pp. 7195

Webb, G. (1992) The Onchocerciasis Control Programme. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* Vol. 86, pp.113-114

Wirth, M. C.; Georghiou, G. P.; Federici, B. A. (1997). CytA enables CryIV endotoxins of *Bacillus thuringiensis* to overcome high levels of CryIV resistance in the mosquito, Culex quinquefasciatus. *Proceedings of the National Academy of Sciences,* Vol.94, pp.10536-10540

Wojciech J. J. & Korsten L. (2002). Biological Control of Postharvest Diseases of Fruits. *Annual Review of Phytopathology* Vol. 40, pp. 411-441.

World Health Organization (1999). *World Health Report*. Geneva: World Health Organization.

Wu, D. & Chang, F.N (1985). Synergism in mosquitocidal activity of 26 and 65 kDa proteins from *Bacillus thuringiensis* subsp. *israelensis* crystal. FEBS Letters, Vol.190, pp.232-236

Xu,Y.; Nagai, M.; Bagdasarian, M.; Smith,T.W. & Walker, E.D. (2001). Expression of the *P20* Gene from *Bacillus thuringiensis* H-14 Increases Cry11a Toxin Production and Enhances Mosquito- Larvicidal Activity in Recombinant Gram-Negative Bacteria. *Applied And Environmental Microbiology*, Vol.67, p.p.3010–3015

Wada Y. (1989). Control of Japanese encephalitis vectors. *Southeas*t *Asian journal of tropical medicine and public health,* Vol.20, pp. 623–626

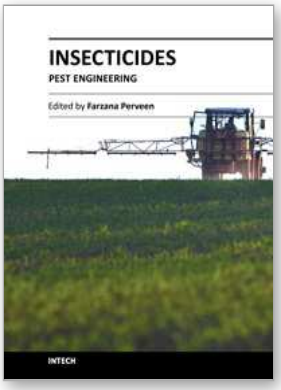
Yamagiwa, M., Sakagawa, K., & Sakai, H. (2004). Functional Analysis of two processed fragments of *Bacillus thuringiensis* cry11A toxin. *Bioscience Biotechnology and Biochemistry*, Vol.68, pp.523–528

Yamamoto, T.; IWatkinson, I.A.; Kim, L.; Sage, M.V.; Stratton, R.; Akande, N.; Li, D.; D.P. & Roe, B.A. (1988). Nucleotide sequence of the gene coding for a 130-kDa mosquitocidal protein of *Bacillus thuringiensis israelensis*. *Gene*, Vol 66, pp.107-120.

Yamamoto, T.; Iizuku, T. & Aronson, J.N. (1983). Mosquitocidal protein of *Bacillus thuringiensis* subsp. *israelensis*: identification and partial isolation of the protein. *Current Microbiology* Vol.9, pp.279-284.

Yu-Tien, L.; Men-Ju, S.; Dar-Der, J.; Wang, W.I.; Chin-Chi, C. & Cheng-Chen, C. (1993). Protection from ultraviolet irradiation by melanin mosquitocidal activity of *Bacillus thuringiensis* var. *israelensis*. *Journal of Invertebrate Pathology,* Vol 62:131-136.

Zeigler, D.R. (1999). *Bacillus thuringiensis Bacillus cereus*. *Bacillus Genetic Stock Center*. Catalog of strains 7th. ed. Vol.2



Insecticides - Pest Engineering

Edited by Dr. Farzana Perveen

ISBN 978-953-307-895-3
Hard cover, 538 pages
Publisher InTech
Published online 15, February, 2012
Published in print edition February, 2012

This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by Bacillus thuringiensis, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of Aedes aegypti, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of Anopheles gambiae P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mario Ramírez-Lepe and Montserrat Ramírez-Suero (2012). Biological Control of Mosquito Larvae by Bacillus thuringiensis subsp. israelensis, Insecticides - Pest Engineering, Dr. Farzana Perveen (Ed.), ISBN: 978-953-307-895-3, InTech, Available from: http://www.intechopen.com/books/insecticides-pest-engineering/biological-control-of-mosquito-larvae-by-bacillus-thuringiensis-subsp-israelensis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821