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BACILLUS THURINGIENSIS: THE BIOCONTROL AGENT IN A FOOD WEB PERSPECTIVE

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ABSTRACT

Bacillus thuringiensis (Bt) is a facultative anaerobic, motile, gram-positive, spore-forming soil bacterium. The spores have parasporal inclusions made of different insecticidal crystal proteins (ICP), predominantly comprising one or more *Cry* and/or *Cyt* proteins (also known as δ-endotoxins) that have potent and specific insecticidal activity. The insecticidal properties of Bt have been known for over a century and commercial products based on this organism have been available for 70 years, occupying >90% of the biopesticide market. The microbe formulations have great potential in IPM programmes as has become the leading biopesticide in commercial agriculture, forest management and mosquito control. This bacterium is also a key source of genes for transgenic expression to provide pest resistance in plants and microorganisms as pest control agents in genetically modified organisms. Bt may persist as a component of the natural microflora after application to an ecosystem. Owing to their specific mode of action, Bt products are unlikely to pose any hazard to vertebrates or to the great majority of nontarget invertebrates. Yet many carnivorous arthropods and other non target organisms come into contact with Bt toxins not via target herbivore, but via nontarget herbivores.. Understanding its role in the ecosystem is crucial in deriving the best out of this great biocontrol agent.

Key words: *Bacillus thuringiensis;* Lepidoptera; Biological control; Parasitoids; non-target organisms; multitrophic

INTRODUCTION

Despite significant increases in per capita agricultural production worldwide over the last decades, the challenge of producing sufficient food supply safe from pests and to keep it safe in transit and storage as well remains daunting. Even more challenging is to develop suitable methods of pest control in accordance of philosophy and methodology of modern integrated pest management (IPM) programme in an increasingly environmentally conscious world of ours. There are an estimated 67,000 pest species worldwide that damage agricultural crops, of which approximately 9,000 species are insects and mites (Ross and Lembi, 1985). The biopesticide *Bacillus thuringiensis* Berliner, the most successful among other microbial pesticides, has attained wide commercial use against major insect pests as a major biocontrol agent. The microbe formulations have great potential in IPM programs. This bacterium is also a key source of genes for transgenic expression to provide pest resistance in plants and microorganisms as pest control agents in so-called genetically modified organisms (GMOs) (WHO, 1999).

Bacillus thuringiensis (Bt) is one of the most widely used entomopathogenic microorganism among many for the biological control of insect pests (Samsonov *et al.* 1997). Historical account interestingly shows that the insecticidal properties of Bt were recognized many years before the bacterium was identified, with some suggesting that Bt spores may have already been in use in ancient Egypt. In the modern era, the bacterium *Bacillus thuringiensis (Bt)* was first isolated in 1901 by the Japanese biologist Shigetane Ishiwatari during an investigation into wilt disease in silk worms, and he named it *Bacillus sotto*. Ten years later, the same bacterium was isolated by Ernst Berliner from a diseased Mediterranean flour moth (*Ephestia kuehniella*) in the German province of Thuringia, and it was named *Bacillus thuringiensis* (Siegel, 2000).

Bt is a Gram positive, spore-forming soil bacteria and is closely related to *Bacillus cereus* (Bc), *B. mycoides* and *B. anthracis*. Bt can only be distinguished from *Bacillus cereus* by the production, during the sporulation process, of one or more inclusion bodies, which have been found to be toxic for invertebrates, primarily insect species in the orders *Coleoptera*, *Diptera* and *Lepidoptera* (de Barjac, 1981b; Andrews et al., 1987). Occasionally the bacteria lose their ability to form crystals and then become indistinguishable from *B. cereus* itself. Investigations into transformation of *B. cereus* to Bt has revealed that the genes conferring crystal formation resides on a plasmid (WHO, 1999).

The defining feature of Bt is its ability to produce crystalline proteinaceous inclusions. The characteristic crystalline proteinaceous parasporal inclusions formed by Bt are composed of different insecticidal crystal proteins (ICP). The existence of parasporal inclusions in Bt was first noted in 1915 (Berliner, 1915), but their protein composition was not delineated until the 1950s (Angus, 1954). Bt subspecies can synthesize more than one inclusion, adjacent to the endospore during sporulation, which may contain different ICPs. ICPs, also called δ-endotoxins, have various shapes (bipyramidal, cuboidal, flat rhomboid, spherical or composite with two crystal types), depending on their ICP composition. A partial correlation has been established between crystal morphology, ICP composition, and bioactivity against target insects (Bulla et al., 1977; Höfte & Whiteley, 1989; Lynch & Baumann, 1985).

The classification of Bt subspecies based on the serological analysis of the flagella (H) antigens was introduced in the early 1960s (de Barjac & Bonnefoi, 1962). This classification by serotype has been supplemented by morphological and biochemical criteria (de Barjac, 1981a). Until 1977, only 13 Bt subspecies had been described, and at that time all subspecies were toxic to Lepidopteran larvae only. The discovery of other subspecies toxic to Diptera (Anand Kumar, 2013; Goldberg & Margalit, 1977), Coleoptera (Krieg et al., 1983) and apparently Nematoda (Narva et al., 1991) enlarged the host range and markedly increased the number of subspecies. Up to the end of 1998, over 67 subspecies based on flagellar H-serovars had been identified. Now use of various criteria such as, phage susceptibility and plasmid profiles along with serotyping has resulted in the classification of approximately 100 subspecies (Sanahuja, 2011). This review does not include (possible) interactions of Bt with other microbial organisms in an ecosystem.

Genetics of Bt toxins and its Mode of action:

In the early 1980s, it was established that most genes coding for the ICPs reside on large transmissible plasmids, of which most are readily exchanged between strains by conjugation (González & Carlton, 1980; González et al., 1981). Since these initial studies, numerous ICP genes have been cloned, sequenced and used to construct Bt strains with novel insecticidal spectra (Höfte & Whiteley, 1989). δ-endotoxins are encoded by the C*ry* and C*yt* genes These genes become active during sporulation because they are controlled by a dedicated RNA polymerase that is also synthesized specifically while spores are forming. Up to 20% of the spore protein content is represented by these Cry ⁄ Cyt toxins (Aronson, 2002). These δ-endotoxins have molecular weights between 14-160 kDa and can be visualized under light microscopy as inclusion bodies (Schnepf *et al*. 1998). In addition, *Bt* has other insecticidal proteins like *Vips* that are secreted during its vegetative cycle (Estruch *et al.* 1996, Schnepf *et al*. 1998).

C*ry* genes were classified into 40 families according to their amino acid sequence similarities (Samsonov *et al.* 1997) and the sequence of more than 160 *cry* genes are known. The specificity of the toxic effect of the δ-endotoxins against certain species makes them environmentally friendly tools for the control of insects that are plagues of important agricultural crops. The currently known crystal (*cry*) gene types encode ICPs that are specific to either *Lepidoptera* (*cryI*), *Diptera* and *Lepidoptera* (*cryII*), *Coleoptera* (*cryIII*), *Diptera* (*cryIV*), or *Coleoptera* and *Lepidoptera* (*cryV*) (Höfte & Whiteley, 1989). A separate designation is used for the cytolytic (*cyt*) genes that encode a nonspecific cytolytic factor, present in Bti ICP and some other Bt subspecies.

The sporulated Bt with ICP or spore-ICP complexes must be ingested by a susceptible insect larva to be effective (Visser et al., 1993). The mode of action of Bt has been reviewed by Schnepf et al. (1998) and can be summarized in the following stages: 1) ingestion of sporulated Bt and ICP by an insect larva; 2) solubilization of the crystalline ICP in the midgut; 3) activation of the ICP by proteases; 4) binding of the activated ICP to specific receptors in the midgut cell membrane; 5) insertion of the toxin in the cell membrane and formation of pores and channels in the gut cell membrane, followed by destruction of the epithelial cells (Cooksey, 1971; Norris 1971; Fast, 1981; Huber & Lüthy, 1981; Lüthy & Ebersold, 1981; Smedley & Ellar, 1996); and 6) subsequent Bt spore germination and septicaemia may enhance mortality (WHO,1999).

In the midgut of the target larva the parasporal crystalline ICP is dissociated to the protoxin form, and the protoxin is then activated to a biologically active holotoxin by the proteolytic enzymes and specifically the alkaline environment of the gut (Warren et al., 1984; Jaquet et al., 1987; Aronson et al., 1991; Honée & Visser, 1993). Shortly afterwards, the gut becomes paralysed and the larva ceases to feed. Experimental data suggest that the C-terminal and middle domains of the toxin are involved in epithelial cell receptor binding and structural functions, while the N-terminal domain is primarily involved in ion channel and pore formation (Huber et al., 1981; Schnepf et al., 1998; Dean et al., 1996). Pore or ion channel formation occurs after the binding to the receptor and insertion of the N-terminal domain into the membrane, whereby the regulation of the trans-membrane electric potential is disturbed. This can result in colloid-osmotic lysis of the cells, which is the main cytolytic mechanism that is common to all ICPs (Knowles & Ellar, 1987; Slatin et al., 1990; Schwartz et al., 1991; Schnepf et al., 1998). When the midgut epithelium of the larva is damaged, the haemolymph and gut contents can mix. This results in favourable conditions for the Bt spores to germinate. The resulting vegetative cells of Bt and the pre-existing microorganisms in the gut proliferate in the haemocoel causing septicaemia, and may thus contribute to the mortality of the insect larva.

Bt as a biocontrol agent:

Biological control is the deliberate use by man of biotic agent to suppress and/or regulate a pest population (Rabb et al., 1974). Biocontrol by natural enemy introductions has its basis in the fact that when organisms invade new environments they often leave their adapted natural enemies behind and released of this pressure, they may erupt in great abundance (Keane and Crawley, 2002; van den Bosch, 1971). This idea was formalized into the ‘‘enemy release hypothesis’’ (Keane and Crawley, 2002) and the success of biocontrol programs has been used as a support for this hypothesis. Biological control holds several distinct advantages over other pest control methods such as self perpetuation (Yamada & Griffiths, 1987), lack of resistance (Van Roermond & Lenteren, 1993) and lack of adverse side effects. Biological control agents generally are quite specific, attacking on several phytophagus pest species without disturbing beneficial forms. Biological control in an ecological sense can be defined "the regulation by natural enemies viz parasitoids, predators and/or pathogens of another organism population at a lower average than would otherwise occur" (DeBach, 1964a, 1964b).

The Bt subspecies occur naturally in most ecological niches and can be added to an ecosystem to achieve insect control (Andrews et al., 1987; Stahly et al., 1991). When nutrients are available the spores of Bt persist in soil, and vegetative growth occurs (DeLucca et al., 1981; Akiba, 1986; Ohba & Aizawa, 1986; Travers et al., 1987; Martin & Travers, 1989). It has also been found extensively on plant surfaces especially in the phylloplane. Numerous Bt subspecies have been isolated from dead or dying insect larvae and in most cases the isolate has toxic activity to the insect from which it was isolated (Goldberg & Margalit, 1977; de Barjac, 1981b; Hansen et al., 1996). The dead insect carcass of such infected insect larva usually contains relatively large quantities of spores and crystals that may be released into the environment (Prasertphon et al., 1973; Grassi & Deseö, 1984; Aly, 1985; Aly et al., 1985). Thus it efficiently presents itself as a major biocontrol agent.

B. thuringiensis-derived bioinsecticides are used in conventional and organic farming (Gustafson *et al.* 1997), representing 90% of the formulated biopesticide products that are available in the market (Bernhard & Utz, 1993), yet it represents less than 1% of insecticides used on a global basis. Commercial *Bacillus thuringiensis* (Bt) products are microbial pest control agents (MPCAs) containing specific insecticidal crystalline proteins (ICPs) and most often living spores as well as formulating agents. They are processed fermentation products. They are advantageous in terms of their safety, specificity and potency compared to chemical sprays, and are also biodegradable, which provides for a large and competitive market. However the advantages are conditional as Bt is only effective when present on the plant organs on which insects feed. Usually Bt is applied when early instar larvae are present, because older larvae are more tolerant. Bt sprays persist for only a few days on the leaf surface because UV light, weather, the chemical environment of the leaf surface contribute to the degradation of Cry proteins. Also spores may get washed off the leaf surface into the soil in inclement weather. Thus *Bt*-based biopesticides also have several disadvantages (discussed in McGaughey and Whalon, 1992). A potential solution to the problem of short window of effectiveness of topical Bt pesticides was developed in the mid-1980s when scientists introduced Bt cry genes into tobacco and tomato plants and expressed the proteins directly in plant tissues.

Despite these drawbacks the microbe formulations have great potential in integrated pest management (IPM) programs. They may be used to complement the effects of other biological control agents because of their environmental safety and pest selectivity (King and Coleman, 1989). The combination of microbial insecticides with entomophagous control is an effective strategy in IPM programs which is used widely in bio-organic agriculture (Navon, 1993). Wallam and Yendol (1976) reported satisfactory control of lepidopteran pests by integrating *B. thuringiensis* with a parasitoid. The suitability of combining Bt and other biological control agents, such as an insect parasitoid, for pest management of stored cereals have also been evaluated using laboratory assays (Oluwafemi et al., 2009). Since Bt do not prevent parasitoid development a combined treatment with Bt and parasitoid release could produce better protection against insect pest than either used singly. Thus combinations of pest antagonists can result in synergistic, additive, or inhibitory effects on target performance compared to the effect of each antagonist alone.

Bt toxins in environment*:*

Commercial *Bacillus thuringiensis* (Bt) products are microbial pest control agents (MPCAs) containing specific insecticidal crystalline proteins (ICPs) and often living spores as well as formulating agents. They are advantageous in terms of their safety, specificity and potency compared to chemical sprays, and are also biodegradable. Bt sprays persist for only a few days on the leaf surface because UV light, weather, the chemical environment of the leaf surface contribute to the degradation of Cry proteins. Also spores may get washed off the leaf surface into the soil in inclement weather.

Several studies have shown that Bt toxins bind to the soil, as are the toxins released from transgenic plants (Crecchio and Stotzky, 1997; Koskella and Stotzky, 1997; Palm et al., 1996; Saxena et al., 1999; Sims and Holden, 1996; Tapp and Stotzky, 1995a; Tapp and Stotzky, 1995b; Tapp and Stotzky, 1998; Tapp et al., 1994; West et al., 1984). Free toxins purifed from Dipel (66 kDa) (Dipel being a commercial formulation of Bt based on Bt var. *kurstaki*) were readily utilized as a carbon source by a mixed microbial culture of *Proteus vulgaris* and *Enterobacter aerogenes* (both Proteobacteria, Enterobacteriales), while soil bound toxins remained toxic after exposure to the microbes (Koskella and Stotzky, 1997). The toxicity of bound toxins has also been established in bioassays, where insects were exposed to free, adsorbed or bound toxins, which were diluted and distributed over the surface of a food medium (Crecchio and Stotzky, 1998; Koskella and Stotzky, 1997; Sims and Holden, 1996; Tapp and Stotzky, 1995a). Studies also show that bound toxins from Bt (66 kDa) (Bt var. *kurstaki*) purifed from Dipel remained toxic to *Manduca sexta* even after 234 days (Tapp and Stotzky, 1995a; Tapp and Stotzky, 1998; Koskella and Stotzky, 1997). Thus there seems a possibility of Bt toxins, even though biodegradable, entering other trophic levels.

Effects of Bt on different food web components*:*

Agro-ecosystems consist of organisms that interact in so called food webs (Dicke and Vet, 1999; Janssen et al., 1998; Mayse and Price, 1978; Poppy, 1997; Price, 1981; Price, 1997; Price et al., 1980; Weires and Chiang, 1973). Usually the food web extend beyond the limits of an agro-ecosystem and thus may incorporating broader life forms. δ-endotoxins have potent and specific insecticidal activity against species of insect larvae belonging to the orders *Coleoptera*, *Diptera* and *Lepidoptera*. Also, many species of arthropods are not phytophagous but carnivorous or saprophagous, and a number of them are important biological control agents. Non-target arthropods comprise non-target Lepidoptera, other non-target herbivorous pests, pollinators, and parasitoids and predators. Effects of different Bt sprays on target and non-target arthropods have been reviewed by Krieg and Langenbruch (1981), Flexner et al. (1986), and Glare and O'Callaghan (2000).

Effects on non-target Lepidopterans and others

Monarch larvae are affected by the Bt toxin Cry1A(b). In forests in the US and Canada, Bt sprays are used against the forest defoliator, the gypsy moth *Lymantria dispar*, and therefore non-target lepidopterans in these areas may be affected as well. When first and early second instar larvae of the three non-target Lepidoptera were placed on host trees that were or were not sprayed with Bt, significantly fewer caterpillars were alive on the Bt-treated trees after 5 days. Non-target lepidopterans that ingest but are not affected by the toxins may subsequently act as intermediates through which these toxins are passed on to the third trophic level, their predators and parasitoids.

Melin & Cozzi (1990) summarized a number of studies on the effects of various Bt subspecies on non-target terrestrial arthropod species and arthropod populations in the laboratory and field. Field studies of the effect of Bt on arthropodan order *Acarina* revealed no significant effects (Weires & Smith, 1977; Horsburgh & Cobb, 1981). Field and laboratory experiments on members of order *Coleoptera* showed no effect (Harding et al., 1972; Buckner et al., 1974; Johnson, 1974; Wallner & Surgeoner, 1974; Asquith, 1975; Wilkinson et al., 1975; Obadofin & Finlayson, 1977). Laboratory experiments with species of order *Dermaptera* gave similar results (Workman, 1977). Similar studies in *Diptera* showed no ill effects of Btk (Hamed, 1978–1979; Horn, 1983; Dunbar et al., 1972; Fusco,1980). Similar were the results in order *Heteroptera* in laboratory and field studies (Hamed, 1978–1979; Harding et al., 1972; Elsey, 1973; Jensen, 1974; Wallner &

Surgeoner, 1974). Laboratory studies on members of order *Neuroptera* failed to show any effect of Btk (Wilkinson et al., 1975; Hassan, 1983). Similar were the results with Mantis of *Dictyoptera* (Yousten, 1973).

As Bt toxins may persist in the soil for at least as long as 234 days (the longest period analysed using *Manduca sexta*; Tapp and Stotzky, 1998), soil organisms such as Collembola and Carabidae are exposed to the toxins for prolonged periods of time (WHO,1999).

Effects on Pollinators*:*

Pollinators like honey-bees are essential for most fruit and vegetable growers and plays a crucial role in ecosystem. Among order *Hymenoptera* Krieg (1973) observed mortality in adult honey-bees (*Apis mellifera*) that were fed non-sporulated broth cultures of Btk. The mortalities attributed to the thermolabile alpha-toxin would not present a problem in sporulated commercial Btk products since it is inactivated during sporulation. Thus no harmful effects were observed when Krieg et al. (1980) fed fully sporulated cultures of Btk to adult honey-bees.

Effects on Parasitoids and predators:

When the third trophic level is considered, primary parasitoids and predators may feed on one or several herbivorous insect species, depending on their degree of specialization. Parasitoids are usually specialists and thus will mostly parasitize only a few species belonging to one family (e.g. Hawkins, 1994). Effects of Bt on Hymenopteran parasitoids have also been investigated. Shorter life spans were reported in the field collected adult parasitoids (*Cardiochiles nigriceps*) fed on commercial Bt product (Dunbar & Johnson, 1975). Since the investigators could not be sure whether feeding actually took place, starvation may have been the cause of death. Hassan & Krieg (1975) observed no adverse effects on adult chalcid wasps (*Trichogramma cacoeciae*) that were fed suspensions of a commercial Bt product. Wallner & Surgeoner (1974) observed no effect on parasitoids following treatments with commercial Bt products for control of the notodontid moth (*Heterocampa manteo*). Wallner et al. (1983) reported an indirect effect on the braconid *Rogas lymantriae* when it parasitized gypsy moth (*Lymantria dispar*) hosts fed Bt. The sex ratio of the parasitoid offspring was skewed towards males in the treated larvae, as the female parasitoids lay more fertilized eggs in larger, untreated host larvae. Weseloh & Andreadis (1982) reported synergism in laboratory tests with gypsy moth larvae (*Lymantria dispar*) fed a commercial Btk product and exposed to the braconid (*Cotesia melanoscelus*). The percentage of parasitism was increased in Bt-intoxicated larvae since these grew more slowly and were at the approximate size suitable for parasitism for a longer time. Dunbar et al. (1972), Fusco (1980) and Wallner & Surgeoner (1974) reported an increase in the percentage of parasitism on pests when treated with a commercial Bt product.

Mantis of *Dictyoptera* showed no effect of Bt (Yousten, 1973). Effects on carnivorous arthropods may especially be expected when Bt toxins are used in crops against dipteran and coleopteran pests, because many Diptera and Coleoptera are important generalist predators. Spiders are also important generalist predators of a large number of insect species, although their role is not always recognized (Bogya, 1999; Mayse and Price, 1978; Riechert and Lockley, 1984; Schmaedick and Shelton, 2000). In determining effects of δ-endotoxins on carnivorous arthropods, the results of many of these studies are of limited use, as β-exotoxins, found to be toxic to many species including mammals, were present in many of the Bt products that were used until the late 1980s (Glare and O'Callaghan, 2000). Bt sprays are now required to be free from β-exotoxins in most European countries, the US and Canada (Glare and O'Callaghan, 2000). In conclusion, positive effects of Bt toxins on the performance of parasitoids and predators were found when these toxins caused prolonged development in target hosts or prey.

Non-target arthropods of which many of them beneficial may come into contact with Bt toxins either through feeding on target or non-target herbivorous insects, or via the environment, i.e.

the soil where Bt and its toxins persist and do not lose their toxicity after plant parts or insects have died. Carnivorous arthropods may also ingest the Bt toxins indirectly when they feed on herbivorous insects that have ingested the toxins. In target insect herbivores, the toxins are bound to receptors in the midgut epithelium, after which they are structurally rearranged (De Maagd et al., 1999; De Maagd et al., 2001; Gazit et al., 1998; Masson et al., 1999) and thus most likely lose their toxicity to natural enemies. In non-target herbivores, however, the toxins do not bind to midgut receptor cells or do not cause pore formation. They may or may not be digested by proteolytic enzymes in the digestive tract, so that the toxins may remain active and subsequently affect entomophagous natural enemies. Thus it is seen that natural enemies may not come into contact with Bt toxins via target herbivores, but via nontarget herbivores (WHO,1999).

***Effects on other invertebrates**:*

Impact of Bt has been examined on various aquatic invertebrates, which included bivalve molluscs (oyster larvae, *Crassostrea gigas, Ostrea edulis*), copepods, decapods, flatworms, isopods, gastropods and ostracods. Of these organisms, only a few demonstrated any adverse effects. The toxins from Bt retain their toxicity when bound to the soil, so accumulation of these toxins is likely to occur. Benz & Altwegg (1975) studied the impact of Bt treatment at 100 times the recommended rate on populations of the earthworm *Lumbricus terrestris* and found no effect. However, earthworms, though unaffected on ingesting the bound toxins, do ingest Bt toxin bound to the soil. Saxena and Stotzky (2001) detected Cry1A(b) in the guts and casts of earthworms. Hence, similar to non-target herbivores, these earthworms may serve as intermediates through which Bt toxins may be passed on to organisms feeding on these earthworms.

***Effects on vertebrates**:*

Various studies have been done to study the impact of Bt on aquatic invertebrates such as frogs (*Hyla regilla*, *Rana temporaria*), goldfish (*Carassius auratus*), mosquito fish (*Gambusia affinis*), newts (*Taricha torosa*, *Triturus vulgaris*), rainwater killifish (*Lucania parva*) and toads (*Bufo* species). No adverse effects were reported. A number of studies, with birds such as young bobwhite quail (*Colinus virginianus*) and young mallards (*Anas platyrhynchus*), were done to test the acute toxicity and pathogenicity of commercial Bt formulations for (Beavers et al., 1989a,b; Lattin et al., 1990a,b,c,d; Beavers, 1991a,b). The Bt-treated birds showed no apparent toxicity or pathogenicity. In Canada, Innes & Bendell (1989) studied the effect of a commercial Btk formulation on small mammal populations in woodland. Populations of eight species of rodents and four species of shrew were studied by trapping over a 3-month period and shown to be unaffected when compared to populations from untreated areas. This suggests that the ingestion of infected insects by shrews had no immediate effects on their populations. Mammalian toxicity studies on Bt-containing pesticides demonstrate that the tested isolates are not toxic or pathogenic (McClintock et al., 1995), as they occur in the products. In studies conducted with a single oral dose of laboratory grown Bt and commercial Bt formulations, there was no mortality associated with ingestion of Bti or Btk in mice and rats (Fisher & Rosner, 1959; de Barjac et al., 1980; Shadduck, 1980; Siegel et al., 1987). Similar experiments for inhalation exposure showed no effect (De Barjac et al., 1980; Siegel et al., 1987; Fisher & Rosner, 1959) (WHO,1999).

DISCUSSION

As members of the *Bacillus cereus* group Bt can be found in most ecological niches. Insecticides formulated with Bt are being manufactured and used worldwide being applied as an insecticide to foliage, soil, water environments and food storage facilities. After application of Bt to an ecosystem, the organism may persist as a component of the natural microflora. Their specific mode of action makes them safe to use in an ecosystem without bothering much of its effects on non-target organisms. However, Bt toxins are structurally rearranged when they bind to midgut receptors, so that they are likely to lose their toxicity inside target herbivores. What happens to the toxins in non-target herbivores, and whether these herbivores may act as intermediaries through which the toxins may be passed on to the natural enemies, remains to be studied. Understanding its role in the ecosystem is crucial in deriving the best out of this great biocontrol agent (Groot & Dicke, 2002).

As components of food web arthropods have important roles to play. Many of them are phytophagous, pollinators, parasitoids and predators. In the context of biopesticide usage in biological pest control, natural enemies of insect pests, such as parasitoids, have received increasing attention, because they, along with other carnivorous arthropods, can act synergistically and thereby, are an important component of insect pest control. Sustainable pest management will only be possible when negative effects on non-target, beneficial arthropods are minimized. Below-ground organisms such as Collembola, nematodes and earthworms should also be included in risk assessment studies, but have received little attention. So far, most studies have concentrated on natural enemies of target herbivores (Groot & Dicke, 2002).

The range of non-target species of insects that have been found to be susceptible to direct toxic action of Bt has remained small. In more than 30 years of commercial use, no serious, direct effects on non-target insects have been reported as arising from Bt based microbial pest control agents. Commercial applications of Bt have been directed mainly against lepidopteran pests of agricultural and forest crops; however, in recent years strains active against coleopteran pests have also been marketed. Strains of Bt var *israelensis* active against dipteran vectors of parasitic disease organisms have been used in public health programmes. Owing to their specific mode of action, Bt products are unlikely to pose any hazard to humans or other vertebrates or to the great majority of nontarget invertebrates provided that they are free from non-Bt microorganisms and biologically active products other than the ICPs. Bt products may be safely used for the control of insect pests of agricultural and horticultural crops as well as forests. They are also safe for use in aquatic environments including drinking-water reservoirs for the control of mosquito, black fly and nuisance insect larvae (WHO, 1999).

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