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Research Article

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The Effect of *Bacillus thuringiensis* and Bt Transgenics on Parasitoids during Biological Control

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ABSTRACT

Bacillus thuringiensis (Bt) has emerged as the major ecofriendly biopesticide and a key source of genes for transgenic expression of δ-endotoxins to provide pest resistance in plants and microorganisms, the so-called genetically modified organisms (GMOs). As components of food web, parasitoids are non-target arthropods that coming into contact with Bt toxins directly via the environment or indirectly through target or non-target herbivorous arthropods which act as intermediates through which Bt toxins can passed on to the third trophic level i.e. their predators and parasitoids. Parasitoids, being important natural enemies of pest lepidopteran larvae, has been investigated for the effects of commercial Bt formulations and GMOs, such as Bt plants. The ecological safety of Bt formulations and transgenically expressed δ-endotoxins and their effect on the interaction of crop pests with their natural enemies are critically important for regulation of pest populations. Studies done so far highlight the advantages and superiority of Bt over other pest control methods. This review highlights the investigations on the interaction of Bt toxins and non-target beneficial insects in the food web and a possible combined pest control approach..

Key words: *Bacillus thuringiensis, genetically modified organisms (GMOs), Parasitoids*

INTRODUCTION

Developing suitable methods of pest control in accordance of philosophy and methodology of modern integrated pest management (IPM) programme is a daunting task in an increasingly environmentally conscious world of ours[43]. The use of microorganisms has assumed a prominent position among the options that seek to control insect pests without the use of chemicals and with high specific toxicity applied in agroecosystems[67]. For the biological control of insect pests, *Bacillus thuringiensis* (Bt) has emerged as the oldest[15] and one of the most widely used entomopathogenic microorganism among many[61].

Bt is a member of the Bc (*Bacillus cereus)* group of Gram positive, spore-forming soil bacteria. During the sporulation process, it produces one or more characteristic crystalline proteinaceous inclusions adjacent to the endospore, which have been found to be toxic for invertebrates, primarily insect species in the orders *Coleoptera*, *Diptera* and *Lepidoptera*, distinguishing it from *Bacillus cereus*[14,2]. These parasporal inclusions are formed by different insecticidal crystal proteins (ICP) or δ-endotoxins. Though, the existence of parasporal inclusions in Bt was first noted in 1915[6], their protein composition was not delineated until the 1950s[3]. These δ-endotoxins, encoded by the C*ry* and C*yt* genes, have molecular weights between 14-160 kDa and can be visualized under light microscopy as inclusion bodies[65]. Bt subspecies can synthesize more than one inclusion, which may contain different ICPs. These crystals have variously shaped depending on their ICP composition. A partial correlation between crystal morphology, ICP composition, and bioactivity against target insects has been established[11,29]. Bt has attained a wide commercial use against major Lepidopteran pests and has emerged as the most successful microbial pesticide having great potential in IPM programmes[9]. This is the leading biopesticide used in commercial agriculture, forest management and mosquito control.

Bacillus thuringiensis is also a key source of genes for transgenic expressions to provide pest resistance in plants[44,45]. Bt crops offer great promise in controlling lepidopteran pests. A decrease in synthetic insecticide use in Bt transgenic crops could increase beneficial arthropod diversity and abundance. Among the spray formulations, Bt var. kurstaki (Btk) HD-1-based products are widely used in many crop ecosystems against over 100 insect species worldwide including[39].

Once ingested by the target larva, the parasporal crystalline ICP is dissociated to the protoxin form in the midgut, and the protoxin is then activated to a biologically active holotoxin by the proteolytic enzymes and specifically the alkaline environment of the gut[77,30,4]. Shortly afterwards, the gut becomes paralysed and the larva ceases to feed. Pore or ion channel formation occurs after the binding of the toxin to the receptor and the subsequent failure of trans-membrane electric potential. This results in colloid-osmotic lysis of the cells[36], which causes vegetative cells of Bt and the pre-existing microorganisms in the gut to proliferate in the haemocoel causing septicaemia, and may thus contribute to the mortality of the insect larva.

The Bt toxins in Formulations and those expressed by transgenic plants that are commercially grown have a narrow range of activity, and no direct negative effects have been reported on natural enemies belonging to other orders than the one targeted by a specific Bt toxin[59]. Microbial Bt formulations applied orally or to the host are generally non-toxic against parasitoids, because most hymenopterans lack receptors in their midgut necessary for binding of Cry toxins. However, some laboratory studies using Bt sprays have reported adverse effects[24].

GLOBAL STATUS OF BT INSECTICIDES AND Bt CROPS

The first *Bt* microbial product registration in U.S. was in 1961 and by 1998, there were approximately 180 products registered in the U.S. Environmental Protection Agency[19,20]. Hammond and Koch[26] have reported at least 120 microbial products in the European Union and Huang et al.[31] approximately 276 registered *Bt* microbial formulations in China. The success and extensive use of *Bt* microbial pesticides worldwide can be attributed to their high specificity against target insect species while greatly limiting the negative impacts to beneficial and non-target organisms, and lack of environmental persistence of Bt toxins[80,51,21,8].

Transgenic cotton was first commercially cultivated in 1996. Since then the increase of transgenic crop cultivation has seen a remarkable 100 fold increase from a mere 1.7 million hectares in 1996. In 2014, the area under Bt crops had increased to 181.5 million hectares with a sustained growth of 3% to 4% (6.3 million ha) annually[33]. These Bt transgenic crops (Bt corn and Bt cotton) have been overwhelmingly successful and beneficial, leading to higher yields and reducing the use of traditional synthetic insecticides pesticides and fossil fuels. Recent reports indicate the success of Bt crops which has resulted in economic benefits to growers and reduced the use of conventional insecticides[10]. In 2009, China made a landmark decision that approved the safety of two Bt-transgenic rice cultivars[32]. Bt corn and Bt cotton, along with Bt soyabean and Bt canola, have been adopted by farmers in 28 countries to control lepidopteran pests such as corn borers (mainly *Ostrinia nubilalis*) in corn and the budworm-bollworm complex (*Heliothis virescens*, *Helicoverpa* spp., *Pectinophora gossypiella*) in cotton[68,33].

Bt TOXINS IN FOOD WEB

Agro-ecosystems consist of organisms, occupying various trophic levels, that are nutritively interdependent and interact in so called food webs[16,34,53,54,55.] Being an open system, the food web extends beyond the limits of an agro-ecosystem and thus may incorporating broader life forms in and around it. Commercial *Bacillus thuringiensis* (Bt) products contain specific insecticidal crystalline proteins (ICPs) and often living spores as well as formulating agents. Though advantageous in terms of their safety, biodegradablility, specificity and potency compared to chemical sprays, when applied to foliage, Bt sprays persist for only a few days because UV light, weather, the chemical environment of the leaf surface contribute to the degradation of Cry proteins. Inclement weather also cause the spores to get washed off the leaf surface into the soil[62,80]. Bt proteins will be incorporated into soil with plant tissue postharvest, with sloughing of root cells, and potentially through the release of exudates from roots. The soil fate of the Bt protein is a key parameter governing exposure of nontarget organisms in the environment. However, conflicting results have been found in assessing Bt protein persistence with varying estimates[12]. Persistence in the environment can be expressed in different ways such as DT_{50} and half-life are used to describe the time until the amount of a substance remaining is 50% of the original amount. Persistence can also be discussed in terms of detectable residues and bioactivity. In addition to differences in expressing persistence, differences in dissipation/persistence of Bt proteins in soil can depend on the soil type, environmental conditions, the protein source (purified versus plant-produced), and the particular Cry protein examined[12].

Studies have indicated that Bt toxins bind to the soil, as are the toxins released from transgenic plants[13,37,52,63,69,71,72,73,79]. Soil bound Bt toxins has found to remain toxic even after exposure to the microbes[37]. 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[37,69,72,13]. Studies also show that bound toxins from Bt var. *kurstaki* purifed from Dipel remained toxic to *Manduca sexta* even after 234 days[37,71]. Thus there seems a possibility of Bt toxins, even though biodegradable, entering other trophic levels.

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[38], Flexner et al.[22], and Glare and O'Callaghan[24]. Although, relationship between Bt and the parasites of some insect pests have been investigated, its effect on other trophic level, such as parasitoids of target insects, are still poorly understood[50,5].

EFFECT OF Bt TOXINS ON PARASITOIDS

Parasitoids, along with predators, feeding on herbivore insect species usually comprise the third trophic level and act as important biological control agents of insect pest population. Bt is considered to have less of an impact on natural enemies, like parasitoids, of pests[41,58,57,1]. Moreover, Bt may be used to complement the effects of other biological control agents because of their environmental safety and pest selectivity[35]. The combination of microbial insecticides with entomophagous control is an effective strategy in IPM programs which is used widely in bio-organic agriculture[49]. Wallam & Yendol[74] reported satisfactory control of lepidopteran pests by integrating *B. thuringiensis* with a parasitoid. Effect of Bt on other trophic level, such as parasitoid of target insects, are still poorly understood[50,5]. Experiments investigating the effects of conventional Bt sprays on natural enemies of pest insects have shown a range of effects from synergism, repellency, toxicity to no effect[46,64]. This highlights the importance of research in this direction.

Primary parasitoids 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[28]. Although many field studies have shown negligible impact on non-target organisms in comparison with conventional insecticides[42,48,59], negative effects of Bt toxins on parasitoids have been reported with Bt formulations and Bt plants both in laboratory[56,7] and field studies[40]. Effects of Bt toxins from Bt formulations on Hymenopteran parasitoids have also been investigated. Dunbar & Johnson[18] reported shorter life spans in the field collected adult parasitoids (*Cardiochiles nigriceps*) fed on commercial Bt product. Since the investigators could not be sure whether feeding actually took place, starvation may have been the cause of death[80]. No adverse effects were observed on adult chalcid wasps (*Trichogramma cacoeciae*) that were fed suspensions of a commercial Bt product[27]. Similarly, Wallner & Surgeoner[75] observed no effect on parasitoids following treatments with commercial Bt products for control of the notodontid moth (*Heterocampa manteo*). Wallner et al.[76] 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 found to be skewed towards males in the treated larvae, as the female parasitoids lay more fertilized eggs in larger, untreated host larvae. Weseloh & Andreadis[78] reported synergism in laboratory tests with gypsy moth larvae (*Lymantria dispar*) fed a commercial Btk product and exposed to the braconid (*Cotesia melanoscelus*). Dunbar et al.[17], Fusco[23] and Wallner & Surgeoner[75] reported an increase in the percentage of parasitism on pests when treated with a commercial Bt product. 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[80].

One route of Bt exposure to nontarget organisms, like parasitoids, is predation upon herbivores consuming transgenic plant material. A limited number of studies have examined the effects of Bt proteins on parasitoids that utilize herbivorous hosts feeding on transgenic plants. Reduced emergence and development of the parasitoid wasp (*Zele chlorophthalmus*) reared on Bt-fed *S. littoralis* was observed by Salama and Zaki. This is not entirely unexpected, as *S. littoralis* is somewhat susceptible to Bt protein, and the parasite may not have been able to develop in the intoxicated host due to its reduced fitness. In another study, effect on diamondback moth parasitoid (*Cotesia plutellae*) was investigated in Bt-resistant and –susceptible host diamondback moths (*Plutella xylostella*) that were fed Bt oilseed rape (*Brassica napus*)[66]. The developmental process of the parasitoid was significantly impaired when reared on susceptible *P. xylostella* that had consumed Bt rape. The choice tests demonstrated that the parasitoids were less likely to choose the susceptible larvae. This effect was probably due to the reduction in herbivore-induced volatile compounds, used by the parasitoid females to locate hosts, in the treatments in which *P. xylostella* was intoxicated by the Bt protein[12].

Sublethal effects of Bt formulations found to affect growth and development of parasitoids could be attributed to the reduced host–prey size, poor nutritional quality and toxin per se contained in the host–prey itself. Thus, the Bt intoxicated host larvae are small in size with poor nutritional quality to support the proper growth and development of parasitoids[59]. Some of the sublethal effects of Bt on natural enemies include prolonged development, reduced weight and altered behaviour. A study on the predator green lacewing (*Chrysoperla carnea*) is a typical example of indirect effects due to a reduction of the quality of the prey[46]

Studies with Bt plants indicated no direct effect on insect predators and parasitoids. Adverse effects on parasitoids are most likely due to reduced host quality and quantity[59]. Bt intoxicated host larvae are small in size with poor nutritional quality to support the proper growth and development of parasitoids. Yang et al.[81] reported that population density of the parasitoids *Trichogramma confusum*, *Campoletis chlorideae* and *Meteorus pulchriconis* were significantly lower in the transgenic cotton fields than in the non-transgenic conventional cotton fields.

The lower parasitoid population in transgenic fields was probably due to the reduced density of the host insect *Helicoverpa armigera*. It is not surprising that all insect control measures including Bt transgenic crops are aimed at reducing insect pest population and thereby inevitably decrease the availability of hosts for specialist natural enemies like parasitoids[46].

DISCUSSION

Bt formulations and Bt crops is far better suited for an uninterrupted food web function in an agro-ecosystem and should be suitably promoted to reduce negative impact on natural enemies. On the contrary, the applications of broad-spectrum synthetic insecticides cause not only parasitoid mortality indirectly through premature host death, but also have direct contact toxicity against adult parasitoids[47,70]. Their introduction and proliferation in agro-ecosystems present a unique challenge in determining or predicting the environmental fate and effects of pesticides or other products incorporated in these crops and formulations. Their widespread adoption represents a shift in how insect control is conducted and should be investigated with the ecology of the agroecosystem in mind. It is evident from studies that the advantages of using Bt formulations and Bt crops far outweighs its subtle negative impacts on the food web. 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 and an understanding of ecological factor in context can result in synergistic, additive, or inhibitory effects on target performance compared to the effect of each antagonist alone.

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[43]. 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[25].

Finally, for a sustainable pest management in the spirit and philosophy of IPM, it is imperative for future studies to develop, compare and scrutinize Bt biopesticides and transgenics in the light of the food web dynamics. They have generally been found to be more protective of beneficial insects and secondary pests. It is therefore important for the studies, aiming at assessing risk, to be placed within the context of the agro-ecosystem, during biological control of insect pests.

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REFERENCES

1. Abedi, Z., Lethal and sub-lethal effects of azadiactin, pyridalil, cypermethrin and methoxyfenozide on biological parameters of Habrobracon hebetor Say (Hym.: Braconidae)., M.Sc. thesis, University of Maragheh, Iran, 104 (2012).
2. Andrews, R.E., Jr. Faust, R.M., Wabiko, H., Raymond, K.C. and Bulla, L.A. Jr., The biotechnology of *Bacillus thuringiensis*, CRC Crit Rev Biotechnol., **6**: 163–232 (1987).
3. Angus, T.A., A bacterial toxin paralysing silkworm larvae, Nature (Lond), **173**: 545–546 (1954).
4. Aronson, A.I., Han, E.S., McGaughey, W. and Johnson, D., The solubility of inclusion proteins from *Bacillus thuringiensis* is dependent upon protoxin composition and is a factor in toxicity to insects, Appl Environ Microbiol, **57**: 981–986 (1991).

5. Atwood, D.W., Young III, S.Y. and Kring, T.J., Development of *Cotesia marginiventris* (Hymenoptera: Braconidae) in tobacco budworm (Lepidoptera: Noctuidae) larvae treated with *Bacillus thuringiensis* and Thiodicarb, *Journal of Economic Entomology*, **90**: 751–756 (1997).
6. Berliner, E., About the sleep sickness of the *Ephestia kühniella Zell*. and its vector *Bacillus thuringiensis*, Z Angew Entomol, **2:** 29–56 (1915). (in German)
7. Bernal, J.S., Griset, J.G. and Gillogly, P.O., Impacts of developing on Bt maize-intoxicated hosts on fitness parameters of a stem borer parasitoid, *J Entomol Sci*, **37**: 27–40 (2002).
8. Betz, F.S., Hammond, B.G. and Fuchs, R.L., Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests, Regul Toxicol Pharmacol, **32**: 156–177 (2000).
9. Blumberg, D., Navon, A., Keren, S., Goldenberg, S. and Ferkovich, S.M., Interactions among *Helicoverpa armigera* (Lepidoptera: Noctuidae), its larval endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae), and Bacillus thuringiensis, *Journal of Economic Entomology*, **90(5)**: 1181-1186 (1997).
10. Brookes, G., Barfoot, P., Global impact of biotech cops: Socio-economic and environmental effects in the first ten years of commercial use, Ag Bio Forum, **9**: 139–151 (2006).
11. Bulla, L.A., Jr. Kramer, K.J. and Davidson, L.I., Characterization of the entomocidal parasporal crystal of *Bacillus thuringiensis*, *J Bacteriol*, **130**: 375–383 (1977).
12. Clark, B.W., Phillips, T.A. and Coats, J.R., Environmental fate and effects of Bacillus thuringiensis (Bt) proteins from transgenic crops: a review, *Journal of agricultural and food chemistry*, **53(12)**: 4643-4653 (2005).
13. Crecchio, C. and Stotzky, G., Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subspecies *kurstaki* bound to humic acids from soil, Soil Biol Biochem, **30**: 463-470 (1998).
14. de Barjac, H., Insect pathogens in the genus *Bacillus*. In: Berkley RCW & Goodfellow M ed. The aerobic endospore-forming bacteria: Classification and identification. New York, London, Academic Press Inc., 241–250 (1981).
15. de Maagd, R.A., *Bacillus thuringiensis*-Based Products for Insect Pest Control. In Principles of Plant-Microbe Interactions, Springer International Publishing, 185-192 (2015).
16. Dicke, M. and Vet, L.E.M., Plant-carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. In: Herbivores: Between Plants and Predators (Olff, H., Brown, V.K. and Drent, R.H., eds), Oxford: Blackwell Science, 483–520 (1999).
17. Dunbar, D.M., Kaya, H.K., Doane, C.C., Anderson, J.F. and Weseloh, R.M., Aerial application of *Bacillus thuringiensis* against larvae of the elm spanworm and gypsy moth and effects on parasitoids of the gypsy moth, Connecticut Experiment Station, (Bulletin No. 735). (1972).
18. Dunbar, J.P. and Johnson, A.W., *Bacillus thuringiensis*: Effects on the survival of a tobacco budworm parasitoid and predator in the laboratory, Environ Entomol, **4**: 352–354 (1975).
19. EPA R.E.D FACTS: *Bacillus thuringiensis*. EPA-738-F-98-001. Washington, DC: United States Environmental Protection Agency. (1998a).
20. EPA. . Reregistration Eligibility Decision (RED): *Bacillus thuringiensis*. EPA738-R-98-004 (1998b).
21. Federici, B.A. and Siegel, J.P., Safety assessment of *Bacillus thuringiensis* and Bt crops used in insect control, Food Safety of Proteins in Agricultural Biotechnology, ed. B. G. Hammond (Boca Raton, FL: CRC Press) (2008).
22. Flexner, J.L., Lighthart, B. and Croft, B.A., The effects of microbial pesticides on non-target, beneficial arthropods, Agric. Ecosyst. Environ, **16**: 203–254 (1986).
23. Fusco, R.A., Field evaluation of a commercial preparation of *Bacillus thuringiensis*, DIPEL 4L: Progress report. Pennsylvania Bureau of Forestry, Gypsy Moth Pest Management Methods Development Project (1980).

24. Glare, T.R. and O'Callaghan, M., *Bacillus thuringiensis*: biology, ecology and safety. Wiley, Chichester, (2000).
25. Groot, A.T. and Dicke, M., Insect-resistant transgenic plants in a multi-trophic context, The Plant Journal, **31**:387–406 (2002).
26. Hammond, B.G. and Koch, M.S., A review of the food safety of Bt crops. Bacillus thuringiensis Biotechnology, ed. E. Sansinenea (New York: Springer Science+Business Media B.V.), 305–325 (2012).
27. Hassan, S. and Krieg, A., *Bacillus thuringiensis* preparations harmless to the parasite *Trichogramma cacoeciae* (Hym.: Trichogrammatidae). Z Pflanzenkr Pflanzenchutz, **82**: 515–521 (1975). (in German)
28. Hawkins, B.A., Pattern and Process in Host-parasitoid Interactions. Cambridge University Press, Cambridge, UK. (1994).
29. Höfte, H. and Whiteley, H.R., Insecticidal crystal proteins of *Bacillus thuringiensis*, Microbiol Rev, **53**: 242–255 (1989).
30. Honée, G. and Visser, B., The mode of action of *Bacillus thuringiensis* crystal proteins, Entomol Exp Appl, **69**: 145–155 (1993).
31. Huang, D.F., Zhang, J., Song, F.P., and Lang, Z.H., Microbial control and biotechnology research on *Bacillus thuringiensis* in China, J Invertebr Pathol, **95**:175–180 (2007).
32. James, C., Executive Summary of Global Status of Commercialized Biotech/GM Crops: ISAAA Briefs No. 34, Ithaca, New York, USA (2009).
33. James, C., Global Status of Commercialized Biotech/GM Crops: 2014. ISAAA Brief No. 49. ISAAA: Ithaca, NY. (2014).
34. Janssen, A., Pallini, A., Venzon, M. and Sabelis, M.W., Behaviour and indirect interactions in food webs of plant inhabiting arthropods, Exp Appl Acarol, **22**: 497–521 (1998)
35. King, E.G. and Coleman R.J., Potential for biological control of *Heliothis* species, Annu Rev Entomol, **34**: 53–75 (1989).
36. Knowles, B.H. and Ellar, D.J., Colloid-osmotic lysis is a general feature of the mechanisms of action of *Bacillus thuringiensis* δ-endotoxins with different insect specificity, Biochim Biophys Acta, **924**: 509–518 (1987).
37. Koskella, J. and Stotzky, G., Microbial utilization of free and clay-bound insecticidal toxins from Bacillus thuringiensis and their retention of insecticidal activity after incubation with microbes, Appl Environ Microbiol, **63**: 3561–3568 (1997).
38. Krieg, A. and Langenbruch, G.A., Susceptibility of arthropod species to Bacillus thuringiensis. In: Microbial Control of Pests and Diseases 1970–80 (Burges, H.D., ed.). London: Academic Press, 837–896 (1981).
39. Lee, H.K., Cheng, H., Gill, S.S., Microbial control of insects. In: Dhaliwal GS, Heinrichs EA (eds) Critical issues in insect pest management. Commonwealth Publishers, New Delhi, 87–117 (1998).
40. Liu, Y.F., He, L., Wang, Q., Hu, S.Q., Liu, W.H., Chen, K.G. and You, M.S., Evaluation of the effects of insect-resistant *cry1Ac/sck* transgenic rice on the parasitoid communities in paddy fields, Acta Entomol Sin, **49**: 955–962 (2006).
41. Mahdavi, V., Residual toxicity of some pesticides on the larval ectoparasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae), Journal of Plant Protection Research, **53(1)**: 27-31 (2013).
42. Marvier, M., McCreedy, C., Regetz, J. and Kareiva, P., A meta-analysis of effects of Bt cotton and maize on non-target invertebrates, Science, **316**: 1475–1477 (2007).
43. Mathew, I.L., Singh, D., Singh, R.P. and Tripathi, C.P.M., *Bacillus thuringiensis*: The biocontrol agent in a food web perspective, Biolife, **2(1)**: 348-362 (2014).

44. McGaughey, W.H. and Whalon, M.E., Managing insect resistance to *Bacillus thuringiensis* toxins, Science, Wash, **258**: 1451-1455 (1992).
45. Meadows, M.P., *Bacillus thuringiensis* in the environment: Ecology and risk assessment. *Bacillus thuringiensis* an Environmental Biopesticide: Theory and Practice (eds. P.F. Entwistle, J.S. Cory, M.J. Bailey and S. Higgs), John Wiley and Sons, New York. 193–220 (1993).
46. Mohan, M., Sushil, S.N., Bhatt, J.C., Gujar, G.T. and Gupta, H.S., Synergistic interaction between sublethal doses of *Bacillus thuringiensis* and *Campoletis chlorideae* in managing *Helicoverpa armigera*, BioControl, **53(2)**: 375-386 (2008).
47. Musser, F.R., Shelton, A.M., *Bt* sweet corn and selective insecticides: impact on pests and predators, J Econ Entomol., **96**:71–80 (2003).
48. Naranjo, S.E., Head, G. and Dively, G.P., Field studies assessing arthropod non-target effects in *Bt* transgenic crops: Introduction, Environ Entomol, **34**: 1178–1180 (2005).
49. Navon, A., Control of lepidopteran pests with *Bacillus thuringiensis.* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds.], *Bacillus thuringiensis,* an environmental biopesticide: theory and practice. Wiley, New York, 125-146 (1993).
50. Nealis, V. and van Frankenhuyzen, K., Interactions between *Bacillus thuringiensis* Berliner and *Apanteles fumiferanae* Vier. (Hymenoptera: Braconidae), a parasitoid of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), Canadian Entomologist, **122**: 585–594 (1990).
51. OECD. Consensus document on safety information on transgenic plants expressing Bacillus thuringiensis–derived insect control proteins. ENV/JM/MONO(2007)14. Proceedings of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Paris. (2007).
52. Palm, C.J., Schaller, D.L., Donegan, K.K. and Seidler, R.J., Persistence in soil of transgenic plant produced *Bacillus thuringiensis* var. *kurstaki* δ-endotoxin, *Can J Microbiol,* **42**: 1258-1262 (1996).
53. Poppy, G.M., Tritrophic interactions: improving ecological understanding and biological control, Endeavour, **21**: 61-65 (1997).
54. Price, P.W., Insect Ecology. New York. Academic Press, (1997).
55. Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. and Weis, A. E., Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies, Annual Review of Ecology and systematics, 41-65 (1980).
56. Prütz, G. and Dettner, K., Effect of Bt corn leaf suspension on food consumption by *Chilo partellus* and life history parameters of its parasitoid *Cotesia flavipes* under laboratory conditions, Entomol Exp Appl, **111**: 179–186 (2004).
57. Rafiee-Dastjerdi, H., Hejazi, M.J., Nouri-Ganbalani, G.H. and Saber, M., Effects of Some Insecticides on Functional Response of Ectoparasitoid, *Habrobracon hebetor* (Say) (Hym.: Braconidae), *J Entomol,* **6(3)**: 161–166 (2009).
58. Rasool, Khan, R.., Ashfaq, M. and Rana, S.A., Some studies on the toxicity of conventional and new chemistry insecticides against *Bracon hebetor* Say (Hym.: Braconidae) under laboratory conditions, Pak Entomol, **27(1)**: 19–21 (2005).
59. Romeis, J., Meissle, M., Bigler, F., Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control, Nat Biotechnol, **24**: 63–71 (2006).
60. Salama, H.S. and Zaki, F.N., Interaction between *Bacillus thuringiensis* Berliner and the parasites and predators of *Spodoptera littoralis* in Egypt, Z Angew Entomol, **95**: 425-429 (1983).
61. Samsonov, P., Padrón, R.I., Pardo, C., Cabrera, J. and De al Riva, G.A., *Bacillus thuringiensis* from biodiversity to biotechnology, *J. Ind. Microbiol. Biotech,* **19**: 202-219 (1997).
62. Sanahuja, G., Banakar, R., Twyman, R., Capell, T. and Christou, P., *Bacillus thuringiensis*: a century of research, development and commercial applications, *Plant Biotech J,* **9**: 283–300 (2011).

63. Saxena, D., Flores, S. and Stotzky, G., Insecticidal toxin in root exudates from *Bacillus thuringiensis* corn, Nature, 402–480 (1999).
64. Sayyed, A.H. and Wright, D.J., Genetic diversity of Bt resistance: implications for resistance management, *Pakistan J Biol Sci*, **5**: 1330-1344 (2002).
65. Schnepf, E., Crickmore, N., van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean, D.H., *Bacillus thuringiensis* and its pesticidal crystal proteins, Microbiol Mol Biol Rev, **62**: 775–806 (1998).
66. Schuler, T.H., Potting, P.J., Denholm, I. and Poppy, M.G., Parasitoid behavior and Bt plants, Nature, **400**: 825-826 (1999).
67. Schünemann, Rogério, Neiva Knaak, and Lidia Mariana Fiuza. Mode of action and specificity of *Bacillus thuringiensis* toxins in the control of caterpillars and stink bugs in soybean culture, ISRN microbiology, (2014).
68. Shelton, A.M., Zhao, J.Z. and Roush, R.T., Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants, Annu Rev Entomol, **47**: 845–881 (2002).
69. Sims, S.R. and Holden, L.R., Insect bioassay for determining soil degradation of *Bacillus thuringiensis* subsp. Kurstaki CryIA(b) protein in corn tissue, Environ Entomol, **25**: 659–664 (1996).
70. Singh, S.P., Ballal, C.R. and Poorani, J., Old world bollworm *Helicoverpa armigera*, associated Heliothinae and their natural enemies. Project Directorate of Biological control, Bangalore, India Tech Bull, **31**: 135 (2002).
71. Tapp, H. and Stotzky, G., Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil, Soil Biochem, **30**: 471–476 (1998).
72. Tapp, H. and Stotzky, G., Insecticidal activity of the toxins from *Bacillus thuringiensis* subsp. *kurstaki* and tenebrionis adsorbed and bound on pure and soil clays, *Appl Environ Microbiol*, **61**: 1786–1790 (1995).
73. Tapp, H., Calamai, L. and Stotzky, G., Adsorption and binding of the insecticidal proteins from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *tenebrionis* on clay minerals, Soil Biol Biochem, **26**: 663–679 (1994).
74. Wallam, J.D. and Yendol, W.G., Evaluation of *Bacillus thuringiensis* and a parasitoid for suppression of the gypsy moth, J Econ Entomol, **69**:113-118 (1976).
75. Wallner, W. and Surgeoner, G., Control of oakleaf caterpillar, *Heterocampa manteo*, and the impact of controls on nontarget organisms. Chicago, Illinois, Abbott Laboratories (Unpublished document) (1974).
76. Wallner, W.E.. Dubois, N.R. and Grinberg, P.S., Alteration of parasitism by *Rogas lymantriae* (Hymenoptera: Braconidae) in *Bacillus thuringiensis*-stressed gypsy moth (Lepidoptera: Lymantriidae) hosts, *J Econ Entomol*, **76**: 275–277 (1983).
77. Warren, R.E., Rubenstein, D., Ellar, D.J., Kramer, J.M. and Gilbert, R.J., *Bacillus thuringiensis* var.*israelensis*: Protoxin activation and safety, Lancet, **24**: 678–679 (1984).
78. Weseloh, R.M. and Andreadis, T.G., Possible mechanism for synergism between *Bacillus thuringiensis* and the gypsy moth (Lepidoptera: Lymantriidae) parasitoid, *Apanteles melanoscelus* (Hymenoptera: Braconidae), Ann Entomol Soc Am, **75**: 435–438 (1982).
79. West, A.W., Burges, H.D., White, R.J. and Wyborn, C.H., Persistence of *Bacillus thuringiensis* parasporal crystal insecticidal activity in soil, *J Invert Pathol*, **44**: 128–133 (1984).
80. WHO/IPCS. Microbial Pest Control Agent: *Bacillus thuringiensis*. Geneva: Environmental Health Criteria 217. (1999).
81. Yang, Y.Z., Yu, Y.S., Ren, L., Shao, Y.D., Qian, K. and Myron, P.Z., Possible in compatibility between transgenic cottons and parasitoids, *Aust J Entomol.*, **44**:442–445 (2005).