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Impact of *Bt* Cotton on Animal Health: A Review

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Abstract: *Bacillus thuringiensis (Bt)* cotton is commonly grown in all over the world to control wide range of pests. *Bt* cotton have several advantages over conventional chemical fertilizers and biological control methods as it provide safe, quick, efficient and long term resistance against diverse range of cotton insects. With the passage of time several technical, socio-economical, ethical and biosafety issues arises with use of *Bt* cotton. As *Bt* cotton adversely affects a variety of non targeted organisms including many beneficial animals. Several researchers have been reported that *Bt* toxins affect several different species of animals such as cows, buffaloes, model mice, goats, pigs, chickens, herbivores and human. The effect of *Bt* toxin is more lethal on Gastro Intestinal Tract (GIT) than other organs in all tested mammals that feed on *Bt* cotton seeds. Besides its usefulness, *Bt* toxin also interrupt normal biochemical and biological processes of many important animals. However some findings revealed that *Bt* toxins affect human lymphocytes and other physiological characters when used in higher concentration. Therefore, the present review is designed to describe the possible lethal effects of different types of *Bt* proteins on non-target animal species. The present study will be useful to minimize the toxicity associated with *Bt* cotton on wide ranges of animals.

Key words: *Bt* Cotton · Toxin · Biosafety · Non-Target Animals

INTRODUCTION

Insect is one of the major plant enemies that damage about 15% of important crops in the world [1, 2]. *Bacillus thuringiensis* (*Bt*) is one of the important genetic engineered gram positive bacterium that is used to control major crops pests. *Bt* produced a specialize type of crystalline proteins against a wide range of insects such as A, D and E- endotoxins. The *cry* genes also encode ä-Endotoxins (*Cry* toxins) that form a crystalline appearance during sporulation time that cause death of insect larvae [3-5]. *Bt* genes have been transformed to many important crops including cotton that provide short and long term tolerance against a large number of insects from order Lepidoptera, Diptera and Coleoptera [6,7]. Genetic engineered (*Bt)* crops have several advantages over chemical pesticides as it is environmental friendly, remains for short time in soil and provide durable resistance against wide range of insects [1, 8, 9]. *Bt* cotton plants have been widely adopted by many developed and developing countries of world such as North and South America, Africa and Asia due to its quick and efficient mode of action against a wide range of pests. Since last decades several technical, socio-economical and environmental issues arise from the use of *Bt* crops as it affect a large number of innocent non-target organisms including animals [11, 12]. Vertical gene flow of *Bt* genes through pollen or seeds to non-target organism produce some serious biosafety problems [13-15]. Therefore the present review provides a baseline to describe the negative effects of *Bt* cotton on wide range of animal species. The major effects of various *Bt* toxin alone or in combination on non-target organisms are mentioned below.

Effects of *Bt* Toxin on Various Tissues and Organs of Animals: Gastro Intestinal Tract (GIT) is an important entry system for foreign molecules in animals. The epithelial lining of GIT gives specific route to the foreign DNA and protein fragments that comes from animal feeds [16]. The foreign DNA-fragments of many important plant genes were found in blood, muscles

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tissues and many other internal organs of many agriculture important animals such as broiler chickens, calves, pigs and cattles [17-19]. Two fragments of *cry1Ab* gene such as *P35S* and *cp4epsps, cry1Ab* gene were found in liver, kidney, heart and muscle tissues of goats [20]. Sajjad *et al.* [21] studied the presence of *cry1AC* gene of cotton in digestive system of model animal mice. The mice were fed with normal feed along with 50% mixture of crushed *Bt* cotton seeds. The tissue samples were taken from stomach, intestines, blood, liver, kidney, heart and brain. The isolated DNA from all the tested samples was screened through Polymerase Chain Reaction (PCR) with a set of specific primer of *cry1AC* gene and *tnos* promoter. The targeted gene was found only in intestinal tissues that affect the inner lining of intestine. They also reported that the acidic medium of stomach degrade the foreign *Bt* DNA fragments.

Effects of *Bt* Toxin on Lactating Animals: Several researchers investigated the effects of *Bt* genes on nutrient utilization, blood composition and other performance of dairy lactating animal that feeds on cotton seeds. For example Mohanata *et al.* [22] studied that effect of *cry1Ac* gene on important nutrient utilization, blood biochemical composition and other performance of lactating dairy cows. The tested animals were fed on both non-transgenic and transgenic cotton seeds for 4 weeks. From the result they revealed that nutrient uptake, digestion process, milk yield, composition, body physiology and blood composition were not varied in control and non-control tested animals. The *Bt* protein (*Cry1Ac*) was not found in both milk and plasma. They concluded that *Bt* protein (*Cry1Ac*) have no adverse effect on qualitative and quantitative characters of lactating cows. Similar findings were noted by Singhal *et al.* [23] for lactating cows that fed on *Bt* cotton seeds. Singhal *et al.* [23] and Castillo *et al.* [24] envisaged the effect of *Cry*1Ac alone or in combination with *Cry*2Ab on lactating cows. The milk saturation content and milk quality was similar in both control and treated experimental cows and no adverse morpho-physiological effects were found. The milk and blood of ruminates, tissues of pigs and other poultry are free from any *Bt* gene after feeding on *Bt* seeds, as it shows safer food for all animals [25-29]. Moreira *et al.* [30] found no toxic effect of *Bt* toxins on digestion process of animals. While, Sullivan *et al.* [31] noted that low level of digestibility in lactating cows feeding was similar or having higher level of *Bt* cotton seeds. Higher concentration of haemoglobin and other serum compositions were noted in lactating buffalo feeding on transgenic cotton seeds carrying *Cry1Ac* gene. Blood urea N and creatinine concentrations were also found similar in cows both controlled and experimental lactating cows groups after feeding on *Bt* cotton seeds for 430 days [32].

***Bt* Toxins in Animal Excretion:** The lethal concentration of *Cry1Ab* toxin from animals faeces come to our environment both directly and indirectly that affect target and non-target organisms. Certain animals like pigs and cattle that feed on *Bt* crops to excrete toxic proteins in their wastes by effecting targeted and non-targeted organisms [33]. Foreign DNA fragments of *Bt* cotton was also found in the muscles of many types of chickens [34].

Influence of *Bt* Cotton on Other Non-target Animals: Several researchers have studied the effect of *Bt* cotton on non-target herbivores. Zhang *et al.* [35] studied the effect of *Bt* cotton on non-target *Aphis gossypii* that feed on both *Bt* and non-*Bt* cotton. The enzyme-linked immunosorbant assay (ELISA) was used to screen the presence of *Bt* proteins in *A. Gossypii*. Results showed that a minute amount (=10 ng/g) of *Bt* protein was detected in *Bt* fed *A. Gossypii*. So, only small amount of *Bt* protein was ingested during feeding on *Bt*-cotton. Lawo *et al.* [36] performed similar type of experiment by feeding *A. gossypii* on *Bt c*otton expressing *Cry1Ac* protein. 11 out of 12 samples showed the presence of *Bt* antigen through ELISA. Liu *et al.* [37] studied the effect of *Bt* and Cowpea trypsin inhibitor (*CpTI*) genes in combination on *Aphis gossypii*. From the results they concluded that *Bt* gene along with *CpTI* gene leads lower survival and reproductive rates in all tested organisms. But, in second and third generation the aphid population gain immunity and fitness. *Bt* toxins effect five major groups of herbivores species such as *Spodoptera littorals*, *Apis mellifera*, monarch butterfly, spider mites *Rhopalosiphum padi* and two important predators *Chrysoperla carnea* and *coleomegilla maculate* [38-40]. The long term application of *Bt* protein at pollen stage adversely affect the larvae of monarch butterfly [41].

Many researchers proved that *Bt* cotton is safe for other living organisms. Farag Dahi [42] studied the effect of two *Bt* genes *Cry 1Ac* and *Cry 2Ab* of Egyptian *Bt* cotton on non- target organisms i.e. arthropods (aphids, whiteflies, leafhopper green bugs and spider mites) and other beneficial arthropods (green lacewing, ladybird *coccinella,* rove beetle*,* Orius *bugs* and true spider). No significant differences were found in all tested

organisms after feeding on control and *Bt* cotton. Romeis *et al.* [43] developed a new method of direct application of *Bt* toxin to the larva of green lacewing (*Chrysoperla carnea*). Their finding showed no toxic effects of *Cry1Ab* protein on *C. carnea* larvae. Genetically engineered cotton plants have no adverse effects on non-targeted organisms like coccinellids and spiders [44]. Romeis *et al* [45] treated *C. Carnea* with *Cryl Ab* toxin at higher concentration but no adverse effect was observed in all tested samples.

Effects of *Bt* Cotton on Human Health: Several antibiotics are used as marker gene to screen transgenic plants. Several bacterial species tolerate antibiotics. So, it is a major concern to people who excessively use antibiotic for controlling many lethal human diseases but on the other hand, it is used in plant transformation experiments. If, these pathogens produce tolerance against antibiotics so, it will no longer to be used for controlling human diseases. Similarly, the horizontal transfer of marker genes or other lethal genes to other pathogens further produce serious problem to human health and other non-target organism [46-49]. There are several reports that *Bt* genes cause some serious problem to human health. Bhat *et al.* [50] studied the cytotoxic and genotoxic effects of *Cry1Ac* toxin from *Bt* cotton (RCH2) on human lymphocytes. The MIT test, cytokinesis blocked micronucleus and erythrolysis tests showed that high dose of *Cry1Ac* toxin decreased the cell survival ability up to 47.08% after 72 hour of incubation period. Only 2.52% of micronuclei were found in test samples. The *Cry1Ac* toxin also showed lethal effect on human leukocytes by their haemolytic action. They concluded that *Cry1Ac* toxin at higher concentration have lethal cytotoxic and genotoxic effects on the human lymphocytes.

CONCLUSION

The evidences clearly reveal that acreage and popularity of *Bt* cotton is increasing day by day as it plays a vital role to provide durable resistance against a wide range of insect species. *Bt* cotton has played important role to sustain agriculture in all over the world for their maximum yield and other agronomic practices as well. With the passage of time, several biosaefty and environmental issues arise with the use of different *Bt* genes. Several researchers have reported the toxic effects of *Bt* proteins of cotton and other crops on diverse range of non-target animal species including human being. Now it is the responsibilities of the scientists to bring awareness in people to develop new *Bt* cotton cultivars that assure no or very low toxicity on non-target organisms to minimize risks associated with *Bt* cotton technology.

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