Conclusion
Mosquitoes transmit some of the world's most serious diseases, including malaria, filariasis, yellow fever and encephalitis. With the emergence of pesticide and drug-resistant mosquitoes, and the increased concern over chemical pesticide toxicity, the use of biological control agents, such as Bacillus thuringiensis is more important than ever. The serotype H. 14 of B. thuringiensis described as the new subspecies israelensis differs from all formerly known serotype of B. thuringiensis by the high toxicity of its crystal toxin, namely δ-endotoxin to mosquito and black fly larvae. When crystals are solubilized, they exhibit toxic effects including cytolysis of cultured insect cell lines, haemolysis of rabbit erythrocytes and lethality for mice.

As the δ-endotoxin has considerable significance in term of vector control and public health, it is important to find out an appropriate simple and easy method instead of laborious and time consuming conventional biological assay method for quantitations of δ-endotoxin as well as to know its effect on biological systems.

Firstly, a simple haemolytic assay method for quantitative estimation of the δ-endotoxin of B. thuringiensis subsp. israelensis (serotype H. 14) from a crude preparation has been developed [302]. The method has several advantages over mosquito larvicidal assay methods as it is inexpensive, highly sensitive and easier to run and can be used for performing a reasonably large number of assays rapidly with high precision and with a coefficient of variation that does not exceed 1.96%. It has also been observed that other haemolytic factors, if present, do not affect the result due to higher dilution of the sample. The yield of δ-endotoxin increases with sporulation of the bacterial culture.

Secondly, the δ-endotoxin at a dose level of 70 and 140 kitu/kg body weight were administered intraperitoneally to inbred swiss mice with a group of physiological saline control for six weeks to study its effect on different biological systems as well as skin and eye irritancy potential on rabbits.

Haematological study, the first parametric study showed decrease of total count of RBC, neutrophils, monocytes, platelets, serum calcium content, haemoglobin
concentration, ESR, haematocrit readings and MCHC whereas total count of WBC, lymphocytes, basophils, clotting time, MCV and MCH increased in endotoxin treated mice. A massive bone marrow depression is found with a elevation of splenic cells count. Reduction in haemoglobin content is due to the reduction in the total count of RBC [220]. Haemopoietic cells of bone marrow were injured by endotoxin resulting anaemia which is confirmed by the value of MCH, MCV, MCHC, ESR and haematocrit readings [217]. Increased clotting time is found to be related to low platelet count and low blood calcium content. Most of the clotting factors are synthesized in liver [221]. The δ-endotoxin causes liver injury an thereby increases clotting time of blood. Bone marrow depression in consequence of the deficiency in megakaryocytes, is responsible for low platelet count. Decrease count of neutrophils may be direct action on the bone marrow of animals. High splenic cell count also indicates anaemic condition. Liver injury due to δ-endotoxin is also confirmed by the functional tests of liver.

The second parametric study is based on the functional and histopathological studies of liver and kidney. Increased value of blood glucose, serum GPT, bilirubin content, cholesterol, free fatty acids and alkaline phosphatase with a depletion of glycogen content indicate the hepatotoxic condition of liver [303]. The functional test of kidney exhibits that δ-endotoxin causes increment of blood urea, NPN and globulin along with total protein content. Decrease of albumin is observed. NPN is raised in those conditions in which blood urea is raised [255]. A low serum albumin with marked increment of globulin results decrease of A/G ratio which indicates liver injury [255]. In liver injury total serum proteins also increase [258]. Histopathologically it has been observed that δ-endotoxin damages both liver and kidney.

Next parametric study is to find out a relationship between the δ-endotoxin and catecholamines level in brain and liver of mice. Adrenaline in brain and noradrenaline in brain and liver are increased whereas dopamine and noradrenaline in brain and dopamine in liver are decreased. Adrenaline and noradrenaline are excitatory substances but both catecholamines often have depressant action [304].
The fourth parametric study includes teratological as well as dominant lethal mutation studies of δ-endotoxin in mice. The endotoxin causes embryocidal and possible fetotoxic effect in pregnant mice with mutagenic effect in male mice.

The last parametric study represents the irritancy potential studies in rabbits which reveals a mild to moderate skin irritation and a reversible eye irritation.

Considerable time and energy were spent to develop a haemolytical method of assay for quantitation of δ-endotoxin to avoid the time consuming and tedious biological assay involving mosquito larvae. Highly precise microbiological procedures under rigorous controlled environment were undertaken to get good yield of toxin (δ-endotoxin) from *B. thuringiensis* subsp. *israelensis*.

From the above facts it can be concluded that δ-endotoxin at a dose level of 140 kitu/kg to inbred swiss mice over a period of six weeks is haemotoxic, hepatotoxic, nephrotoxic, neurodepressant and toxic to reproductive system.