SUMMARY

The control of insects by humans has existed about as long as humans themselves have. People are usually disturbed by the insects as they bite, sting and usually get in the way. The protection of food crops from destruction by the insects needs urgent attention as the world's population increases. The chemical substances were commonly used in the middle of 18^{th} century for the control of pests. These synthetic chemicals can cause environmental hazards and pose serious risks to human health.

Bacillus thuringiensis is a ubiquitous, sporulating bacterium. Its shape is rod-like and it is gram positive. It mostly inhabits soils of different localities all around the world. *Bacillus thuringiensis* most commonly known as *Bt* and its insecticidal crystal protein i.e δ-endotoxin has a toxic effect on insects. *Cry2Aa* shows dual nature and is a rare insecticidal crystal protein. It is active against both dipteran (mosquitoes) and lepidopteran (moths and butterflies) insects

The aim of this research work was to isolate the *B.t.* from different habitats and the molecular characterization of *cry2* gene from local isolates of *B.t.* For the isolation of *Bacillus thuringiensis* containing *cry 2* gene, the soil samples from different locations were collected. The habitats selected for isolation of soil samples were leaf litter, leaf surfaces of broad leaf trees, National parks, insect habitat, alluvial soil and wild life sanctuaries. From these 25 samples 38 bacterial colonies were isolated by sodium selection test and growth on LB agar medium.

First of all morphological characterization of these isolates was done. For this purpose Gram's staining, Endospore staining and Motility tests were performed. On the basis of this morphological characterization the isolates were characterized as Bacilli, spore forming and motile.

Biochemical characterization was performed for all 38 isolates which include carbohydrate fermentation (glucose, sucrose and lactose), Catalase test, indole, nitrate reduction, citrate test and V-P test, starch agar test, determination of urease, gelatin and amylase activity.

Optimal conditions were determined for biochemically characterized isolates that include Optimum temperature, pH, inoculum size and incubation time. For the *B.t.* isolates optimum temperature was 37°C optimum pH was 7 and 9% inoculum size was found to be optimum. In case of incubation time *B.t.* isolates showed maximum growth at 21st hour and after that their growth was started to decline.

Molecular characterization was performed to accurately identify the *B.t.* isolates. For molecular characterization PCR was performed for the amplification of the 16S rDNA of these isolates. After ribotyping, 16S rDNA gene of the isolates demonstrated the maximum homology with *Bacillus thuringiensis* strain HD682 complete genoeme(RAH-Bt 1.5/2),, *Bacillus thuringiensis* strain 2PR56-10 16S ribosomal RNA gene, partial sequence(RAH-Bt 3.1/2).

After ribotyping *B.t.* isolates were further screened for the presence of *cry2* gene. RAH-*Bt* 1.5/2 showed 99% homology with *Bacillus thuringiensis* serovar kurstaki str. YBT-1520 plasmid pBMB293, complete sequence, accession number CP007615. RAH-*Bt* 3.1/2 showed 98% homology with KM588296.1 *Bacillus thuringiensis* serovar kurstaki strain MSS8.4 Cry2Aa (cry2Aa) gene, complete cds.

There is huge requirement of target-oriented insect pest control. We should keep in view the development of resistance. The use of chemical insecticides and synthetic pesticides should be limited to control the contamination of environment. We need to exploite better strains of *Bacillus thuringiensis* that help in making cotton crops better and also save our environment through use of bio-pesticides.