SUMMARY

Excessive use of synthetic organic pesticides for the insects and crop pests has resulted in many problems like environmental pollution, killing of non-targeted organisms and food chain contamination. To overcome these problems traditional approaches suggested the use of *Bacillus thuringiensis* as a biopesticide due to presence of toxin crystal proteins in it that exhibit the specificity only to targeted pests and not harmful for other non-targeted organisms.

Bacillus thuringiensis commonly represented as *B.t.* is a gram-positive, rod-shaped and endospore forming soil bacterium found almost in all the environments. Its insecticidal activity is based on the presence of insecticidal crystal proteins (ICP) or called as δ-endotoxins, produced during sporulation and specific in their action against insects.

The aim of this research work was to isolate the *Bacillus thuringiensis israelensis(B.t.i.)* from different habitats and the screening of *cry4* gene from local isolates of *B.t.i.* For the accomplishment of this research work total thirty five samples were collected from different areas of Punjab like Lahore, Sheikhupura and Muridke. Isolates were from soil (dry and moist), fresh foliage and leaf litter, insect habitat and animal faeces, stored grains dust (wheat and rice fields). From these 32 samples 44 bacterial colonies were isolated by sodium acetate selective medium and grown on LB agar medium.

First of all these isolates were processed morphological characterization in which Gram's staining, endospore staining and motility tests were performed. On the basis of this morphological characterization the isolates were considered as Bacilli, spore forming and motile.

Then these isolates were screened through biochemical characterization. Biochemical tests include carbohydrate fermentation (glucose, sucrose and lactose), Catalase test, dextrose agar test, citrate test and V-P test, determination of urease, gelatin and amylase activity. Four strains were characterized as *B.t.* through these biochemical tests.

Biochemically characterized isolates were further processed for the determination of the optimum conditions for their growth. Optimal conditions include Optimum temperature, pH, inoculum size and incubation time. For the *B.t.* isolates optimum temperature was 37°C optimum

pH was 7 and 8% inoculum size was found to be optimum. In case of incubation time *B.t.* isolates showed maximum growth at 20th hour and after that their growth was started to decline.

After the morphological and biochemical characterization, isolated bacterial strains were further characterized by performing the crystal staining. Because it is a useful approach for the identification of *B.t.i.* strains.

Antibiotic resistance was also determined for these *B.t.* isolates. These isolates showed maximum resistance against ampicillin and penicillin.

For the accurate identification of *B.t.* isolates, these strains were characterized at molecular level. 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* Bt407 (M-2), *Bacillus thuringiensis* strain BAB 2592 (M-4), *Bacillus thuringiensis* strain EAPL24 (M-7) and *Bacillus thuringiensis* strain ATCC 10792 (M-8).

After ribotyping *B.t.i.* isolates were further screened for the presence of dipteran specific *cry4* gene. M-2, M-4 and M-7 *B.t.i.* isolates were found positive for the presence of *cry4* gene. All these strains showed the maximum homology with DAB BT2 Cry4 (*cry4*) gene, partial cds, *Bacillus thuringiensis israelensis* bt8 gene for 130kDa crystal protein (mosquito-specific toxin), (M-2), DAB BT6 Cry4 (*cry4*) gene, partial cds, *Bacillus thuringiensis* serovar *israelensis* strain BRC-LLP29 putative cry4A gene, (M-4), isolate DAB BT5 Cry4 (*cry4*) gene, partial cds, *Bacillus thuringiensis* strain PBT602 130kDa crystal protein (cry) gene, complete cds, (M-7).