SUMMARY

The objective of this study was to isolate the *Bacillus thuringiensis* strains from soil sample and then to identify the *cyt2B* protein gene from their isolates. Twenty five samples were collected from the different ecological localities of Lahore under sterilized conditions.

Two approaches were used and applied for the isolation of *B. thuringiensis*, Sodium acetate selection and heat shock methods. For the molecular characterization of *Cyt* genes of *B. thuringiensis,* polymerase chain reaction (PCR) technique was used.

Seven isolates showed *B. thuringiensis* like colony morphology and sub-terminal spores were seen after staining with malachite green during endospore staining. Eight isolated bacterial cultures were gram positive bacilli because they were rod shaped and showed purple color after staining. Other bacterial cultures GCU-DAB-M.K 4 and GCU-DAB-M.K 9 were gram negative.

Various biochemical tests like catalase test, indole test, motility test, lecithinase test, starch hydrolysis test, and gelatin hydrolysis test were performed and seven isolates gave positive results for these tests

Optimization of *B. thuringiensis* was done at different temperatures and pH conditions. Graph was plotted between O.D values and temperature or pH ranges. The bacterial isolates showed maximum growth at 37°C and more prominent growth was noted at pH 7.

The nucleotide sequences of full length 16S rRNA gene for the identification of *B. thuringiensis* isolates were determined through PCR. Presence of *Cyt2B* gene in *B. thuringiensis* was confirmed by PCR. Bioassays of four selected bacterial isolates (GCU-DAB-M.K 1, GCU-DAB-M.K 6, GCU-DAB-M.K 10 and GCU-DAB-M.K 15) carried out to evaluate the mosquitocidal activity of *B.t* against mosquito larvae. Isolates have biotoxicity activity as follows. GCU-DAB-M.K 1 (654.211$\pm$1.30 µg/ml), GCU-DAB-M.K 6 (1007.33$\pm$1.3µg/ml), GCU-DAB-M.K 10 (763.731$\pm$ 1.2 µg/ml) and GCU-DAB-M.K (522.027$\pm$0.17 µg/ml).