SUMMARY

Synthetic pesticides have been widely used to control insects and crop pests which often negatively affect humans in a variety of ways. These chemicals target a wide spectrum of organisms. However, they cause insect resistance, kill beneficial insects when used improperly. Therefore, the need of alternate, more effective and environment-friendly control agents become imperative.

For this, a total of twenty five soil samples were collected from different ecological habitats of Pakistan. Two approaches were used and applied for the isolation of *Bacillus thuringiensis* (*B.t.*): sodium acetate selection method and heat shock method. These methods yielded 120 morphologically distinct *B.t.* like colonies on LB agar medium. Staining and biochemical characterization, yielded 25 *Bacillus thuringiensis* isolates. Eight isolates were found positive on the basis of ribotyping of conserved region (550bp) 16S rRNA whereas the full-length (1.6Kb) 16S rRNA ribotyping demonstrated maximum homology of six isolates with *Bacillus thuringiensis* serovar israelensis, *Bacillus thuringiensis* serovar chinensis, *Bacillus thuringiensis* serovar kurstaki, *Bacillus thuringiensis* serovar tolworthi and *Bacillus thuringiensis* serovar Indiana. Three of the isolates were found positive for cry4A gene.

All *B.t.* isolates showed typical growth curves at 37°C, pH 07, and inoculum size of 10% of the total bacterial culture.

Bioassays of *B.t.* spores of isolates positive for cry4A gene against third instar larvae of *Aedes aegypti* showed that four *B.t.* isolates (GCU-DAB-TK-04, GCU-DAB-TK-13, GCU-DAB-TK-06, and GCU-DAB-TK-12) were most toxic to the target insects. Among these the most toxic *B.t.* isolate, GCU-DAB-TK-04 (*LC50=104μg/ml*) was isolated from moist and sticky soil from field area, Kasur, GCU-DAB-TK-13 (*LC50=602μg/ml*) was isolated from dry and sandy soil from field area Cantt. road, Lahore, GCU-DAB-TK-06 (*LC50=812μg/ml*) was isolated from moist soil from field farm Johar Town, Lahore, GCU-DAB-TK-12 (*LC50=1230μg/ml*) was isolated from Canal area, Gujranwala and GCU-DAB-TK-09 (*LC50=7585μg/ml*) was found to be least toxic and isolated from Nursery farm Canal view society, Lahore. These isolates have the potential
to develop into a biopesticidal formulation for the control of different species of mosquitoes.