

## 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 ( $LC_{50}=104\mu\text{g/ml}$ ) was isolated from moist and sticky soil from field area, Kasur, GCU-DAB-TK-13 ( $LC_{50}=602\mu\text{g/ml}$ ) was isolated from dry and sandy soil from field area Cantt. road, Lahore, GCU-DAB-TK-06 ( $LC_{50}=812\mu\text{g/ml}$ ) was isolated from moist soil from field farm Johar Town, Lahore, GCU-DAB-TK-12 ( $LC_{50}=1230\mu\text{g/ml}$ ) was isolated from Canal area, Gujranwala and GCU-DAB-TK-09 ( $LC_{50}=7585\mu\text{g/ml}$ ) 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.

## INTRODUCTION

Insects are one of Earth's most abundant organism's groups. They often have a negative impact on humans, damage crops and are the vectors of various diseases of both humans and animals. These insects spread different diseases like yellow fever, malaria, dengue fever, filariasis and viral encephalitis etc. in humans. Human efforts to control insects over the passage of time have shifted from naturally used substances to artificially synthesized chemicals. Pesticides are now widely used to control pests. These are the chemicals which kill the harmful pests. The use of chemical pest control drugs began in the middle of 19<sup>th</sup> century. The insecticides used in the earliest 19<sup>th</sup> century include organic and inorganic compounds, organochlorides, carbamates, and pyrethroids etc. (Gillies and Nirajda, 1995). Many of these chemicals are currently used. These chemicals have certain characteristics which make them useful for a broad range of organisms, such as residual action and toxicity. However, many environmental issues have been created by chemical pesticides. They cause resistance to insects, kill beneficial insects when used improperly. These insecticides harm liver, cause cancer, infertility, kidney and reproductive issues in both insects and humans (Shankya, 2002; Kozay and Wink, 1994). In spite of deleterious and few beneficial effects of all these insecticides, a huge amount of these insecticides are being used from the world every year to kill the insects and other pests as they provide the easiest solution to enhance agricultural yield (Shankya *et al.*, 1998; Ahmed and Shrivast, 2013).