Summary

Chemical insecticides are widely used to control the insects, these insecticides are not environmentally healthy as they are not biodegradeable and hence are biomagnified. These insecticides are also not host specific; they also kill the beneficial insects. So there is a need to search for a control agent which should not harm the environment and also to human beings. Several different methods have been used in recent past to control the insects which include use of pheromones for trapping or disruption of mating behavior, insect growth regulators that interfere with larval development, parasitoids, fungi, viruses and bacteria, which debilitate or cause death in the infected insect.

One of the most successful biological control organisms is a naturally occurring bacterial pathogen, *Bacillus thuringiensis* generally known as *B.t.* Formulations based on *B.t.* have been used for decades as biological insecticides for agriculture and forestry, as well as for vector control against mosquitoes and black flies. Interest in *B.t.* proteins has increased during the last two decades because of their unique qualities which are unmatched by any conventional insecticide. Of the 297 genes known to encode *B.t.* proteins, some share a high degree of homology, while others have diverse nucleotide sequences. Because of the interest in *B.t.*, the list of new *B.t.* subspecies is growing as is the group of economically important target insects. *B.t.* produces *cry*stal proteins during sporulation. These crystal proteins are of two types Cry and Cyt. Both of these types of proteins are different in their mode of action. Cytolytic proteins have an additional property of having cytolytic activity

against different cells and also against mammalian erythro*cyte*s. These proteins especially Cyt proteins are active against mosquito larvae.

Recent studies reported the development of resistance in mosquitoes against Cry toxins. Researchers tried different methods to overcome this resistance and they found that when Cyt proteins are used in combination with Cry proteins they greatly reduced the resistance of mosquitoes against *B.t.* toxins. This indicated that Cyt proteins work synergistically with Cry proteins.

In the present study, soil samples collected from different areas of Lahore, Kasur and Faisalabad. A total of 50 soil samples were collected, these soils were rich in organic manure. *B.t.* like bacteria were isolated from these soil samples using differential medium containing sodium acetate buffer. These isolates were then subjected to biochemical characterization by performing biochemical tests. The expected *B.t.* like isolates were screened for the presence of *cyt* genes. After confirmation of presence of *cyt* 2B gene, mosquitocidal activity of these isolates were checked by using *B.t.* spores and total *B.t.* cell proteins against 3rd instar larvae. From the bioassays, it was found that NB *B.t.*4 was found to be most toxic with LC_{50} value of 400±1.15 µg/ml and 68±0.46 µg/ml for its spores and total cell protein, respectively. After bioassays, six most toxic *B.t.* isolates were then selected for further study.

Ribotyping of these isolates was done to amplifying 16S rRNA gene to identify these isolates. Protein profile of these isolates was checked to confirm the presence of 29 kDa protein band. Full length *cyt* 2B gene was amplified, cloned in pTZ57RT cloning vector, and pET22b vector was used for expression. IPTG induction of 1 mM was found good for expression ranging incubation time of 4-6 hours. Expressed protein was then purified by anion exchange chromatography.

Bioassays were performed using recombinant organism (*E. coli* transformed with *cyt* 2B gene), expressed crude protein and purified protein. It was found that the purified protein was most toxic with LC_{50} Value of 50±1.68 µg/ml.