Summary

Bacillus thuringiensis has undeniably been the most effective microbial agent for biological insect control of all time. The present study aimed to control resistant insects by isolating soil samples from different areas of Pakistan intended for effective mosquitocidal *cry 11* positive *Bacillus thuringiensis* (*Bt.*). Fifty samples were collected from different areas of Pakistan's ecosystem. It was noticed that 36%, 22%, 20%, 12%, and 10%, *Bt.* isolates were quarantined from dry soil, moist soil, soil containing cattle waste, garden soil, and sandy soil samples, respectively. The major source of *cry 11* positive *Bt.* isolates were dry soil and dung-containing soil. Genomic DNA was isolated, and a DNA fragment of 650 bp, full length 1.9 kb *cry 11* gene was amplified by PCR, respectively. Fourteen *Bt.* were brought to be positive for the *cry 11* gene. The 16S rDNA study exposed that these screened *Bt.* confirmed 99% homology with *Bt. kurstuki* RKD12, *Bti* strain AM65-52, *Btko.*MC28, *Bt.YB.T.*-1518, *Bt.* NCIM *coreaensis*, *gallerie*, strain, *Fdaargos_*796. *Bt.* serovar *tolworthi*, *Bt.* str. *Al -Hakam* and *Bt.* serovar *Chinensis*. *Bt.* serovar *Indiana.* The toxicity bioassays with *Bt.* spores, and protein diet proved that eleven *Bt.* isolates harboring *cry 11* genes (*viz.,* NF1*Bt.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11) were most toxic to 3rd instar larvae of mosquito, *Aedes aegypti*, *Anopheles stephensii*, and *Culex pipens*. The first six stains were selected on the basis of toxicity in descending order. Finally, 9NF, a highly toxic strain out of the three most toxic *Bt.* isolates, was selected for cloning and characterization of the full-length *cry 11* gene. It was isolated from dry soil animal dung at Kashmir Neelam Valley having spore diet LC50 327.8 ±846 µg/ml against *A. aegypti* (3rd instar larvae) after 24 hours and showed 100% mortality at 0.7mg of spores/ml. The positive control HD-500

showed 94% mortality. It was found that LC50 (327.8, 442.7, 460.8 µg/ml) of 9, 4, and 6NF is quite less than HD500 LC50 (683 µg/ml). So, 9NF the most toxic strain as compared to HD500. The LC50 of 9NF is 644.105±45 against *Culex pipens*, which shows less toxicity against spore diet as compared to the genera *Aedes* and *Anopheles*. Protein LC50 of 9, 4, 6NF is 69.130±5, 84.1±5, 95.1±407 µg/ml against *A. aegypti* harboring *cry 11* gene. All isolates did not show the same level of toxicity, which reflects the variation in expression level.

The present study describe the screening and characterization of toxic *Bt.* (9NF) locally isolate was done which showed 99% homology with already reported *B. thuringiensis* (AXJ97553.1). *Cry 11* gene from the most toxic *Bt.* strain 9NF was cloned and subcloned in pTZ57R/T and pET30a (+) for expression. The optimized conditions for good expression of the *cry 11* gene were found to be 1mM IPTG, 3.5-4 hours incubation time, and 37°C. Biotoxicity assays revealed that partially purified *Bt.* protein is highly toxic against *A. aegypti* larvae with LC50 value of 42.883±6 µg/ml. Further screening from different ecological habitats must be necessary to search for a novel *cry 11* gene to form biopesticides and overcome insect resistance. This research may lead to applications in the field to control the insect expression level of the *cry 11* gene present in local *Bt.* isolates.