

TOWARDS SAFE AND EFFICIENT *Bt* MOSQUITOCIDES; DETERMINING CRY AND CYT PROTEIN PROFILES AND THE PRODUCTION OF TYPE I B EXOTOXIN BY LOCAL *Bt* STRAINS 4 AND 6E

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Bacillus thuringiensis is a ubiquitous, gram positive and spore forming bacterium which has been used to control certain insect species among the orders Lepidoptera, Diptera and Coleoptera. The genetic diversity of *Bacillus thuringiensis* (*Bt*) strains show differences according to the regions where they have been isolated. Hence, each habitat may contain novel *Bt* strains, which have toxic effect on target spectra of insects. The aim of this study was to identify the crystalline protein content of the local isolates with reference to *Bacillus thuringiensis* subsp. *israelensis* (*Bti*). To detect the protein profiles of crystal proteins obtained from *Bti*, *Bt4* and *Bt6e* (local isolates), SDS-PAGE analysis was optimized. To identify the presence of *cyt* genes, the local strains were cultured on blood agar and observed for hemolysis. Both the local strains were subjected to HPLC analysis on a C-18 column for type 1 β exotoxin. The mobile phase was 50 mM KH₂PO₄ (pH 3.0) in double distilled water at a flow rate of 2 mL/min at 25 °C. It was found that *Bti* exhibited three main bands, 125-135kDa (Cry 4A and Cry 4B), 68kDa (Cry 11Aa) and 29kDa (Cyt 1Aa) and all the three including *Bt4* and *Bt6e* contained Cyt1Aa protein. *Bt4* showed a distinct band at 49kDa and *Bt6e* showed two distinct bands at 112kDa and 45kDa concluding that the *Bt* strains differed in their content of proteins. Both the local strains showed β hemolytic character, indicating the presence of *cyt* genes. Type 1 β exotoxin was not produced by *Bt6e*, but *Bt4* produced a significant quantity (114.15 μ g/ml). Accordingly *Bt6e* could be integrated into mosquito control programs, whereas *Bt4* is not ideal due to the production of type 1 β exotoxin. This work has identified, that these novel *Bt* isolates differ in their protein profiles, driving them to be used as potential candidates for the industrial production of mosquito pathogenic *Bt* isolates.

Keywords: *Bacillus thuringiensis*, *Bti*, *Bt4*, *Bt6e*, Cry proteins