



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student : DHANASINGH M

Roll Number : 136106005

Programme of Study : Ph.D.

Thesis Title: **Studies on engineering entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* to express heterologous insect-specific toxins**

Name of Thesis Supervisor(s) : Prof. Gurvinder Kaur Saini

Thesis Submitted to the Department/ Center : BSBE

Date of completion of Thesis Viva-Voce Exam : 23/12/2019

Key words for description of Thesis Work : Entomopathogenic fungi, insect toxins, biopesticides

SHORT ABSTRACT

M. anisopliae and *B. bassiana* are the environmentally friendlier insect pathogenic fungi, being utilized as an alternative to chemical synthetic insecticides, to control major economically crucial agricultural insect pests globally. Nevertheless, in the duration to complete the procession of pathogenic invasion, insects cause stellar temporal damages to the food crops, thus necessitated an increase in the virulence and reduction in the lethal timing of entomopathogenic fungi. In the present study, heterologous proteins from scorpion *Leiurus quinquestriatus quinquestriatus* excitatory toxin β -BUTX Lq1a, defense protein from snowdrop plant *Galanthus nivalis* agglutinin (GNA), and *Bacillus thuringiensis* insecticidal toxins (Cyt1Aa and Cry1Ac) chosen to empower the fungi pathogenicity towards insect pests. Initial studies with the recombinantly purified β -BUTX Lq1a on insect cell line revealed the cytotoxic effects such as cell shrinkage, membrane blebbing and apoptotic structures. Intra-hemocoellic injection bioassay on major agricultural pests resulted in immediate paralysis and time dependent mortality. Similarly, recombinant fusion protein β -BUTX Lq1a/GNA and GNA alone resulted in dose dependent reduction in cell viability of various cell lines. Toxicological evaluation revealed the cytotoxic effects such as membrane compromised cells, reduction in mitochondrial membrane potential, chromosomal condensation and fragmentation, increased expression of caspases and G2/M phase cell cycle arrests. When the *M. anisopliae* expressing β -BUTX Lq1a was tested based on topical application against sugarcane white grub *Phyllophaga smithi* resulted in 24.4 % reduction in lethal time. Similarly, fusion protein β -BUTX Lq1a/Cyt1Aa expression in *M. anisopliae* exhibited 17 % reduction in LT_{50} value against *P. smithi*. These results are paving the way to the development of novel *M. anisopliae* expressing BUTX Lq1a, which thereby increases the mortality of infected insect pest. This present study provides a potential alternative to the chemical pesticides, in principle to enhance the effectiveness of biological control agents when expressed and delivered through an appropriate system.