INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **"Studies on the cloning and expression of a spider neurotoxin, Mu-diguetoxin-Dc1a, in entomopathogenic fungi for enhanced bioactivity"**

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

Extensive use of generic chemical insecticides to control insect pest population poses potential risks to human health and the environment because of the appearance of insect resistance. Insect pathogenic fungi are considered as a promising alternative to chemical insecticides for efficient as well as environmentally benign insect pest control. However, the slow killing speed and requirement of high conidial concentrations are limiting this approach to become viable. Thus, the primary aim of this thesis was to engineer transgene encoding highly potent insecticidal spider venom-peptide (ISVP) Mu-diguetoxin-Dc1a (Dc1a) into *Metarhizium* and *Beauveria* to enhance its efficacy in controlling insect pests of different orders. To facilitate this, an efficient *Escherichia coli* periplasmic expression system was developed to produce sufficient amounts of rDc1a for characterisation and structural studies. Injection bioassays revelled that the intrinsic insecticidal activity of rDc1a was maintained and caused a spastic paralysis with LD_{50} for tobacco cutworm was 0.416 nmol/g and for Cotton bollworm was 0.397 nmol/g, conclude that rDc1a has a similar lethality in lepidopteran larvae when compared with native toxin. Moreover, feeding bioassays showed a significant level of oral toxicity in housefly (>75 % mortality at 48 h post-feeding) with LD_{50} of 85.39 nmol/g against adults of the dipteran insect vector. In contrast, rDc1a showed ~1000-fold less cytotoxicity effects towards cultured insect cells *in situ* compared to lepidopteran pests *in vivo*. To achieve the primary aim of this thesis, the potent ISVP Dc1a was successfully expressed in *M. anisopliae* strains 892 & 3210 and *B. bassiana* 984. Using the promoter of MCL1 for genetically engineering *Metarhizium* and *Beauveria*, the expression of the target genes was limited to the hemocoel of the target insects. Hemolymph induced expression of transgene Dc1a started within 20 min of induction and moderated until 12 h. Insect bioassays of genetically engineered strains against lepidopteran and dipteran insect orders revealed a significant improvement in virulence compare to wild type strain, with the engineered strains requiring less time to kill insect pests. The LT_{50} values for genetically modified *Beauveria* and *Metarhizium* strains expressing ISVP Dc1a were 20–35 fold lower than wild-type strains. In summary, the work described in this thesis will serve as a useful guide for future studies aimed towards the development of engineered mycoinsecticides from *Beauveria* and *Metarhizium* strains with the potential to control insect pests of different orders.