- Toxicity of Cry toxin was enhanced by domain swapping approach. The coding region of domain I of *cry1Ac* was amplified from plasmid pKK-223 *cry1Ac* and expected band ~ 802 bp was confirmed by 2 % Agarose gel electrophoresis (AGE).
- Also, domain II & III coding region of Cry9Aa was amplified from pSB1402-*cry9Aa* plasmid and desired PCR band was confirmed ~1107 bp in 0.8 % AGE.
- The recombinant *cry1Ac-cry9Aa* construct was prepared by overlap extension PCR by taking equivalent concentration amplicons. The fusion product was ~1893 bp which was confirmed by 0.8 % Agarose gel electrophoresis (AGE).
- In order to further improvement in toxicity, 144 nucleotides bp coding region of alpha helix-1 from N terminal of *cry1Ac-cry9Aa* construct deleted by PCR. The deletion was observed size of approx 1757 bp by 0.8 % agarose gel electrophoresis.
- *cry1Ac-cry9Aa* and *cry1Ac-cry9AaMod* amplicons were blunt end ligated to EcoRV site of pBSKS^{+}. Recombinants *cry1Ac-cry9Aa* and *cry1Ac-cry9AaMod* were confirmed by releasing insert after digested by XhoI and NheI restriction enzymes.
- *cry1Ac-cry9Aa* and *cry1Ac-cry9AaMod* constructs were sub- cloned in pET-28a(+) at XhoI and Nhe I sites. ORF of constructs was confirmed by sequencing. For expression analysis, pET-28a (+)-*cry1Ac-cry9Aa* and pET-28a (+)-*cry1Ac-cry9AaMod* were transformed into *E.coli* strain BL21(DE3) plysS.
- The expression of hybrid toxins Cry1Ac-Cry9Aa and Cry1Ac-Cry9AaMod observed with induction of 1mM IPTG final conc. in shaking condition at 10 °C after 12 h incubation.
- SDS-PAGE analysis of His-tag purified hybrid proteins observed an around ~74kD band of *cry1Ac-cry9Aa* and ~ 68 kDa of Cry1Ac-Cry9AaMod toxin respectively.

- Insect bioassay was demonstrated against *Helicoverpa armigera*, Cry1Ac-Cry9Aa toxin exhibited LC_{50} value 0.725 ng/cm^2 (95 % C.I. - 0.493-1.620) and Cry1Ac-Cry9AaMod shown 0.696 ng/cm^2 (95 % C.I. - 0.404 - 1.519) against *H. armigera* and standard toxin Cry1Ac toxin showed 3.564 ng/cm^2 (95 % C.I.- 1.822- 3.780).
- Nanoencapsulated formulation of *Bacillus thuringiensis* hybrid toxin Cry1Ac-Cry9Aa was prepared by ionotropic gelation method.
- Nanoparticles with different ratios of chitosan: TPP were investigated for entrapment efficiency, size, zeta potential and PDI (poly dispersion index) analysis. Chitosan: TPP ratio (0.3/0.06) observed entrapment efficiency 30.63 ± 0.41%, size 177.96 ± 6.9 nm, zeta potential 30.92 ± 0.50 mv and PDI: 0.281 employed for insecticidal study to *Helicoverpa armigera*.
- Upon insect bioassay analysis, the LC_{50} value of Cry1Ac-Cry9Aa hybrid toxin encapsulated nanoparticles was 2.352 ng /cm^2 (95 % C.I.- 1.684 -3.195 ng/cm^2) and storage stability of encapsulated Cry1Ac-Cry9Aa toxin nanoparticles showed 51.66 ± 5.77 % insecticidal activity on 14 days at room temperature.
- Box-behnken design in response surface methodology (RSM) was performed to determination process parameters of microencapsulation process. Upon verification of model, the optimized formulation condition was 20 mg/ml chitosan concentration, 10 mg /ml *Btk HD-1* (SCA), and 1% (v/v) cross linker and the response parameter encapsulation efficiency found to be 86.97 ± 7.63 %.
- Scanning electron Microscopy (SEM) analysis raveled that capsules were spherical in shape, rough integrity and presence of pores on the surface. Encapsulated bypyramidal shape of Cry1 protoxin and cuboidal shape of Cry2 was confirmed by Transmission electron microscopy (TEM).

- The microcapsule diameter was optimised with different stirring speed (2000, 3000, 5000 RPM) with different chitosan concentration (1%, 1.5%, 2%). The capsules size 17.69 ± 3.56 µm formed with 2% chitosan polymer on 5000 RPM selected for bioefficacy analysis against UV irradiation.
- Chitosan polymer encapsulated *Btk HD-1* spores retained 89.86 ± 8.1 % survival ($F=8.90$, $df = 9$, $P < 0.05$) on 0.2 J (UV-C, 254 nm) irradiation while free spores observed viability 3.11 ± 1.5 % ($F= 30.84$, $df = 9$, $P < 0.01$).
- In UV-B (365 nm) exposure analysis concluded that unformulated spore viability was 3.5 ± 0.90 % ($F= 82.69$, $df = 9$, $P < 0.01$) at 43.2 Joule. After long time UV-B irradiation, microencapsulation spore viability retained 17.30 ± 7.7 % ($F= 39.41$, $df = 17$, $P < 0.01$) at 311.04 Joule (J).
- Bioassay against *H.armigera* larva showed that irradiation on 259.2 J, free spore-crystal *Btk HD-1* has 5.55 ± 2.4 % larvicidal activity while formulated spore-crystal of *Btk HD-1* retained 47.22 ± 2.4 % insecticidal activity.
- Bioefficacy of chitosan based emulsion formulation evaluated on sunlight exposed *C. cajan* pots. The pot bioassay analysis suggested plant treated with 0.210 µg/ml dose observed near to 60 % control population of larva on the fourth day and approx average 95 % control of larval population exhibited at the end of the sixth day. The LC_{50} of formulated *Btk HD-1* calculated to be 126.93 µg/ml after six days.
- Field level efficacy of W/O based emulsion formulation of *Btk HD-1* was determined to pod bearing *C.cajan* plants. The treatment included three doses W/O chitosan microspheres *Btk HD-1* SCA emulsion with 35 µg L^{-1} (Treatment-1), 105 µg L^{-1} (Treatment -2) and 210 µg L^{-1} (Treatment -3) in Tween -80 (0.1% v/v) surfactant and chemical insecticide Indoxacarb (14.5%, SC) used as Treatment – 4. Tween -80 (0.1% v/v) in water used as Treatment – 5. Different treatment of doses showed a

significant reduction ($P < 0.01$) and across the day ($P < 0.001$). The reduction of the population of larva *H.armigera* with 44.66 to 79.05 % on ninth day after spray.

- Green chemistry way silver nanoparticles were synthesized by supernatant of *Bacillus thuringiensis krustaki* HD-73. UV-Visible spectra analysis was performed each of the sun light exposed sample exhibited λ_{max} of silver nanoparticles around 440 nm. The size of synthesized nanoparticles in the range of 50-80 nm. FTIR spectrum analysis revealed that protein act as capping agent surrounding AgNPs.
- XRD analysis showed four distinct diffraction peaks at 38.06°, 44.32°,64.14° and 77.33° and can be indexed 2 θ values of (111), (200), (220), (311) crosspond to Ag plane.
- Comparative synthesis of silver nanoparticles carried out by synthesis of AgNPs occurred in dark condition with different concentration of reducing agent of Bt supernatant. The O.D. reached only 0.3 to 0.4 after 24 h incubation.
- Insect bioassay of silver nanoparticles against *Spodoptera litoralis* concluded *that AGNPs has* strong insecticidal nature and the LC_{50} to be 1.348 mg/ml (95% confidence interval 0.784 -1.560 mg/ml) on ninth day of the experiment