

# INDEX

No.	Contents	Page No
<b>Chapter -1</b>	<b>Review of literature</b>	<b>1-38</b>
1	Introduction	1
1.1	History of Agriculture	1
1.2	<i>Bacillus thuringiensis</i> as biocontrol agent	2
1.3	Ecology and Diversity of <i>Bacillus thuringiensis</i>	7
1.4	Different virulence factors of <i>Bacillus thuringiensis</i>	7
1.4.1	Cry toxin	7
1.4.2	Cyt toxins	8
1.4.3	Vip toxins	8
1.4.4	Exotoxins	9
1.4.5	Proteases and Chitinases	9
1.5	Classification of insecticidal Cry protein (Cry)	10
1.6	Nomenclature of insecticidal Cry/Cyt/Vip toxins and <i>cry/cyt/vip</i> genes of Bt	10
1.7	Insect toxicity spectrum of <i>Bacillus thuringiensis</i> toxins	11
1.8.1	Advantages of <i>Bt</i> being Biocontrol agent	13
1.8.2	Disadvantages of <i>Bt</i> being Biocontrol agent	14
1.9	Three domains structure of Cry protein	14
1.10	Non three dimensional structure of Cry toxins	16
1.11.	Mechanisms of Cry toxin action	18
1.12	Mechanisms of resistance to Cry toxin in different insects	19
1.13	Bt toxins as insect resistance transgenic plants	21
1.14	Resistance development in insect against transgenic Bt crops	23
1.15	Insect resistance management against Cry toxin	24
1.16	Microbial formulation for sustainable agriculture	25
1.16.1	Dry solid products	25

1.16.1.1	Dusts	26
1.16.1.2	Granules	26
1.16.1.3	Briquettes	26
1.16.1.4	Wettable powders (WP)	26
1.16.2	Liquid suspension	27
1.16.2.1	Suspension concentrates (Flowables)	27
1.16.2.2	Emulsion	27
1.16.2.3	Encapsulation	27
1.17	Bt spore-crystal formulation for biocontrol of pests	28
1.17.1	Criteria for ideal Bt spore-crystal formulation	30
1.18	Nanotechnology in crop protection	30
1.19	Application of nanotechnology as pesticides delivery	32
1.20	Chitosan polymer in crop protection and production	35
	<b>Aim and scope of present investigation</b>	37
<b>Chapter- 2</b>	<b>Genetic engineering of Bt toxin to improve insecticidal activity</b>	39-67
2.1	Introduction	39
2.2	Materials & Methods	45
2.2.1	Primer designing	45
2.2.2	PCR amplification of domain coding region	46
2.2.3	Construction of hybrid <i>cryIAc-cry9Aa</i> gene by Overlap extension PCR	46
2.2.4	Deletion of alpha helix-1 coding region of <i>cryIAc-cry9Aa</i>	47
2.2.5	Cloning, Expression and Purification of hybrid Cry toxins	47
2.2.6	Insect Bioassay of Cry1Ac-Cry9Aa and Cry1Ac-Cry9AaMod hybrid Bt toxins	48
2.3	<b>Results</b>	49
2.3.1	Construction of <i>cryIAc-cry9Aa</i> hybrid Bt toxin	49

2.3.2	Alpha helix deletion of <i>cry1Ac-cry9Aa</i> toxin	49
2.3.3	Expression analysis of recombinant <i>cry1Ac-cry9Aa</i> toxin in BL21 (DE3) plysS	49
2.3.4	Purification of hybrid Bt toxins	50
2.3.5	Insect bioassay analysis	50
2.4.	Discussion	50
2.5	References	60
<b>Chapter- 3</b>	<b>Formulation of Recombinant Bt toxin with nanoparticles</b>	68-87
3.1	Introduction	68
3.2	Materials & Methods	70
3.2.1	Materials	70
3.2.2	Preparation of chitosan nanoparticles	70
3.2.3	Optimization of chitosan and TPP concentrations	71
3.2.4	Entrapment efficiency of Cry1Ac-Cry9Aa Bt toxin nanoencapsulated in chitosan nanoparticles	71
3.2.5	Particle size and zeta potential of Cry1Ac-Cry9Aa loaded chitosan nanoparticles	72
3.2.6	Morphological analysis of chitosan-TPP nanoparticles	72
3.2.7	Insect bioassay of nanoencapsulated Cry1Ac-Cry9Aa toxin against <i>Helicoverpa armigera</i>	72
3.2.8	Stability study of nanoparticles of chitosan-TPP-Cry1Ac-Cry9Aa	73
3.3	Results	73
3.3.1	Optimization entrapment efficiency of Cry1Ac- Cry 9Aa insecticidal protein	73
3.3.2	Size analysis of hybrid Cry toxin loaded chitosan nanoparticles	74

3.3.3	Zeta potential and polydispersion index analysis	74
3.3.4	TEM analysis	75
3.3.5	Insect bioassay analysis	76
3.3.6	Stability analysis of Bt toxin loaded nanoparticles	76
3.4	Discussion	78
3.5	References	81
Chapter -4A	<b>Box behneken design for formulation of Bt spore-crystal</b>	88-96
4A.1	Introduction	88
4A.2	Materials & Methods	89
4A.2.1	Experimental design	89
4A.2.2	Microencapsulation <i>Btk HD-1</i> spore-crystal by suspension cross linking	90
4A.2.3	Measurements of encapsulation efficiency	91
4A.3	Results	92
4A.3.2	Verification of Model	93
4A.4	Discussion	94
4A.5	References	95
<b>Chapter -4B</b>	<b>Formulation of Bt spore-crystal by microencapsulation</b>	97- 126
4B.1	Introduction	97
4B.2	Material and Methods	99
4B.2.1	Enrichment of <i>Bacillus thuringiensis krustaki</i> HD-1	99
4B.2.2	Preparation of chitosan microspheres of <i>Btk HD-1</i>	99
4B.2.3	Microcapsules diameter optimization	100
4B.2.4	Morphology analysis of microcapsules by SEM (Scanning Electron Microscopy)	100

4B.2.5	TEM analysis of encapsulated Cry proteins	101
4B.2.6	Insect bioassay of microencapsulated <i>Btk-HD1</i> against lepidopteran pests	102
4B.2.7	Release analysis of microencapsulated spores	102
4B.2.8	Effect of UV irradiation on Bt formulation	102
4B.2.9	Effect of UV-C (254nm) and UV-B (365 nm) irradiation to Bt formulation	103
4B.2.10	Insect bioassay of UV-B exposed <i>Btk HD-1</i> spore-crystal formulation	104
4B.2.11	Efficacy and persistence of formulation on sun-exposed potted <i>C .cajan</i> plant	104
4B.2.12	Experimental design and field level efficacy of formulation	105
4B.3	Results	105
4B.3.1	Effect of stirring speed (RPM) to size of microcapsules	105
4B. 3.2	Morphological analysis of microcapsules	107
4B. 3.3	Insect bioassay analysis of microencapsulated <i>Btk-HD1</i>	107
4B. 3.4	Release analysis of microencapsulated <i>Btk HD-1</i> spores	109
4B. 3.5	Spore survival upon exposure to UV-C irradiation	109
4B. 3.6	Effect of UV-B exposure on spore survival	110
4B. 3.7	Insect bioassay analysis of UV-B exposed <i>Btk HD-1</i>	112
4B.3.8	Efficacy of formulation on sunlight exposed <i>C. cajan</i> pots	113
4B.3.9	Bioefficacy of <i>Btk HD-1</i> formulation on pigeon pea ( <i>C.cajan</i> ) field	115
4B.4.	Discussion	116
4B.5	References	121

<b>Chapter -5</b>	<b>Rapid synthesis of silver nanoparticles by <i>Bacillus thuringiensis</i></b>	127-139
5.1	Introduction	127
5.2	Material & Methods	128
5.2.1	Sunlight based synthesis of AgNPs with supernatant of <i>Btk-HD73</i>	128
5.2.2	UV -Visible spectroscopy analysis of synthesized AgNPs	129
5.2.3	FTIR analysis of samples	129
5.2.4	Transmission electron microscopy (TEM) analysis	129
5.2.5	XRD measurement	129
5.2.6	AgNPs synthesis in dark condition	130
5.2.7	Bioassay of synthesized AgNPS against <i>Spodoptera littoralis</i>	130
5.3	Results	130
5.3.1	UV-visible spectroscopy analysis of synthesized AgNPs	130
5.3.2	TEM analysis of synthesized AgNPs	132
5.3.3	FTRI analysis of silver nanoparticles	132
5.3.4	XRD analysis of lyophilized AgNPs	133
5.3.5	Comparative analysis of formation of silver nanoparticles in dark condition	134
5.3.6	Insect bioassay of silver nanoparticles against <i>S.littoralis</i>	135
5.4	Discussion	135
5.5	References	137
<b>Chapter -6</b>	<b>Materials and Methods</b>	140-153
6.1	Materials	140

6.1.1	<i>E. coli</i> strains harbouring plasmids	140
6.1.2	<i>E. coli</i> strains for cloning and expression	140
6.1.3	<i>Bacillus</i> reference strains	140
6.1.4	Culture growth conditions and maintenance	140
6.1.5	Insect sources and rearing	141
6.1.6	Media	141
6.1.7	Antibiotics stocks	142
6.2	Protocols	143
6.2.1	SDS-PAGE	144
6.2.2	Agarose gel Electrophoresis	145
6.2.3	Blunt end cloning in pBluescript KS (+)	145
6.2.4	Plasmid extraction	145
6.2.5	Elution by glass solution method	147
6.2.6	Concentration of DNA by alcohol precipitation	149
6.2.7	Competent cell preparation	150
6.2.8	Transformation using CaCl <sub>2</sub>	151
6.2.9	PCR system and program for OE-PCR of <i>cryIAa</i> and <i>cry9Aa</i> genes	152
6.2.10	Ligation of hybrid genes in pET- 28a (+)	152
6.2.11	Acetone based co-precipitation method to prepare spore crystal aggregate	153
<b>Chapter-7</b>	Summary	154-157
	Conclusion	158
	Bibliography	159-175
	Publication and Presentations	176-177