	Dev	-	ent of Wide Spectrum Biocontrol Actinomy ainst Fungal Phytopathogens of <i>Cajanus caj</i>	-	
			TABLE OF CONTENTS		
No			TITLE	Pages	
	DEC	LARAT	IONS	Ii	
	DED	ICATIO	NS	Iii	
	ACK	NOWLE	EDGEMENTS	Iv	
	LIST	OF AB	BREVIATIONS	Ix	
	LIST	OF TAI	BLES	Xi	
	LIST	OF FIG	URES	Xii	
	TAB	LE OF C	CONTENTS	Xvi	
	ABS	FRACT		Xxi	
1	Review of Literature				
	1.1	Food S	ecurity and Plant Diseases	1	
	1.2	Contro	l Strategies of Fungal Disease in Plants	3	
		1.2.1	Biological control of plant diseases	3	
	1.3	Types	and Mechanisms of biological control	4	
		1.3.1	Types of Biological Control	4	
		1.3.2	Mechanisms of Biocontrol	7	
	1.4	Limitat	tions of Biocontrol Agents	14	
		1.4.1	Biological and ecological factors	14	
		1.4.2	Social and economic aspects	15	
		1.4.3	Strategies to overcome the limitation of BCAs	15	
		1.4.4	Combination of BCAs	15	
		1.4.5	Integration of BCAs with chemical control measures	16	
		1.4.6	Mass production of BCAs	17	
		1.4.7	Formulation of BCAs	17	
	1.5	Future	of Biocontrol	18	
	1.6	Pigeon Pea (Cajanus cajan)			
		1.6.1	Fungal Diseases of Pigeon Pea	20	

Г

	1.7	Stress	Ethylene and Plants	21	
		1.7.1	Role of ACC Deaminase in the regulation of ethylene biosynthesis	23	
	1.8	Mycorr	hiza	24	
		1.8.1	Formation of Mycorrhizae	27	
		1.8.2	Mycorrhizal Plant Interactions	27	
		1.8.3	Mycorrhizal and Bacteria	29	
	1.9	Genom	e-Based Taxonomic Classification of Genus Streptomyces	30	
	1.10	Aim an	d scope of present investigation	31	
	1.11	Objecti	ves of the Study	33	
2			entification, and Characterization of Actinomycetes from Soil of <i>Cajanus cajan</i>	36	
	2.1				
	2.2	Materia	and Methods	40	
		2.2.1	Sampling Sites	40	
		2.2.2	Collection of soil samples	41	
		2.2.3	Isolation and Characterization of Actinomycetes	41	
		2.2.4	Genomic DNA sequencing and phylogenetic analysis	41	
		2.2.5	Characterization of Actinomycetes for Biocontrol and PGP traits	42	
	2.3	Result		45	
		2.3.1	Isolation and characterization of actinomycetes from Rhizspheric soil	45	
		2.3.2	Identification of Actinomycetes by 16S rRNA Amplification	47	
		2.3.3	Characterization of Streptomyces isolates on different ISP media	48	
		2.3.4	Phenotypic characterization of <i>Streptomyces</i> sp. S-9	52	
		2.3.5	Plant Growth Promoting (PGP) Potential of Studied Actinomycetes Isolates	52	
	2.4.	Discuss	sion	54	

3		Screening of antifungal actinomycetes against fungal phytopathogens of <i>Cajanus cajan</i>		
-	3.1			58
-	3.2	Material and Methods		60
		3.2.1	Screening of Actinomycetes	60
		3.2.2	Phenotypic characterization of Streptomyces sp.	60
		3.2.3	Scanning Electron Microscopy	61
		3.2.4	Production and Extraction of Antifungal Metabolites from S-9	61
		3.2.5	Identification and Purification of Bioactive Compounds	62
	3.3	Results	8	63
		3.3.1	Phenotypic characterization of <i>Streptomyces</i> sp.	63
		3.3.2	Effect of Strain S-9 on Fusarium udum morphology	64
		3.3.3	Bioautography and purification of bioactive compounds	65
		3.3.4	LC-MS Analysis	66
		3.3.5	Proton nuclear magnetic resonance (¹ H NMR) of purified bioactive compounds	72
		3.3.6	FTIR Analysis	73
	3.4	Discus	sion	74
ļ	Statistical optimization of antifungal compound production by Streptomyces sp. S-9			77
	4.1	Introdu	uction	77
-	4.2	Materia	al and Methods	80
		4.2.1	Seed Stock Preparation	80
		4.2.2	Mycelial Biomass Determination	80
		4.2.3	Determination of antifungal activity by agar well diffusion method	80
		4.2.4	Selection of basal nutrient medium	81
		4.2.5	Optimization of selected media components by Response surface methodology	82

	4.3	Results	3	83
		4.3.1	Statistical optimization of culture medium	83
		4.3.2	Optimization of selected media components by Response surface methodology	90
		4.3.3	Optimization and Experimental Validation	94
	4.4	Discuss	sion	96
5	Gree	nhouse s	study and Field trials of <i>Streptomyces</i> sp. S-9 as a Biocontrol	0.0
	Agen	ıt		98
	5.1	Introdu	ction	98
	5.2	Materia	l and Methods	101
		5.2.1	Development of a powder formulation of <i>Streptomyces</i> sp. S-9	101
		5.2.2	Determining the shelf life of the powder formulation	102
		5.2.3	In vitro antagonism assay of the powder formulation	102
		5.2.4	Pot trials of the powder formulation under natural conditions	102
		5.2.5	Pot trial of the powder formulation S-9 under greenhouse conditions	104
		5.2.6	Field trial of the talcum formulation S-9	105
		5.2.7	Pre-emergence and post-emergence wilt incidence (%)	107
		5.2.8	Interaction between actinomycetes and AM fungi	107
		5.2.9	Quantification of mycorrhizal colonization	107
		5.2.10	Evaluation of plant vegetative parameters, biochemical, and defense enzymes	107
		5.2.11	Estimation of the contents of NPK in soil and plant	109
		5.2.12	Histology of Pigeon pea roots under greenhouse conditions	110
		5.2.13	Statistical analysis	110
	5.3	Results		110
		5.3.1	In vitro seed germination test (Vigor index)	110
		5.3.2	Efficacy of the powder formulation S-9 <i>in vitro</i> antagonism assay and pot experiments	112
		5.3.3	Field trial of the talcum powder formulation of S-9	114
		5.3.4	Compatibility of <i>Streptomyces</i> sp. S-9 with <i>R. irregularis</i>	119

		5.3.5	Mycorrhizal colonization of Pigeon pea	120	
		5.3.6	Ethylene Production	120	
		5.3.7	Proline Accumulation	121	
		5.3.8	Malonaldehyde and H ₂ O ₂ Content	121	
		5.3.9	Histochemical detection of H ₂ O ₂ accumulation	123	
		5.3.10	Determination of Defense-related Enzymes	123	
		5.3.11	Physicochemical Investigations of Cultivated Plants	124	
	5.4	Discuss	sion	127	
6	Who	le-genon	ne sequencing of Streptomyces sp. S-9 and Transcriptome	131	
	analysis of Pigeon pea under control and wilt condition				
	6.1	Introdu	ction	131	
	6.2	Materia	al and Methods	134	
		6.2.1	Genomic DNA extraction	134	
		6.2.2	Whole-genome sequencing of S-9	135	
		6.2.3	Quality control and assembly of the S-9 genome	135	
		6.2.4	Genome Annotation and Assembly	136	
		6.2.5	Antibiotics Resistance	136	
		6.2.6	COG Analysis	136	
		6.2.7	Secondary Metabolites	136	
		6.2.8	Transcriptome analysis and miRNA study	137	
		6.2.9	Plant material used	137	
		6.2.10	RNA Extraction and quality check	137	
		6.2.11	cDNA preparation	137	
		6.2.12	qRT-PCR	138	
		6.2.13	Novel gene prediction	138	
		6.2.14	Functional analysis	138	
		6.2.15	GO Enrichment analysis	138	
		6.2.16	KEGG Enrichment analysis	139	
	6.3	Results		139	
		6.3.1	Whole genome sequencing of S-9	139	
		6.3.2	Phylogenetic analysis and general genome features of S-9	139	
		6.3.3	Genome annotation	140	

	6.3.4	Average Nucleotide identity (ANI)	141
	6.3.5	Average Amino acid identity (AAI)	144
	6.3.6	Subsystem features of S-9	143
	6.3.7	Antibiotic resistance gene prediction analysis	145
	6.3.8	Cluster of orthologus group (COG) annotation	145
	6.3.9	Secondary metabolite and biosynthetic gene clusters	146
	6.3.10	RNA Extraction and quality check	147
	6.3.11	RNA Quality Check	147
	6.3.12	Result of miRNA	147
	6.3.13	qRT-PCR	149
	6.3.14	Transcriptome Analysis	150
	6.3.15	Correlation Analysis	150
	6.3.16	Differential Expression Analysis	150
	6.3.17	Novel genes	150
	6.3.18	Network Statistics	151
	6.3.19	Enrichment Analysis	151
	6.3.20	Coexpression Venn Diagram	152
	6.3.21	Differential Expression Analysis	152
	6.3.22	DEGs in Response to Fusarium infection in Cajanus cajan	153
	6.3.23	Cluster Analysis	155
	6.3.24	GO Enrichment Analysis	156
	6.3.25	KEGG Enrichment Analysis	158
5.4	Discuss	sion	160
Sum	mary		164
Conc	lusion		168
Refe	rences		170
ist (of Publics	ations and Presentations	234