Contents

Title	Contents	Pg.
		No.
	Declaration Research Scholar-Originality of Research	Ι
	Work	
	Certificate of Supervisor	II
	Thesis Approval Form	III
	Declaration by Research-Submission of Thesis	IV
	Acknowledgment	V
	Contents	VI
	List of Figures	
	List of Tables	
	Abstract	
Chapter:1	Introduction	
	1.1 Cumin plant morphology and botany	1
	1.1.1 Origin	1
	1.1.2 Area and Production	2
	1.1.3 International Scenario	2
	1.1.4 Indian Scenario	3
	1.2 Nutritional value of cumin plant	3
	1.3 Medicinal importance of cumin	4
	1.4 Cultivation of cumin	5
	1.4.1 Soil	5
	1.4.2 Climate	5
	1.4.3 Varieties	5
	1.4.4 Time of sowing	6
	1.4.5 Sowing method	6
	1.4.6 Manure and Fertilizers	6
	1.4.7 Irrigation/Watering in Cumin Farming	6

1.4.8 Harvesting time of cumin seeds	7
1.5 Cumin Pest and Diseases	7
1.5.1 Cumin wilt	7
1.5.1.1 Symptoms	9
1.5.2 Cumin Blight Disease	10
1.5.2.1 Economic Impact	10
1.5.2.2 Symptoms	10
1.5.2.3 Disease cycle	11
1.5.2.4 Favorable Circumstance	11
1.6 Management	12
1.6.1 Cultural method	12
1.6.2 Chemical methods	13
1.6.3 Biological control	13
1.7 Purpose of Research	14

Chapter: 2	Review of literature	
	2.1 Introduction	16
	2.1.1 Wilt causing pathogen-Fusarium oxysporum	17
	2.1.2 Blight causing pathogen-Alternaria burnsii	18
	2.2 Isolation, Identification, and Characterization of	19
	F. oxysporum and A. burnsü	
	2.2.1 Morphology of F. oxysporum & A. burnsii	20
	2.2.2 Purification of pathogens	21
	2.3 Koch's Postulates	21
	2.4 Invitro evaluation of pathogens	25
	2.5 Pot trail	26
	2.5.1 Management of Pathogens	28
	2.6 Biochemical parameter	31
	2.6.1 Chlorophyll	32
	2.6.2 Sugar	33
	2.6.3 Protein	34

	2.6.4 Phenolic content	36
Chapter: 3	Objectives of the research	37
Chapter: 4	Isolation, Identification, and Characterization of <i>F</i> . <i>oxysporum</i> and <i>A</i> . <i>burnsii</i> from Cumin Disease Plant	38
	oxysporum and A. burnsu from Cumm Disease Franc	
	4.1 General Laboratory Procedure	38
	4.1.1 Materials	38
	4.1.1.1 Culture media	38
	4.1.1.2 Chemicals and Reagents	38
	4.1.1.3 Apparatus and Equipment	39
	4.1.1.4 Glassware	40
	4.1.2 Glassware Cleaning	40
	4.1.3 Equipment & Requirements	40
	4.1.4 Culture Medium preparation	40
	4.1.5 Sterilization of Glassware and Culture Media	41
	4.1.6 Pouring of Medium	
	4.2 Mehods	41
	4.2.1 Collection of cumin plant samples infected by A.	42
	burnsii	
	4.2.2 Collection of cumin plant Samples infected by	43
	<i>F</i> .	
	oxysporum	
	4.2.3 Isolation, Identification, and Purification of	43
	pathogens from cumin disease plant	
	4.2.4 Storage and Maintenance of Pathogen	45
	4.2.5 Lactophenol Cotton Blue (LPCB) staining	45
	4.2.5.1 Principle of Lactophenol Cotton Blue (LPCB)	45
	Staining	

	4.2.5.2 Preparation of Lactophenol Cotton Blue	45
	Solution	
	4.2.5.3 Procedure for Lactophenol Cotton Blue	46
	Method	
	4.2.5.4 Cultural studies	46
	4.2.5.5 Morphological studies	47
	4.3 Results	47
	4.3.1 A. burnsii was identified on the basis of Cultural	47
	characters	
	4.3.1.1 Morphological characters A. burnsii under	47
	microscopic examination	
	4.3.2 F. oxysporum was identified on the basis of	49
	Cultural	
	characters	
Chapter: 5	Proving pathogenicity (Koch's Postulates) of isolated	51
	and identified fungal cultures	
	5.1. Introduction	51
	5.1.1 Koch's Postulates	52
	5.1.2 Limitation of Koch's Postulates	52
	5.1.3 Limitation when dealing with Root and stem	53
	pathogens	
	5.1.4 Limitation of studies when introducing new	53
	fungal	
	pathogens	
	5.2. Introductions for conducting a plant pathogenicity	54
	test	
	5.2.1 Inoculum preparation	55
	5.2.2 Host preparation	55
	5.2.2.1 Leaf preparation	55
	5.2.2.2 Petioles preparation	55

	5.2.2.3 Stem and Cane Preparation	55
	5.2.2.4 Seed preparation	56
	5.2.2.5 Root Preparation	56
5.2.3	3. Inoculation	57
	5.2.3.1 Leaf Inoculation	57
	5.2.3.2 Root Inoculation	57
5.2.4	4 Infectious Evaluation as well as Reinfection	58
5.3	Methods	58
	5.3.1 Pathogenicity test of A. burnsii on cumin	59
	5.3.2 Pathogenicity Test of F. oxysporum on Cumin	61
5.4 1	Result	61
	5.4.1 Recovering of Alternaria burnsii	61
	5.4.2 Recovering of F. oxysporum	61
6.1]	Introduction	63
	Methods	63
	6.2.1 Collection of native plants from Rajkot City	63
	6.2.1 Collection of native plants from Rajkot City6.2.2 Procedure for preparation of phytoextracts	63 64
	6.2.2 Procedure for preparation of phytoextracts	64
	6.2.2 Procedure for preparation of phytoextracts6.2.3 Using the poison food procedure to assess the	64
	6.2.2 Procedure for preparation of phytoextracts6.2.3 Using the poison food procedure to assess the antifungal	64
	6.2.2 Procedure for preparation of phytoextracts6.2.3 Using the poison food procedure to assess the antifungal activity of medicinal plant preparations.	64 65
6.3 1	 6.2.2 Procedure for preparation of phytoextracts 6.2.3 Using the poison food procedure to assess the antifungal activity of medicinal plant preparations. 6.2.4 Fungal strain 	64 65 65
6.3]	 6.2.2 Procedure for preparation of phytoextracts 6.2.3 Using the poison food procedure to assess the antifungal activity of medicinal plant preparations. 6.2.4 Fungal strain 6.2.5 Calculation Formula and Statistical Analysis 	64 65 65 65
6.3 1	 6.2.2 Procedure for preparation of phytoextracts 6.2.3 Using the poison food procedure to assess the antifungal activity of medicinal plant preparations. 6.2.4 Fungal strain 6.2.5 Calculation Formula and Statistical Analysis 	64 65 65 65 66
6.3]	 6.2.2 Procedure for preparation of phytoextracts 6.2.3 Using the poison food procedure to assess the antifungal activity of medicinal plant preparations. 6.2.4 Fungal strain 6.2.5 Calculation Formula and Statistical Analysis Results 6.3.1 Growth Inhibition of <i>A. burnsii</i> at various	64 65 65 65 66
6.3 1	 6.2.2 Procedure for preparation of phytoextracts 6.2.3 Using the poison food procedure to assess the antifungal activity of medicinal plant preparations. 6.2.4 Fungal strain 6.2.5 Calculation Formula and Statistical Analysis Results 6.3.1 Growth Inhibition of <i>A. burnsii</i> at various concentrations of phytoextract extracted in 	64 65 65 65 66

6.3.1.1 In vitro efficacy of phytoextract extracted in	66
water	
at different concentrations	
6.3.1.2 In vitro efficacy of phytoextract extracted in	72
acetone	
at different concentrations	
6.3.1.3 In vitro efficacy of phytoextract extracted in	78
cow	
urine at different concentrations	
6.3.2 After seven days of incubation at 28°C, the	84
growth of	
F. oxysporum was inhibited through different	
phytoextract amounts obtained with different	
solvents	
in order.	
6.3.2.1 In vitro efficacy of phytoextract extracted in	85
water	
at different concentrations	
6.3.2.2 In vitro efficacy of phytoextract extracted in	91
acetone	
at different concentrations on PDA media	
6.3.2.3 In vitro efficacy of phytoextract extracted in	97
cow	
urine at different concentrations	
Pot trial for evaluation of different botanicals	104
7.1 Information about the workplace	104
7.1.1. Experimental area	104
7.1.2 Climatic Condition	104
7.2 Methods	104

Chapter:7

7.2.1 Antifungal activity of plant extract against cumin	104
blight caused by Alternaria burnsii in the pot experiment	
7.2.1.1 Details of the Experiment.	105
7.2.1.2 Method of pot preparation	105
7.2.1.3 Preparation of plant extracts	106
7.2.1.4 Methods of Application	106
7.2.1.5 Methods of recording observation	106
7.2.1.6 Following the formula, the percentage of the	107
disease severity of Alternaria blight was	
calculated	
in this pot experiment	
7.2.1.7 PDRC (Percent disease reduction over control)	107
is	
calculated by following the equation	
7.2.1.8 Statistical Analysis	107
7.2.2 Evaluation of botanicals against wilt caused by F .	107
oxysporum in cumin plants grown in pots.	
7.2.2.1 Details of the Experiment	108
7.2.2.2 Preparation for Plant Extraction	108
7.2.2.3 Techniques for Recording Observations.	109
7.3. Results	109
7.3.1 Impact of botanicals on control of Alternaria	109
blight	
disease in cumin.	
7.3.1.1 Impact of botanicals on control of Alternaria	109
blight	
disease in cumin in the first and Second weeks	
after	
application of treatments	
7.3.1.2 One and Two weeks after the application of	110

treatments

7.3.1.3 Three weeks after the application of the	112
treatments	
7.3.1.4 Impact of botanicals on control of Alternaria	113
blight	
disease in cumin at four weeks after application	
of	
treatments	
7.3.1.5 Four-week application of the treatments	114
7.3.1.6 Impact of botanicals on control of Alternaria	115
blight	
disease in cumin at five weeks after application	
of	
treatments.	
7.3.1.7. Five weeks after the application of the	116
treatments	
7.3.1.8 Impact of botanicals on control of Alternaria	117
blight	
disease in cumin at six weeks after the	
application of	
treatments	
7.3.1.9 Six weeks after the application of the treatments	118
7.3.1.10 Impact of treatments on the development of	120
Alternaria blight disease of cumin at a	
different	
week	
7.3.2 Impact of botanicals on control of Fusarium	121
wilt	
disease in cumin.	
7.3.2.1 Impact of botanicals on control of Fusarium	121
wilt	

disease in cumin at first week after application	
of	
treatments.	
7.3.2.2 One week after the application of treatments	122
7.3.2.3 Impact of botanicals on control of wilt disease	122
in	
cumin the 2^{nd} week after the application of	
treatments	
7.3.2.4 Two weeks after the application of the	124
treatments.	
7.3.2.5 Impact of botanicals on control of wilt disease	124
of	
cumin at the 3 rd week after application of	
treatments	
7.3.2.6 Three weeks after the treatments were applied.	126
7.3.2.7 Impact of botanicals on control of Fusarium	127
wilt	
disease in cumin at four weeks after application	
of	
treatments.	
7.3.2.8 Four-week application of the treatments	128
7.3.2.9 Impact of botanicals on control of wilt disease	129
in	
cumin at five weeks after the application of	
treatments	
7.3.2.10 Five weeks after the application of the	130
treatments	
7.3.2.11 Impact of botanicals on control of Fusarium	130
wilt	
disease in cumin at six weeks after application	
of	

	treatments	
	7.3.2.12 Six weeks after the application of the	132
	treatments	
	7.3.2.13 Impact of various botanical plant extracts on	133
	control	
	of cumin wilt at different weeks after seed	
	germination.	
Chapter:8	Estimation of Biochemical test associated with resistance	136
	to wilt and blight disease	
	8.1 Introduction	136
	8.1.1 Protein	136
	8.1.2 Phenol	137
	8.1.3 Total sugar	137
	8.1.4 Chlorophyll	138
	8.2 Methods	138
	8.2.1 Collection of Samples	138
	8.2.2 Extraction and estimation of protein	139
	8.2.2.1 Principle	139
	8.2.2.2 Preparation of a pH 7.0 buffer with phosphate	139
	(0.1 M)	
	8.2.2.3 Extraction of protein	140
	8.2.2.4 Quantification of Total Protein Content	140
	8.2.2.5 Calculation formula for protein Estimation	141
	8.2.3 Estimation and extraction of phenol	141
	8.2.3.1. Principle	141
	8.2.3.2 Extraction of total phenol	141
	8.2.3.3 Calculation the concentration of total phenol	142
	8.2.4. Total sugar estimation and extraction	142
	8.2.4.1 Principle	142
	8.2.4.2 Extraction of total sugar	143

	8.2.4.3 Phenol sulphuric acid technique for calculating	143
	the	
	total sugar	
	8.2.4.4 Calculation formula	144
	8.2.5 Extraction and Estimation of Chlorophyll Lenz	144
	and	
	Zeitzchlen Method	
	8.2.6 Statistical Analysis	145
	8.3 Result	146
	8.3.2 Estimation of phenol by Folin-Ciocalteu method	147
	8.3.3 Estimation of total sugar	148
	8.3.4 Estimation of chlorophyll	150
Chapter:9	Discussion	152
Chapter:10	Summary and Conclusion	163
Chapter:11	References	166
Appendix-I	Invitro evaluation of A. burnsii and F. oxysporum	194
Appendix -II	Impact of various botanical treatments on the blight	203
	disease development of cumin in 3 rd week after	
	application	
Appendix-III	Biochemical test	213
Appendix -	Plagiarism Report	
IV		
Appendix -V	Publication	