

CONTENTS

1. INTRODUCTION	1-6
2. LITERATURE REVIEW	7-48
3. MATERIALS AND METHODS	49-77
3.1 Plant material	49
3.1.1 Selection	49
3.1.2 Growth and maintenance	50
3.2 Fungal culture	50
3.2.1 Source	50
3.2.2 Completion of Koch's postulate	52
3.2.3 Maintenance of Stock culture	52
3.2.4 Assessment of mycelial growth	52
3.3 Inoculation technique	53
3.3.1 Detached leaf	53
3.3.2 Cut shoot	53
3.3.3 Whole plant	54
3.4 Assessment of disease caused by fungal pathogens	54
3.4.1 Detached leaf	54
3.4.2 Cut shoot	54
3.4.3 Whole plant	54
3.5 Assessment of incidence of attack by <i>Helopeltis theivora</i>	55
3.6 Inducing agents and their application	55
3.6.1 Plant extract	55
3.6.2 Biocrop	56
3.6.3 Metabass	56
3.6.4 Salicylic acid	56
3.7 Extraction of total soluble proteins	57
3.7.1 Leaf protein	57
3.7.2 Mycelial protein	57
3.8 Estimation of protein content	57
3.8.1 SDS-PAGE analysis of total soluble protein	58

3.8.1.1 Preparation of stock solutions	58
3.8.1.2 Preparation of gel	59
3.8.1.3 Sample preparation	60
3.8.1.4 Electrophoresis	61
3.8.1.5 Fixing and staining	61
3.9 Extraction and assay of defense enzymes	61
3.9.1 Phenylalanine ammonia lyase (PAL)	61
3.9.2 Tyrosine ammonia lyase (TAL)	62
3.9.3 Polyphenol oxidase (PPO)	62
3.9.4 Peroxidase (PO)	63
3.9.5 Chitinase (CHT)	63
3.9.6 β -1, 3 Glucanase (BGLU)	64
3.10 Extraction of phenolics	64
3.11 Estimation of phenol content	65
3.11.1 Total phenol	65
3.11.2 Orthodihydroxy phenol	65
3.12 Extraction of antifungal phenol	65
3.12.1 Chromatographic analysis	66
3.12.2 Bioassay of antifungal phenols	66
3.12.2.1 TLC plate bioassay	66
3.12.2.2 Radial growth	67
3.12.2.3 Spore germination	67
3.12.2.4 UV-spectrophotometric analysis	67
3.12.3 Extraction of catechins from tea leaves	68
3.12.3.1 HPLC analysis of catechins	68
3.13 Preparations of antigens	68
3.13.1 Mycelial antigen	68
3.13.2 Leaf antigen	69
3.14 Production of polyclonal antibody	69
3.14.1 Immunization	69
3.14.2 Bleeding	70

3.15 Purification of IgG	70
3.15.1 Precipitation	70
3.15.2 Column preparation	71
3.15.3 Fraction collection	71
3.16 Immunodiffusion tests	71
3.17 Enzyme Linked Immunosorbent assay	72
3.18 Fluorescence antibody staining and microscopy	74
3.18.1 Fungal mycelia	74
3.18.2 Tea leaves	74
3.19 Dot-immuno binding assay	75
3.20 Western blotting	75
3.21 Scanning Electron Microscope (SEM)	76
3.22 Transmission Electron Microscope (TEM)	77
4. EXPERIMENTAL	78-207
4.1 Assessment of damage potentiality on different tea varieties following <i>H. theivora</i> infestation	78
4.2 Changes in the level of phenol and protein in <i>H. theivora</i> infested tea leaves	84
4.2.1 Total phenol	84
4.2.2 Orthodihydroxy phenol	84
4.2.3 Protein	87
4.3 Determination of activity of defense enzymes in healthy and <i>H. theivora</i> infested tea leaves	87
4.3.1 Phenylalanine ammonia lyase (PAL)	91
4.3.2 Peroxidase (PO)	91
4.3.3 Polyphenoloxidase (PPO)	91
4.3.4 β -1,3-glucanase (β GLU)	96
4.3.5 Chitinase (CHT)	97
4.3.5.1 Dot blot	99
4.3.5.2 Western blotting	102
4.4 Detection of antifungal compounds in tea leaves following <i>H. theivora</i> infestation	102

4.4.1 Antifungal phenols	104
4.4.1.1 Radial growth bioassay	104
4.4.1.2 Slide germination bioassay	105
4.4.1.3 HPLC analysis	106
4.4.2 HPLC analysis of catechins	108
4.5 Application of bioresources for induction of resistance in tea plants against <i>H. theivora</i>	113
4.5.1 Metabass	113
4.5.2 Biocrop	116
4.5.3 Plant extract	116
4.6 Screening of resistance of tea plants against foliar fungal pathogens	123
4.6.1 Detached leaf	133
4.6.2 Cut shoot	135
4.6.3 Whole plant	135
4.7 Immuno-detection of fungal pathogens in tea leaf tissues	138
4.7.1 Plate Trape Antigen Enzyme Linked Immunosorbant Assay (PTA-ELISA)	138
4.7.1.1 Optimization of ELISA	139
4.7.1.1.1 Enzyme dilution	139
4.7.1.1.2 Antisera dilution	139
4.7.1.1.3 Antigen dilution	141
4.7.2 Detection of fungal pathogens in tea leaf tissues	143
4.7.3 Dot immunobinding assay	146
4.7.4 Immunocytochemical staining	146
4.7.5 Immunofluorescence	151
4.7.5.1 Mycelia	154
4.7.5.2 Tea leaf tissues	154
4.8 Changes in the level of phenols and proteins in tea leaves following inoculation with foliar fungal pathogens	160
4.8.1 Total phenol	160
4.8.2 Orthodihydroxy phenol	160
4.8.3 Soluble proteins	165

4.9 Determination of level of defense enzymes in tea plants following inoculation with foliar fungal pathogens	165
4.9.1 Phenylalanine ammonia lyase (PAL)	165
4.9.2 Polyphenoloxidase (PPO)	170
4.9.3 Peroxidase (PO)	172
4.9.4 β -1,3-glucanase (β GLU)	173
4.9.5 Chitinase (CHT)	175
4.10 Accumulation of pyrocatechol and catechins in tea leaves following inoculation with <i>A. alternata</i>	177
4.10.1. Radial growth bioassay	177
4.10.2 TLC plate bioassay	179
4.10.3 Glass slide bioassay	179
4.10.4 HPLC analysis of pyrocatechol	181
4.10.5 HPLC analysis of catechins	184
4.11 Application of plant extracts for induction of resistance in tea plants against <i>Alternaria alternata</i>	187
4.11.1 <i>In vitro</i> test	187
4.11.2 <i>In vivo</i> test	187
4.11.3 Biochemical analysis	191
4.11.3.1 Phenolics	191
4.11.3.2 Defense enzymes	191
4.11.4 Western Blot analysis	200
4.11.5 Immunohistology	200
4.12 Cellular localization of defense enzyme in tea leaf tissues following induction by Salicylic acid	203
4.12.1 Indirect immunofluorescence	205
4.12.2 Immunogold localization	205
5. DISCUSSION	208-230
6. SUMMARY	231-234
7. REFERENCES	235-269