

Contents

1. Introduction	1-3
2. Review of Literature	4-38
3. Materials and Methods	39-66
3.1. Plant material	39
3.1.1. Growth and maintenance	39
3.2. Fungal culture	42
3.2.1. Source of culture	42
3.2.2. Completion of Koch's postulates	42
3.2.3. Maintenance of stock culture	42
3.2.4. Assessment of mycelial growth	43
3.2.4.1. Solid media	43
3.2.4.2. Liquid media	44
3.3. Inoculation technique	44
3.3.1. Inoculum preparation	44
3.3.1.1. Fungal pathogen	44
3.3.1.2. Biocontrol agent	44
3.3.2. Inoculation of healthy tea seedlings in pot	44
3.4. Disease assessment	45
3.5. Soluble protein	45
3.5.1. Extraction	45
3.5.2. Estimation	45
3.6. SDS-PAGE analysis of total protein	46
3.6.1. Preparation of stock solutions	46
3.6.2. Slab gel preparation	47
3.6.3. Sample preparation	48
3.6.4. Electrophoresis	49
3.6.5. Fixing and staining	49
3.7. Extraction and estimation of phenolics	49
3.7.1. Extraction	49
3.7.2. Estimation	49
3.7.2.1. Total phenol	49
3.7.2.2. Ortho-dihydroxy phenol	50
3.8. Extraction of antifungal phenolics	50
3.8.1. Chromatographic analysis	51
3.8.2. Bioassay of antifungal phenols	51
3.8.2.1. Radial growth	51
3.8.2.2. Sclerotial germination	52

3.8.3. UV-spectrophotometric analysis	52
3.8.4. HPLC analysis	52
3.9. Extraction of enzymes	52
3.9.1. Phenylalanine ammonia lyase (EC 4.3.1.5.)	52
3.9.2. Peroxidase (EC 1.11.1.7)	53
3.9.3. Polyphenol oxidase (EC 1.10.3.2)	53
3.10. Assay of enzyme activities	53
3.10.1. Phenylalanine ammonia lyase	53
3.10.2. Assay of peroxidase	54
3.10.3. Polyphenol oxidase	54
3.11. Preparation of antigens	54
3.11.1. Root antigen	54
3.11.2. Mycelial antigen	55
3.11.3. Soil antigen	55
3.12. Serology	55
3.12.1. Maintenance of rabbit	55
3.12.2. Immunization	56
3.12.3. Bleeding	56
3.12.4. Purification of IgG	56
3.12.4.1. Precipitation	56
3.12.4.2. Column preparation	57
3.12.4.3. Fraction collection	57
3.13. Immunodiffusion	57
3.13.1. Preparation of agarose gel plates	57
3.13.2. Diffusion	58
3.13.3. Washing, staining and drying of slides	58
3.14. Enzyme linked immunosorbent assay (ELISA)	58
3.14.1. Plate trapped antigen ELISA	59
3.15. Immunoblotting	60
3.15.1. Dot-immunobinding assay	60
3.15.2. Western blotting	61
3.15.2.1. SDS-PAGE of protein	62
3.15.2.2. Blot transfer process	62
3.15.2.3. Immunoprobng	62
3.16. Immunofluorescence	63
3.16.1. Mycelia	63
3.16.2. Cross section of tea roots	63

3.17. Inducing agents and their application	64
3.17.1. <i>In vitro</i> test	64
3.17.2. <i>In vivo</i> test	65
4. Experimental	67-150
4.1. Sclerotial blight disease occurrence under natural conditions	67
4.2. Factors influencing mycelial growth of <i>S. rolf sii</i>	69
4.3.1. Media	69
4.2.2. Incubation period	69
4.2.3. pH of Medium	71
4.2.4. Carbon sources	75
4.2.5. Nitrogen sources	76
4.3. Varietal resistance of tea against <i>Sclerotium rolf sii</i>	79
4.4. Estimation and analysis of proteins in fungal mycelia and tea roots following infection	83
4.4.1. Protein content in tea roots following infection	83
4.4.2. Protein content in fungal mycelia	83
4.4.3. SDS-PAGE analysis of fungal protein	86
4.5. Detection of cross reactive antigens between <i>Sclerotium rolf sii</i> and tea varieties	86
4.5.1. Immunodiffusion tests	87
4.5.2. Plate trapped antigen – ELISA	92
4.5.2.1. Optimization of ELISA	92
4.5.2.1.1. Enzyme dilution	92
4.5.2.1.2. Antiserum dilution	92
4.5.2.1.3. Antigen dilution	93
4.5.3. Comparison of ELISA reactivity among antigens of different tea varieties against antiserum of <i>S. rolf sii</i>	95
4.5.4. Cellular location of CRA using immunofluorescence	98
4.6. Detection of <i>Sclerotium rolf sii</i> in artificially inoculated tea root tissue	104
4.6.1. PTA-ELISA	104
4.6.2. Dot immunobinding assay	104
4.6.3. Western blot	105
4.6.4. Indirect immunofluorescence	107
4.6.4.1. Mycelia	107
4.6.4.2. Sclerotia	107
4.6.4.3. Tea root tissue	107
4.7. Determination of levels of phenolics in tea roots of resistant and susceptible varieties following inoculation with <i>S. rolf sii</i>	110
4.7.1. Total phenols	110

4.7.2. Ortho-dihydroxy phenols	110
4.7.3. Analysis of antifungal compound in tea roots following inoculation with <i>S. rolfsii</i>	114
4.7.3.1. Bioassay	115
4.7.3.2. UV-spectrophotometric analysis	115
4.7.3.3. HPLC analysis	118
4.8. Determination of enzyme activity in healthy and <i>S. rolfsii</i> inoculated tea roots	120
4.8.1. Phenylalanine ammonia lyase (PAL)	120
4.8.2. Peroxidase (PO)	120
4.8.3. Polyphenol oxidase (PPO)	120
4.9. Management of seedling blight	127
4.9.1. <i>In vitro</i> evaluation	127
4.9.1.1. Plant extract	127
4.9.1.2. Fungicides	129
4.9.1.3. Biocontrol agents	129
4.9.2. <i>In vivo</i> test	133
4.9.2.1. Growth promotion in tea seedlings	133
4.9.2.2. Disease development	137
4.10. Changes associated with induction of resistance in tea plants	139
4.10.1. Biochemical changes	139
4.10.2. Serological changes	145
4.10.2.1. Immunodiffusion test	145
4.10.2.2. PTA-ELISA	146
4.10.2.3. Dot-immunobinding assay	149
5. Discussion	151-166
6. Summary	167-169
7. References	170-194