

CONTENTS

1. INTRODUCTION	1
2. REVIEW OF LITERATURE	5
3. MATERIALS AND METHODS	32
3.1. Plant Material	32
3.1.1. Collection	32
3.1.2. Propagation	32
3.1.2.1. Cutting	
3.1.2.2. Seed germination	
3.1.3. Plantation	33
3.1.4. Maintenance	33
3.2. Fungal culture	34
3.2.1. Source of culture	34
3.2.2. Completion of Koch's Postulate	39
3.2.3. Maintenance of stock culture	39
3.2.4. Assessment of mycelial growth	39
3.2.4.1. Solid media	
3.2.4.2. Liquid media	
3.3. Inoculation technique	41
3.3.1. Detached leaf	41
3.3.2. Cut shoot	41
3.4. Assessment of disease intensity	42
3.4.1. Detached leaf	42
3.4.2. Cut shoot	42
3.5 Preparation of antigen	42
3.5.1 Fungal antigen	42
3.5.1.1. Mycelia	
3.5.1.2. Cell Wall	
3.5.2. Leaf antigen	43
3.5.2.1. Healthy leaf	
3.5.2.2. Artificially inoculated leaf	
3.5.2.3. Naturally infected leaf	

3.6 Purification	44
3.6.1. SAS fraction	44
3.6.2. Ion exchange chromatography	44
3.7. SDS-PAGE of Protein	45
3.7.1. Preparation gel column or slab	45
3.7.2. Sample preparation	47
3.7.3. Electrophoresis	47
3.7.4. Fixing	48
3.7.5. Staining	48
3.8. Cell wall characterization	48
3.8.1. Extraction	48
3.8.2. Protein estimation	49
3.8.3. Carbohydrate estimation	49
3.8.4. SDS-PAGE Analysis	49
3.8.4.1. Gel preparation	
3.8.4.2. Sample preparation	
3.8.4.3. Electrophoresis	
3.8.4.4. Fixing	
3.8.4.5. Staining	
3.8.5. Binding of FITC labelled concanavalin A	51
3.9. Agglutination response of conidia	51
3.9.1. Preparation of conidial suspension	51
3.9.2. Agglutination test	51
3.10. Antisera production	52
3.10.1. Rabbits and their maintenance	52
3.10.2. Immunization	52
3.10.3. Bleeding	52
3.11. Purification of IgG fraction	53
3.11.1. Precipitation	53
3.11.2. Column Preparation	53
3.11.3. Fraction collection	53

III

3.12. Immunodiffusion tests	54
3.12.1. Preparation of agarose slides	54
3.12.2. Diffusion	54
3.12.3. Washing, staining and drying of slides	54
3.13. Immunoelectrophoresis	54
3.13.1. Preparation of agarose slides	54
3.13.2. Electrophoresis	55
3.13.3. Diffusion	55
3.13.4. Washing, staining and drying of slides	55
3.14. Rocket immunoelectrophoresis	55
3.14.1. Preparation of agarose slides	55
3.14.2. Electrophoresis	55
3.14.3. Washing, staining and drying of slides	56
3.15. Enzyme-linked immunosorbent assay	56
3.15.1. Indirect ELISA	57
3.15.2. Direct ELISA	58
3.15.3. Competition ELISA	58
3.16. Estimation of fungal mycelium in leaf tissue	59
3.17. Establishment of callus	59
3.17.1. Culture media	59
3.17.2. Preparation of fragile callus	61
3.18. Fluorescent antibody staining and microscopy	61
3.18.1. Loosened cells	61
3.18.2. Cross sections of tea leaves	62
3.18.3. Mycelia	62
3.18.4. Conidia	62

IV

4. EXPERIMENTAL	63
4.1. Pathogenicity tests of <i>Pestalotiopsis theae</i> on different tea varieties	63
4.1.1. Detached leaf	63
4.1.2. Cut shoot	69
4.2. Cultural conditions effecting growth of <i>P.theae</i>	69
4.2.1. Solid media	71
4.2.2. Liquid media	72
4.2.2.1. Incubation period	
4.2.2.2. pH	
4.2.2.3. Carbon source	
4.2.2.4. Nitrogen source	
4.3. Detection of cross-reactive antigens (CRA) between <i>P.theae</i> and tea varieties	79
4.3.1. Immunodiffusion tests	79
4.3.2. Immunoelectrophoresis	85
4.3.3. Rocket-immunoelectrophoresis	87
4.3.4. Direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA)	87
4.3.4.1. Optimization of ELISA	
4.3.4.1.1. Antiserum raised against antigen preparation from mycelia of <i>P.theae</i>	
4.3.4.1.1.1. Enzyme dilution	
4.3.4.1.1.2. Antiserum dilution	
4.3.4.1.1.3. Antigen dilution	
4.3.4.1.2. Antiserum raised against antigen preparation from cell wall of <i>P.theae</i>	
4.3.4.1.2.1. Antiserum dilution	
4.3.4.1.2.2. Antigen dilution	
4.3.4.2. Comparison of ELISA reactivity among antigens of different tea varieties against antiserum of <i>P.theae</i>	
4.3.4.2.1. Mycelia	
4.3.4.2.2. Cell wall	

4.3.4.3. Comparison of ELISA reactivity among antigens prepared from tea leaves of various ages against antiserum of <i>P.theae</i>	110
4.3.4.3.1. Mycelia	
4.3.4.3.2. Cell wall	
4.3.4.4. Reciprocal cross reaction of antisera of tea varieties and non pathogen with leaf antigens (host and non host) and mycelial antigens (pathogen and non pathogen)	
4.3.5. Double antibody sandwich (DAS)-ELISA	
4.4. Detection of <i>P.theae</i> in infected tea leaf tissues by indirect ELISA	113
4.4.1. Artificially inoculated tea leaves	113
4.4.2. Naturally infected tea leaves	113
4.4.3. Different times after inoculation	116
4.4.3.1. Anti- <i>P.theae</i> antiserum (mycelium)	
4.4.3.2. Anti- <i>P.theae</i> antiserum (cell wall)	
4.4.4. Different antigen concentration	122
4.5. Detection of infection with other pathogens using anti - <i>P.theae</i> antiserum	124
4.5.1. DAC-ELISA	124
4.5.2. Competition ELISA	124
4.6. Determination of cross - reactivity of anti- <i>P.theae</i> antiserum	127
4.7. Estimation of mycelia in tea leaf tissues at different times after inoculation with <i>P.theae</i>	128
4.7.1. Different times after inoculation	128
4.7.2. Different varieties	128
4.8. Purification of cross reactive antigen (s) from mycelia of <i>P.theae</i>	133
4.8.1. Ammonium sulphate fractionation	133
4.8.1.1. Immunodiffusion tests	
4.8.1.2. DAC-ELISA	
4.8.2. DEAE-Sephadex chromatography	133
4.8.2.1. DAC-ELISA	
4.8.2.2. SDS-PAGE	

4.9. Evaluation of antiserum raised against purified mycelial antigens of <i>P.theae</i>	141
4.9.1. Immunodiffusion tests	141
4.9.2. ELISA	141
4.9.2.1. Detection of CRA among selected tea varieties	
4.9.2.1.1. DAC-ELISA	
4.9.2.1.2. DAS-ELISA	
4.9.2.2. Detection <i>P.theae</i> in artificially inoculated tea leaves of selected varieties	
4.9.2.2.1. DAC-ELISA	
4.9.2.2.2. DAS-ELISA	
4.10. Characterization of mycelial wall and conidial wall of <i>P.theae</i>	148
4.10.1. Mycelial wall	148
4.10.1.1. Con A - FITC binding	
4.10.1.2. SDS - PAGE analysis	
4.10.2. Conidial wall	148
4.11. Callus induction	153
4.12. Immunofluorescence	153
4.12.1. Loosened cell	153
4.12.2. Leaf tissues	155
4.12.3. Mycelia and conidia	155
5. DISCUSSION	159
6. SUMMARY	171
7. REFERENCES	175