

## CONTENTS

	PAGE
<b>1. INTRODUCTION .....</b>	<b>1-4</b>
<b>2. LITERATURE REVIEW .....</b>	<b>5-33</b>
<b>3. MATERIALS AND METHODS .....</b>	<b>34-57</b>
<b>3.1 Plant material .....</b>	<b>34</b>
3.1.1. Collection .....	34
3.1.2. Propagation by cuttings .....	35
3.1.3. Maintenance of tea sleeves in nursery .....	35
3.1.4. Maintenance of germplasm in glass house .....	37
3.1.5. Plantation .....	37
<b>3.2. Collection of blister infected tea leaves .....</b>	<b>39</b>
<b>3.3. Inoculation technique .....</b>	<b>39</b>
3.3.1. Collection of spores from infected leaves .....	39
3.3.2. Spore germination test .....	40
3.3.3. Inoculation of healthy tea plants .....	40
<b>3.4. Disease assessment .....</b>	<b>40</b>
3.4.1. Natural infection of field grown plants .....	40
3.4.2. Artificial inoculation of nursery plant .....	41
<b>3.5. Obtaining Meteoriological data .....</b>	<b>41</b>
<b>3.6. Foliar spray .....</b>	<b>41</b>
3.6.1. Systemic fungicide .....	41
3.6.2. Biocide .....	41
<b>3.7. Soluble leaf protein .....</b>	<b>42</b>
3.7.1. Extraction .....	42
3.7.2. Estimation .....	42
<b>3.8. SDS PAGE analysis of total soluble protein .....</b>	<b>42</b>
3.8.1. Preparation of stock solutions .....	42
3.8.2. Preparation of gel .....	43
3.8.3. Sample preparation .....	44
3.8.4. Electrophoresis .....	45
3.8.5. Fixing and staining .....	45

3.9. Preparation of antigens .....	46
3.9.1. Leaf antigen .....	46
3.9.2. Spore antigen .....	46
3.10. Serology .....	46
3.10.1. Rabbits and their maintenance .....	46
3.10.2. Immunization .....	46
3.10.3. Bleeding .....	47
3.11. Antisera production .....	47
3.11.1. Polyspecific .....	47
3.11.2. Polyclonal .....	48
3.12. Purification of IgG .....	48
3.12.1. Precipitation .....	48
3.12.2. Column preparation .....	48
3.12.3. Fraction collection .....	49
3.13. Immunodiffusion tests .....	49
3.13.1. Preparation of agarose slides .....	49
3.13.2. Diffusion .....	49
3.13.3. Washing, staining and drying of slides .....	50
3.14. Enzyme linked immunosorbent assay (ELISA) .....	50
3.15. Establishment of Callus .....	52
3.15.1. Culture media .....	52
3.15.2. Preparation of fragile callus.....	53
3.16. Fluorescence antibody staining and microscopy .....	54
3.16.1. Blister spore .....	54
3.16.2. Cross-section of tea leaf .....	54
3.16.2.1. Healthy leaf .....	54
3.16.2.2. Infected leaf .....	55
3.17. Dot-Blot .....	55
3.18. Western blotting .....	56
3.18.1. Extraction of soluble proteins .....	56
3.18.2. SDS-PAGE analysis .....	56
3.18.3. Transfer process .....	56
3.18.4. Immunoblotting .....	56

<b>4.</b>	<b>EXPERIMENTAL .....</b>	<b>58-144</b>
4.1.	Blister blight disease occurrence under natural conditions.....	58
4.1.1.	Nursery .....	58
4.1.2.	Tea gardens .....	58
4.1.2.1.	Plains .....	59
4.1.2.2.	Hills .....	59
4.2.	Meteorological data (Temperature, Relative humidity and Rainfall).....	66
4.2.1.	Correlation of weather conditions with occurrence of blister blight disease.....	66
4.3.	Pathogenicity test of <i>Exobasidium vexans</i> on different tea varieties.....	77
4.4.	Detection of cross-reactive antigens between <i>E. vexans</i> and tea varieties. ....	81
4.4.1.	Immunodiffusion tests.....	81
4.4.2.	Direct antigen coated enzyme linked immunosorbent assay.....	83
4.4.2.1.	Optimization of ELISA .....	84
4.4.2.1.1.	Polyspecific antibody (PS-I) raised against blister infected tea leaves of Castleton Tea Estate.....	84
4.4.2.1.1.1.	Antigen dilution .....	84
4.4.2.1.1.2.	Antiserum dilution....	86
4.4.2.1.2.	Polyspecific antibody (PS-II) raised against blister infected tea leaves of Hansqua Tea Estate.....	86
4.4.2.1.2.1.	Antigen dilution.....	86
4.4.2.1.2.2.	Antiserum dilution ....	91
4.4.2.1.3.	Polyclonal antibody (PC-I) raised against basidiospores collected from blister infected leaves of Castleton Tea Estate.....	94
4.4.2.1.3.1.	Antigen dilution.....	94
4.4.2.1.3.2.	Antiserum dilution....	94

4.4.2.2.	Comparison of ELISA reactivity among antigens of different tea varieties against antiserum of <i>E. vexans</i> , (PS-I; PS-II, PC-I).....	100
4.4.2.3.	Reciprocal cross reaction of antisera of tea varieties and non pathogen with leaf antigens (host and non host) and fungal antigens (pathogen and non pathogen).....	110
4.5.	Detection of <i>E. vexans</i> in naturally infected tea leaf tissues by indirect ELISA (PS-I & PS-II).....	111
4.6.	Detection of <i>E. vexans</i> in artificially inoculated tea leaf tissues by indirect ELISA (PS-I, PS-II and PC-I).....	115
4.7.	Detection of <i>E. vexans</i> in artificially inoculated tea leaf tissues at different times after inoculation, by indirect ELISA (PS-I, PS-II and PC-I).....	124
4.8.	Immunofluorescence .....	124
4.8.1.	Healthy leaf .....	131
4.8.2.	Fragile Callus .....	131
4.8.3.	Blister infected leaves .....	131
4.8.4.	Basidiospores of <i>E. vexans</i> .....	131
4.9.	Dot immunoblotting.....	138
4.10.	Western blotting .....	138
4.11.	Disease management.....	140
4.11.1.	Systemic fungicide.....	140
4.11.2.	Biocide.....	143
4.12.	DAC-ELISA response of tea leaves after treatment with systemic fungicide and biocides.....	144
5.	DISCUSSION.....	145-153
6.	SUMMARY .....	154-155
7.	REFERENCES.....	156-172