

# Contents

1. Introduction	1
2. Literature Review	8
3. Materials and Methods	39
3.1. Plant materials	39
3.1.1. Host plants	39
3.1.2. Plants for extraction of botanicals used for induction studies	39
3.2. Fungal culture	39
3.2.1. Source	39
3.2.2. Verification of Koch's postulates	43
3.3. Major chemicals used	43
3.4. Composition of media and solutions used	43
3.5. Growth and physiology of the pathogen <i>Lasiodiplodia theobromae</i>	50
3.5.1. Influence of different culture media	50
3.5.2. Assessment of growth and sporulation	50
3.5.3. Influence of light conditions	50
3.5.4. Influence of different pH	51
3.5.5. Influence of temperature	51
3.6. Inoculation with pathogen and disease assessment	51
3.6.1. Leaf inoculation technique	51
3.6.2. Root inoculation technique	51
3.6.3. Leaf disease assessment	52
3.6.4. Root disease assessment	52
3.7. Antigen preparation	53
3.8. Immunization of rabbits and collection of sera	55

3.9. Evaluation of antisera for presence of cross-reactive antigens (CRA)	57
3.9.1. Immunodiffusion (ID)	57
3.9.2. Immunoelectrophoresis (IE)	57
3.10. Screening of commercially cultivated tea varieties by indirect ELISA	59
3.11. Cellular location of CRA	60
3.11.1. Immunofluorescence (IF) staining of host and pathogen	60
3.11.2. Immuno gold labeling and silver enhancement	60
3.12. Application of inducers	61
3. 12. 1. Application of abiotic inducers	61
3. 12. 2. Application of biotic inducers	62
3. 12. 3. Application of plant extracts as inducers	62
3.13. Assay of Phenylalanine ammonialyase (PAL)	64
3.14. Assay of Polyphenol oxidase (PPO)	64
3.15. Assay of Chitinase	65
3.15.1. Estimation of Chitinase	65
3.15.2. Chitinase activity by chitin supplemented plate method	65
3.16. Assay of $\beta$ -1,3-glucanase	66
3.17. Assay of Peroxidase	66
3.18. Polyacrylamide gel electrophoresis (PAGE)	67
3.19. Analysis of isozymes patterns	68
3.19.1. Polyphenol oxidase	68
3.19.2. $\beta$ -1,3-glucanase	68
3.19.3. Peroxidase	69
3.20. Statistical analysis	69
4. Experimental	70

4.1. Chapter I: Studies on morphology and physiology of <i>Lasiodiplodia theobromae</i>	70
4.1.1. Effect of different liquid medium on growth and sporulation of <i>L. theobromae</i>	70
4.1.2. Effect of Light on growth and sporulation of <i>L. theobromae</i>	72
4.1.3. Effect of temperature on growth and sporulation of <i>L. theobromae</i>	74
4.1.4. Effect of pH on growth and sporulation of <i>Lasiodiplodia theobromae</i>	74
4.2. Chapter II: Pathogenicity of <i>Lasiodiplodia theobromae</i> in different tea varieties	77
4.2.1. Pathogenicity on leaves	77
4.2.1. Pathogenicity on roots	78
4.3. Chapter III : Serological studies	83
4.3.1. Serological relationship between different tea varieties and test pathogen ( <i>L. theobromae</i> ) by agar gel double diffusion	84
4.3.2. Serological relationship between different tea varieties and test pathogen ( <i>L. theobromae</i> ) by immunoelectrophoresis	87
4.3.3. Indirect ELISA between <i>L. theobromae</i> and different tea seed varieties	87
4.3.4. Comparative analysis of ELISA and Cross reactive antigens (CRA)	94
4.3.5. Immunofluorescence for cellular location of antigens and cross-reactive antigens	94
4.3.6. Immunogold labelling for cellular location of antigens and cross-reactive antigens	97
4.4. Chapter IV: Studies on defense related enzyme: phenylalanine ammonia-lyase (PAL)	102
4.4.1. PAL activity with abiotic inducers	102
4.4.2. PAL activity with biotic inducers	103

4.4.3. PAL activity with phyto-extracts	105
4.5. Chapter V: Studies on defense related enzyme: polyphenol oxidase (PPO)	109
4.5.1. PPO activity with abiotic inducers	109
4.5.2. PPO activity with biotic inducers	109
4.5.3. PPO activity with phyto-extracts	110
4.5.4. Study of PPO isoform patterns	112
4.6. Chapter VI: Studies on defense related enzyme: $\beta$ -1,3-glucanase	118
4.6.1. $\beta$ -1, 3-glucanase activity with abiotic inducers	118
4.6.2. $\beta$ -1, 3-glucanase activity with biotic inducers	118
4.6.3. $\beta$ -1, 3-glucanase activity with phyto-extracts	122
4.6.4. Study of $\beta$ -1,3-glucanase isoform pattern	122
4.7. Chapter VII: Studies on defense related enzyme: Chitinase	127
4.7.1. Chitinase activity with abiotic inducers	127
4.7.2. Chitinase activity with biotic inducers	128
4.7.3. Chitinase activity with phyto-extracts	128
4.7.4. Chitinase activity by chitin supplemented plate method	130
4.8. Chapter VIII: Studies on defense related enzyme: peroxidase	137
4.8.1. Peroxidase with abiotic inducer	137
4.8.2. Peroxidase activity with biotic inducers	137
4.8.3. Peroxidase activity with phyto-extracts	138
4.8.4. Study of peroxidase isoform pattern	140
4.9. Chapter IX: Foliar application of inducers, for controlling diplovia disease caused by <i>L. theobromae</i> in tea plants	145
5. Discussion	148
6. Summary	168
7. References	172