SUMMARY

1. A review of literature pertaining to this investigation has been presented which deals mainly with serological techniques for the detection of plant pathogenic fungi.

2. Materials used in this investigation and experimental procedures followed have been discussed in detail.

3. Pathogenicity of *Fomes lamaoensis* was tested on twenty five varieties (10 Tocklai, 9 Darjeeling and 6 UPASI) of tea. Among these, TV-18, T-78 and UP-26 appeared to be susceptible and TV-26, BS/74/76 and UP-9 were found to be resistant.

4. Cultural conditions affecting growth of *F. lamaoensis* were studied with special reference to their growth in different media, variable pH and different types of carbon, organic and inorganic nitrogen source. Maximum growth of the pathogen occurred in Potato dextrose agar while minimum growth was noticed in Czapex-dox. Lactose was the most effective carbon source whereas Beef extract was most effective nitrogen source followed by yeast extract and casein hydrolysate. Organic nitrogen sources were found to be better than inorganic nitrogen sources, though no growth was observed in urea as organic and optimum growth in calcium nitrate as inorganic source.

5. Protein content of healthy and artificially inoculated tea root tissues from 25 different tea varieties as well as mycelia and cell wall proteins of *F. lamaoensis was* estimated and analysed in SDS-PAGE.

6. Characterization of the cell wall of *F. lamaoensis* by ConA-FITC binding revealed its glycoprotein nature.

7. Polyclonal antibodies (PAbs) were raised against antigen preparations from mycelia and cell wall of *F. lamaoensis,* mycelia of bioconrol agents (*T. harzianum* and *T. viride*) and tea root tissues (UP-26 and TV- 26). These were purified by ammonium sulphate precipitation followed by DEAE cellulose chromatography. IgG obtained in each case was used for different immunoenzymatic tests.

8. To check the effectiveness of PAbs, Agar gel double diffusion tests were performed using crude antibody as well as purified IgG obtained from different bleeding collected for the pathogen. Strong precipitin reactions were observed in homologous

cross reaction of each case.

9. Optimization of ELISA using PAbs of *F. lamaoensis* and antigen preparations at variable concentrations were performed. ELISA values decreased with the decrease of antigen concentrations ranging from 40 to 0.312mg/ml. However maximum absorbance values was obtained in 3rd bleeding followed by 4th bleedings.

10. DAC-ELISA tests were performed separately using PAbs raised against mycelia and cell wall antigens of *F. lamaoensis* against root antigens prepared from 25 different tea varieties, non-pathogen and non-host and major cross reactive antigens (CRA) shared between tea varieties and *F. lamaoensis* were detected.

11. Detection of *F. lamaoensis* in artificially inoculated tea root tissues using DAC-ELISA and DAS-ELISA formats were standardized.

12. Antigens prepared from soil samples collected different tea field and amended soil, were tested against PAb of *F. lamaoensis* using DAC-ELISA formats and Dot blot analysis. Amended soils gave very high values comparable to homologous values and ELISA values decreased with the increase in days of incubation in field condition after an optimum period.

13. Purification of antigenic protein of *F. lamaoensis* from the crude mycelial extracts by ammonium sulphate saturation was done and detection was done by immunodiffusion, ELISA and Western blot.

14. Antigenic preparation of 60-80% SAS fraction was used for raising antiserum and this antiserum was tested by immunodiffusion test and IgG was purified. CRA between *F. lamaoensis* and tea varieties, non-host and non-pathogen was detected in DAC-ELISA. This antiserum also showed higher reactivity in the susceptible reactions as compared to resistant reaction.

15. PAb raised to 60-80% SAS fraction could also significantly detect infections by DAC-ELISA, Dot blot and Western blot analysis.

16. Cross sections of tea roots (TV-18, UP-26 and T-78) treated with PAb of *F.*

lamaoensis and then labelled with FITC developed a bright fluorescence throughout the sections, extending upto vascular tissues as well as outer surface.

17. Mycelia of *F. lamaoensis* when treated with homologous antisera followed by FITC, bright fluorescence was noticed on young hyphae.

18. Reactions of various antigens (fungal and root) with PAb of *F. lamaoensis* has also been determined through dot-immunobinding as well as Western blot analysis.

19. Specific immunocytochemical stain for detection of hyphal location of *F. lamaoensis* within tea root tissues of susceptible varieties were developed. Hyphal penetration throughout root tissue was evident and presence of fungal mycelia on outer surface was also evident.

20. *In vitro* interaction of *F. lamaoensis* with *T. harzianum* and *T. viride* was studied. Both inhibited the growth of *F. lamaoensis.*

21. Soil amendment of tea rhizosphere with *T. harzianum* and T. viride both in potted conditions and in the field reduced disease intensity significantly.

22. DAC-ELISA of tea root tissues and rhizosphere soil of different treatment with pathogen and biocontrol agents, reacted with PAbs of *F. lamaoensis, T. harzianum* and *T. viride* indicated the reduction of pathogen population in rhizosphere soil and root tissues.

23. The implication of results have been discussed.