6. SUMMARY

1. A review of literature pertaining to this investigation has been presented which deals mainly with phenolics in plant tissue in relation to disease development, phytoalexin accumulation and their role in early defense response; and screening of phylloplane microorganisms and biological control of foliar diseases.

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

3. Pathogenicity of P. theae was tested on ten varieties of tea (TV-18, TV-20, TV-22, TV-23, TV-25, TV-26, TV-27, Teen Ali 17/1/54, TS-449 and CP-1) by detached leaf, cut shoot and whole plant inoculation technique. TV-23 and CP-1 were found to be the most susceptible and resistant varieties respectively.

4. Potato-dextrose agar (PDA) and Richard's medium (RM) supported maximum mycelial growth and sporulation. Conidial morphology was studied under bright field, phase contrast microscope and Scanning Electron Microscope (SEM).

5. Maximum mycelial growth of P. theae occurred after 20 days of incubation and at pH 6.5. Sucrose, ammonium nitrate followed by potassium nitrate and casein were found to be most effective carbon, inorganic nitrogen and organic nitrogen sources respectively.

6. Low concentration (4.8×10^4 spores/ml), 24h incubation period and pH 6.5 from 15-20 day old culture of P. theae were optimum for spore germination.

7. Protein contents of all tested varieties (both susceptible and resistant) increased significantly in the P. theae inoculated

leaves in comparison to healthy ones, and more significantly in four susceptible varieties (TV-23, Teen Ali 17/1/54, TV-18 and TV-26).

8. P. theae had a protein content of 3.42 mg/g fresh weight. SDS-PAGE analysis of mycelial protein revealed twenty one protein bands ranging in molecular weight from 113 to 26 kD.

9. Healthy leaf protein preparations of TV-18 and CP-1 yielded 13 protein bands each ranging from 147.2 to 26.6 kD and 200.6 to 29 kD rspectively, while TV-23 yielded 14 protein bands ranging from 191.7 to 24.2 kD in SDS-polyacrylamide gel electrophoresis. P. theae inoculated leaves of CP-1, TV-18 and TV-23 exhibited one (173.9 kD), two (82.4 and 48.2 kD) and three (173.9, 114.1 and 97 kD) additional protein bands respectively.

10. Phenolics present in healthy leaves were found to be protocatechuic acid, gallic acid, catechol, caffeic acid and p-coumaric acid. Both total and orthodihydroxy phenol content decreased following inoculation with P. theae in susceptible varieties, while there was an increase in resistant varieties following inoculation.

11. Leaf diffusates collected from resistant varieties were more fungitoxic than those from susceptible varieties. Diffusible compound collected from the leaves of all four varieties 48h after inoculation with spore suspension of P. theae exhibited maximum absorption peak at 273 nm which was not evident in case of water drops collected from the healthy leaves after 48h of incubation.

12. Antifungal compounds were extracted separately from healthy and P. theae inoculated tea leaves of resistant and susceptible varieties which inhibited mycelial growth of P. theae in the

solid medium. Two antifungal compounds (I and II) were detected at Rf 0.63 and 0.58 when thin layer chromatograms were developed in chloroform : methanol (9:1, v/v) and sprayed with B. carbonum.

13. Compound I from healthy leaf extracts of all tested varieties showed prominent inhibition zone at Rf 0.63. This compound showed brown colour reaction when sprayed with Vanillin - H_2SO_4. Rf value and colour reaction of this antifungal compound corresponded with catechin.

14. The compound II showed positive colour reaction of phenolics with the chromogenic sprays (Folin-Ciocalteau's reagent and diazotized p-nitroaniline) on TLC plates at Rf 0.58. This compound exhibited prominent inhibition zone on TLC plate bioassay and also inhibited spore germination.

15. UV-spectral analysis of the compound II revealed absorption peak at 274 nm and showed similarity to pyro-catechol. It accumulated in inoculated leaves of resistant varieties in greater amount (477-612 µg/g fresh wt.) in comparison to that in susceptible varieties (292-325 µg/g fresh wt.). Concentration of this compound in healthy leaf tissues was very low (60-96 µg/g fresh wt.).

16. Cell walls from P. theae were isolated, extracted and characterized by SDS-PAGE. Carbohydrate and protein content of the cell wall preparations were 27.37 mg/g and 37.5 mg/g respectively. Six glycoproteins of molecular weight 113, 105, 46, 39, 28 and 26 kD were detected in the mycelial wall extract.

17. ConA-FITC binding of the isolated cell walls showed strong fluorescence under the microscope which confirmed glycoprotein nature of mycelial wall extract.

18. The resistant varieties (CP-1 and TV-27) showed similar disease reaction with the mycelial wall extract as that of the spore suspension of P. theae.

19. Mycelial wall extract elicited the production of antifungal compound in the tea leaves of resistant variety.

20. A large number of microorganisms were isolated from the phyllosphere of tea from different tea estates of Dooars and Hill regions of West Bengal.

21. The isolated fungi were identified on the basis of morphology (mycelia, conidiophore and conidia) and characteristics of spore, while the isolated bacteria were identified on the basis of morphological, physiological and biochemical tests.

22. All isolated microorganisms were paired with P. theae. Acremonium fusidioides, Aspergillus nidulans, A. niger, Penicillium oxalicum, Phoma exigua, Bacillus sp., B. cereus, B. pumilus, B. sphaericus, Micrococcus sp., M. luteus and Coryneform sp. showed antagonistic reaction against P. theae.

23. Antagonistic bacteria were grown with P. theae in liquid medium. The growth of P. theae was inhibited by all the tested bacteria. The fungitoxicity of the bacteria were due to the release of antifungal metabolites into culture medium.

24. Spraying of tea leaves of susceptible varieties with aqueous cell-suspension, washed cell or cell-free culture filtrates of the selected antagonistic bacteria resulted in reduction of disease development.

25. Reduction in disease development by the antagonistic bacteria were also dependent on the ratio of antagonists to pathogen on leaf surface.

26. Culture filtrates of the antagonistic bacteria inhibited spore germination of *P. theae in vitro*. Antifungal compounds were found to be partly thermolabile.

27. Maximum activity of the antifungal compounds were noted in diethyl ether fraction of *B. pumilus* and ethyl acetate and chloroform fractions of *Micrococcus* sp. Serial dilutions of the extracts collected either from diethyl ether fraction of *B. pumilus* cell-free culture filtrate or ethyl acetate fraction of *Micrococcus* sp. reduced their activity.

28. Maximum production of antifungal compounds by *B. pumilus* and *Micrococcus* sp. were at 7 and 4 days of incubation respectively. The compounds were analysed by UV-spectrophotometric analysis.

29. *In vivo* tests with partially purified compound from *B. pumilus* showed it to be very effective in disease reduction.

30. Implications of the results have been discussed.