

SUMMARY

1. A short comprehensive review pertaining to this investigation has been presented which deals mainly detection of plant pathogenic fungi and biochemical changes in plants following fungal infection has been presented below.
2. Materials used in this investigation and experimental procedures followed have been discussed in detail.
3. Screening of resistance on twenty five tea varieties (10 Tocklai, 9 Darjeeling and 6 UPASI) were done. Among the tested tea varieties BSS-2, BS/74/76 and AV-2 were found to be highly susceptible, while UPASI-9, UPASI-2, UPASI-3 were most resistant.
4. Cultural conditions affecting growth of *U. zonata* 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. Lactose was the most effective carbon source while no growth was observed in sorbose. Beef extract was most effective nitrogen source followed by yeast extract and then in peptone. Among the inorganic sources calcium nitrate supported maximum growth. Other inorganic sources supported lower growth than organic sources, though no growth was observed in urea and insignificant growth was noted in basal medium without nitrogen.
5. Protein content of healthy and artificially inoculated tea root tissues from sixteen tea varieties as well as mycelia and cell wall proteins of *U. zonata* was estimated and analyzed in SDS-PAGE. Characterization of the cell wall of *U. zonata* by ConA-FITC binding revealed its glycoprotein nature.
6. Sixteen tea varieties (TV-9, TV-18, TV-22, TV-25, TV-26, S-449, BS/7A/76, CP-1, AV-2, P-1258, K1/1, UPASI-2, UPASI-3, UPASI-8, UPASI-9 and UPASI-26) were selected for biochemical analyses. Total phenol, orthodihydroxy phenol content as well as antifungal phenolics were quantified in resistant (428-512 $\mu\text{g/g}$ fresh wt) and susceptible tea varieties (54-73 $\mu\text{g/g}$ fresh wt) following inoculation with *U. zonata*). Concentration of this compound in healthy root tissues were very low.

Time course accumulation of peroxidase , phenyl alanine ammonia lyase and polyphenol oxidase in sixteen selected tea varieties were determined.

7. Polyclonal antibodies (PABs) were raised against antigen preparations from mycelia and cell wall of *U. zonata*, mycelia of biocontrol agents (*T. harzianum* and *T. viride*) and tea root tissues (UP-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 bleedings collected from *U. zonata* . Strong precipitin reactions were observed in homologous cross reaction of each case.

9. Optimization of ELISA using PABs of *U. zonata* and antigen preparations at variable concentrations ranging from 40 to 0.312mg/ml. However maximum absorbance values were obtained in 3rd bleeding followed by 4th bleedings.

10. PTA-ELISA tests were performed separately using PABs raised against mycelia and cell wall antigens PAB of *U. zonata* against root antigens prepared from sixteen different tea varieties, non-pathogen and non-host and major cross reactive antigens (CRA) shared between tea varieties and *U. zonata* were detected.

11. Detection of *U. zonata* in artificially inoculated tea root tissues using PTA-ELISA and DAS-ELISA formats were standardized.

12. Antigens prepared from soil samples collected from different tea field and amended soil, were tested against PAB of *U. zonata* using PTA-ELISA formats and Dot blot analysis.

13. Purification of antigenic protein of *U. zonata* from the crude mycelial extracts by ammonium sulphate saturation was done and detection was done by immunodiffusion, PTA-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 *U. zonata* and tea varieties, non-host and non-pathogen was detected in PTA-ELISA. This antiserum also showed higher reactivity in the susceptible reactions as compared to resistant reaction. PAb raised to 60-80% SAS fraction could also significantly detect infections by PTA-ELISA, Dot blot and Western blot analysis.

15. Cross sections of tea roots inoculated with *U. zonata* and treated with PAb of *U. zonata* followed by labelling with FITC developed a bright fluorescence throughout the sections, extending up to vascular tissues as well as outer surface.

16. Mycelia of *U. zonata* when treated with homologous antisera followed by FITC, bright fluorescence was noticed on young hyphae.

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

18. Specific immunocytochemical stain for detection of hyphal location of *U. zonata* 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.

19. *In vitro* interaction of *U. zonata* with *T. harzianum* and *T. viride* was studied. Both inhibited the growth of *U. zonata*.

20. Soil amendment of tea rhizosphere with *T. harzianum* and *T. viride* both in potted conditions and in the field reduced disease intensity significantly. PTA-ELISA of tea root tissues and rhizosphere soil of different treatment with pathogen and biocontrol agents, reacted with PABs of *U. zonata*, *T.harzianum* and *T. viride* indicated the reduction of pathogen population in rhizosphere soil and root tissues.