

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 *Sphaerostilbe repens* was tested on twenty five varieties (11 Tocklai, 8 Darjeeling and 6 UPASI) of tea. Among these , TV-26 and TeenAli-17/1/54 were appeared to be susceptible and TV-23, TV-25, UP-9, S-449 were found to be resistant.
4. Cultural conditions affecting growth of *S.repens* were studied with special reference to their growth in different media, variable pH and five different types each of carbon, organic and inorganic nitrogen sources. Maximum growth of the pathogen occurred in carrot juice agar while minimum growth was noticed in Elliot agar. Starch was the most effective carbon source whereas yeast extract followed by casein hydrolysate yielded optimum mycelial growth. Organic nitrogen sources were found to be better than inorganic nitrogen sources.
5. Polyclonal antibodies (PABs) were raised against antigen preparations from mycelia, cell wall and spores of *S.repens* and tea root tissues (TV-26). These were purified by ammonium sulphate precipitation followed by DEAE cellulose chromatography. IgG obtained in each case was used for immunodiffusion and ELISA tests.
6. Agar gel double diffusion tests were performed using crude antibody as well as purified IgG prepared after four different bleedings collected for the pathogen. Strong precipitin reactions were observed in each case.
7. Optimization of ELISA using PABs of *S.repens* and antigen preparations at variable concentrations were performed. ELISA values decreased with the decrease of antigen concentrations ranging from 40 to 0.312 ug/ml . However absorbance values increased with different bleedings.

8. DAC-ELISA tests were performed separately using PABs raised against mycelia, cell wall and spore antigens of *S.repens* against root antigens prepared from 25 different tea varieties, non-pathogen and non-host. Major cross reactive antigens (CRA) shared between tea varieties and *S.repens* were detected.
9. Detection of *S.repens* in artificially inoculated tea root tissues using DAC-ELISA and DAS-ELISA formats were standardized.
10. Antigens prepared from 26 soil samples were tested against PAB of *S.repens* using DAC-ELISA formats and dot blot analysis. Spiked soils gave very high values comparable to homologous values.
11. Protein content of healthy and artificially inoculated tea root tissues from 25 different tea varieties as well as mycelia and cell wall proteins of *S.repens* were estimated and analysed in SDS-PAGE. Mycelial protein of *S.repens* exhibited 23 bands ranging in molecular weight from 97kDa to 14 kDa.
12. Characterization of the cell wall of *S.repens* by ConA-FITC binding and SDS-PAGE electrophoresis revealed its glycoprotein nature, with 6 bands of 62, 56, 33, 30, 17 and 14 kDa molecular weights.
13. Agglutination test of conidia of *S.repens* with different lectins revealed strong agglutination with ConA and HPA followed by WGA and least with UAE-1. The presence of glycoconjugates containing glucose and/or mannose residues, and N-acetylgalactosamine and N-acetyl glucosamine were confirmed on the outer surface of the conidial wall.
14. Cross sections of tea roots (TeenAli-17/1/54 and TV-26) treated with PAB of *S.repens* and then labelled with FITC developed a bright fluorescence throughout, which was concentrated mainly in epidermal cells and cortical tissues.
15. Reactions of various antigens (fungal and root) with PAB of *S.repens* has also been determined through dot- immunobinding as well as Western blot analysis.

16. Mycelia and conidia of *S.repens* when treated with homologous antisera followed by FITC, bright fluorescence was noticed on young hyphae and throughout the surface of conidia.
17. Specific immunocytochemical stain for detection of hyphae of *S.repens* within tea root tissues (TeenAli-17/1/54 and TV-26) were developed. Beneath the bark tissue, rhizomorph development and hyphal penetration throughout root tissue were evident.
18. *In vitro* interaction of *S. repens* with *Trichoderma harzianum* and *T. viride* was studied. Both inhibited the growth of *S.repens*. *T. viride* overgrew the pathogen while *T. harzianum* completely inhibited its growth .
19. Soil amendment of tea rhizosphere with *T. harzianum* and *T. viride* both in potted conditions and in the field reduced disease intensity significantly.
20. DAC-ELISA of rhizosphere soil and tea root tissues as well as competition ELISA of rhizosphere soil with PABs of *S. repens*, *T. harzianum* and *T. viride* indicated the reduction of pathogen population in rhizosphere soil and root tissues.
21. The implications of results have been discussed.