6. Summary

1. The present study deals with "Studies on tea seed mycoflora and resistance of young tea plants against *Rhizoctonia solani*, a soil borne root pathogen of germinating tea seedlings". The study consists of: i) Screening of tea seeds of different varieties for isolation of seed-borne pathogens. ii) Identification of major pathogen (*Rhizoctonia solani*) following Koch's postulates. iii) Pathogenicity of the fungus against seedlings of different tea varieties. iv) Studies on physiological characteristics of the pathogenic fungus. v) Studies on the resistance of tea against *Rhizoctonia solani* following serological techniques. vi) Control of the pathogen (*Rhizoctonia solani*) by antagonistic microorganisms and botanicals.
2. After a short introduction to the work, a brief review of literature related to the present line of investigation has been presented. The review is selective manner rather than comprehensive. Keeping relevance with the present works and also for convenience, the review has been grouped separately under subheadings likes seed mycoflora and seed diseases of tea plants, root diseases of tea plants, diseases caused by *Rhizoctonia Solani*, characteristics of *Rhizoctonia solani* as a pathogen, studies on growth and physiology of the pathogens, antigenic relationship in host and pathogen, disease control by antagonistic organisms and by disease control by botanicals.
3. Details of different experimental procedures and techniques have been described in the materials and methods section. The present study involves investigations on damages of tea seeds due to fungal infection. Initially, tea seed surface fungal flora and seed borne fungal flora were established following isolation and identification of the organisms. Altogether 400 seeds of seven different varieties (TS520, TS462, TS463, TS 449, TS464, TS491 and TS506) of tea were studied and the associated fungal species identified were *Curvularia lunata*, *Rhizoctinia*

solani, *Fusarium* sp., *Alternaria* sp., five species of *Aspergillus*, *Botryodiplodia* sp., two species of *Rhizopus*, *Penicillium* sp., *Trichoderma pseudokoningii* and a sterile fungus.

4. Following isolation, pathogenicity of the fungal isolates was confirmed by verification of Koch's postulates. Degree of pathogenicity of a selected pathogen, *Rhizoctonia solani* was determined by pathogenicity test in the selected varieties of tea which showed TS 449 as moderately resistant and the other varieties as susceptible to the fungus.
5. Growth and sporulation of *R. solani* have been studied on nine different media viz. Potato dextrose agar (PDA), Oat meal agar (OMA), Root extract agar (REA), Czapek dox agar (CDA), Richard's agar (RA), Yeast extract mannitol agar (YEMA), Malt extract agar (MEA), Potato carrot agar (PCA) and Nutrient agar (NA). Important physiological parameters have also been studied. Among these OMA and YEMA were best medium for the vegetative growth of the fungus. PDA medium was also good for growth. Sclerotia formation was very good in PDA and CDA media.
6. Optimum temperature for mycelial growth of *R. solani* was found between 23°C and 33°C. Maximum growth was observed at 28°C. Optimum pH was found to be 6.5.
7. Optimization of nutrient requirements was done where different carbon sources were supplemented in a basal medium. It was found that mannitol was the best carbon source for optimum growth and sclerotia formation of *R. solani* among the six different carbon sources tested. Sorbitol showed second best mycelial growth and sclerotia formation next to mannitol. Lactose showed minimum growth among the carbon sources tested.
8. Similarly, the influence of organic and inorganic nitrogen sources on growth and sclerotia formation of *R. solani* were also tested. Excellent growth and sclerotia formation was observed in beef extract

supplemented medium. Peptone, trypton, sodium nitrate, yeast extract and potassium nitrate were also good for growth and sclerotia formation.

9. The presence and the level of cross reactive antigens (CRA) between the seven different tea seed varieties and the pathogen, *R. solani* was determined in order to study the correlation between host-pathogen compatibility and CRA levels by immunodiffusion, immunoelectrophoresis and ELISA. Common antigens were detected in the form of precipitin arcs in immunodiffusion plates when antigens of susceptible tea varieties (520a, 462a, 464a and 491a) were cross reacted with the antisera of *R. solani* but were absent during similar cross-reactions with resistant variety.
10. In immunoelectrophoretic studies, antigens of susceptible varieties shared at least one precipitin band each in all the cases when reacted with antisera of *R. solani* while antigen of resistant variety (449a) showed no precipitin band. In reciprocal cross reaction, antigen of *R. solani* shared one precipitin band with antisera (520A) of TS-520 (susceptible variety) but no precipitin band with antisera of TS449 variety (resistant variety).
11. Indirect ELISA technique was followed for detecting CRA at very low concentrations. The higher ELISA values in cross reactions revealed the presence of more CRA, which indicated the susceptibility of the variety. Similarly, lower ELISA values revealed lower amount of CRA that indicated resistance. The results obtained by indirect ELISA values i.e. the degree of susceptibility and resistance was in agreement with the results of pathogenicity tests also.
12. Immunocytolocalization studies were conducted to determine cellular locations of CRA in root sections of tea varieties and mycelial cells and propagules of the fungal pathogen *R. solani.* In the present study, immuno-gold labeling followed by silver enhancement was done

specifically to study CRA level in the light microscope. When root section of susceptible variety (TS-520) was treated with antisera of *R. solani* and labelled with immunogold particles enhanced by silver precipitation, CRA was observed mainly in the epidermal regions. Cortical tissues and vascular bundle elements also showed marginal darkening which indicate presence of CRA in these areas also. When root section of resistant variety (TS-449) was treated with the antisera of pathogen, no such precipitation was observed. When the fungal pathogen, *R. solani* was treated with antisera of susceptible host followed by immunogold labelling and silver enhancement, dense blackish colour was observed mainly in the hyphal tips indicating presence of CRA. When similar treatment was done with antisera of resistant variety, no darkening was observed indicating disparity in the antigens.

13. Considering the capacity of mycoparasitism and also its ability to destroy the fungal pathogens by several cell wall degrading enzymes, three different strains which included *Trichoderma harzianum*, *T. viride* and *Gliocladium virens* (isolates 1 and II) was tested in the present study to control *R. solani*. Results revealed that *T. harzianum* was the most effective fungi by *in vitro* dual culture tests. Similarly 23 different plant extracts were also tested for their ability to control the growth of the pathogen by agar cup assay which showed that *Allium sativum*, *Polyalthia longifolia* and *Leucas cephalotes* were 100% effective in inhibiting the growth of *R. solani*.
14. Extract of *Polyalthia longifolia* (20%) and 25% culture filtrate of *T. harzianum* was tested individually and also in combination in various seed treatment studies for determining the effect of the extracts *in vivo*. In all treatments, the pathogen-population was reduced. Vigour index of seeds treated with sporulated culture of *T. harzianum* was highest and

that of seeds treated with 25% culture filtrate of *T. harzianum* was lowest in TS 449 variety.

15. For controlling root disease caused by *R. solani*, the tea seedlings were treated with the fungus *T. harzianum* by soil inoculation method. Two different formulations were tested by adopting pre inoculation, post inoculation and simultaneous inoculation method. Seedling mortality percentage was highly reduced in pre-inoculation method than in post inoculation and simultaneous inoculation method.
16. Thus the present study, identifies the problem of low percentage of seed germination, establishes a pathogen of the seedlings and also designs the suitable control measures of the disease using bio-control agents, botanicals and also suggests suitable formulations for controlling seed borne pathogen *R. solani.*