Summary

A review of literature has been presented in combination with serological techniques for the detection of cross reactivity between host and parasite, effective integrated disease management practices and biochemical changes following induced resistance in plants.

The materials used in this investigation and the experimental procedures followed have been discussed in detail. Factors influencing mycelial growth of *S. rolfsii* were studied with special reference to their growth in different media, variable pH and variable sources of carbon as well as organic and inorganic nitrogen sources. Maximum growth of pathogen occurred after 8 days of inoculation at pH 6. Dextrose was the most effective carbon source and of the nitrogen sources, yeast extract (organic source) was found most optimum for growth of *S. rolfsii*. Organic nitrogen sources were found to be better than inorganic nitrogen sources.

Resistance of eighteen tea varieties (TV-17, TV-18, TV-22, TV-25, TV-26,TV-30, UP-2, UP-3, UP-8, UP-26, BSS-2, B-157, AV-2, T-78, T-135, K-1/1 and HV-39) were screened against *S. rolfsii*. Among these B-157, UP-8 and TeenAli-17/1/54 were found to be highly susceptible, whereas K1/1 and HV-39 were found to be resistant.

Protein content of healthy and artificially inoculated tea root tissues from 18 different tea varieties as well as mycelia of *S. rolfsii* was estimated. Mycelial protein of *S. rolfsii* exhibited 24 bands with molecular weights ranging from 205 to 6.5 kDa in SDS-PAGE analysis.

Polyclonal antibodies (PAbs) were raised against antigen preparations from mycelia of *S. rolfsii* and tea roots. Serological cross reactivity among tea varieties and *S. rolfsii* isolates (Sr-1, Sr-2 and Sr-3) were determined following immunodiffusion test, enzyme linked immunosorbent assay, dot immunobinding assay and indirect immunofluorescence. Optimum conditions for PTA-ELISA reaction with PAb of *S. rolfsii* were determined. The antiserum dilution 1:125 and enzyme (alkaline phosphatase) dilution 1:10,000 proved to be most optimum. PTA-ELISA detected antigen up to a concentration of 10 μg/ml in homologous reaction. Major cross reactive antigens (CRA) shared between isolates of *S. rolfsii* and tea

varieties were determined following PTA-ELISA using PAb of *S. rolfsii*. Cellular location of major CRA was determined following indirect immunofluorescence test. Detection of pathogen (*S. rolfsii*) in artificially inoculated tea roots (AV-2 and B-157) using PTA-ELISA formats and immunofluorescence tests were developed. The reaction of mycelial antigens prepared from various fungi has also been determined on nitrocellulose papers following dot immunobinding assay using PAb of *S. rolfsii*. Specificity of PAb of *S. rolfsii* against all three isolates of *S. rolfsii* was also determined through western blot analysis.

Biochemical changes following inoculation with *S. rolfsii* were investigated. Both total and ortho-dihydroxy phenol contents increased following inoculation with *S. rolfsii* in resistant varieties while there was a decrease in susceptible varieties. Among 5 resistant varieties tested tea roots of K 1/1, followed by TV-26 and BSS-2 showed maximum increase in orthodihydroxy phenol content after inoculation with *S. rolfsii* Antifungal compounds were extracted separately from healthy and *S. rolfsii* inoculated tea roots of resistant tea variety that inhibited mycelial growth of *S. rolfsii* in solid media. UV-analysis and HPLC profile clearly showed the presence of antifungal compounds in infected tea roots. It is interesting to note that extracts from *S. rolfsii* inoculated root tissue gave a peak at 274 nm. Maximum absorption peak measured at 274nm was identical to an authentic sample of pyrocatechol. In resistant varieties, higher accumulation (525-678 µg/g fresh wt) of pyrocatechol was detected, than in the susceptible varieties (212-290 µg/g fresh wt) following 96h of inoculation with *S. rolfsii*. Concentration of this compound in healthy root tissues were very low (60-93 µg/g fresh wt). Phenylalanine ammonia lyase (PAL) activity increased after 4 days of inoculation in TV-18, TV-25, TV-30, UP-26, AV-2, T-78, T-135, UP-2, BSS-2, K-1/1 and HV-39 markedly. Peroxidase (PO) and polyphenol oxidase (PPO) activities in tea roots increased markedly after 4 days of inoculation with *S. rolfsii* in all the varieties tested.

In vitro interaction of *S. rolfsii* with *Trichoderma harzianum* and *T. viride* was studied. Both bioagents inhibited the growth of *S. rolfsii*. The efficacy of fungicides and plant extracts were also tested against *S. rolfsii in vitro*. Thiodan and calixin completely arrested the growth of the pathogen at a concentration as low as

0.0125%. Increase in growth of the plant was evident when grown in soil amended with organic additives. Phenol contents were found to be high in treated plants in comparison to untreated healthy control plants. Effective integrated management practices were adopted using plant extract, biocontrol agents, organic additives along with selected fungicides for control of seedling blight disease . *In vivo* trials demonstrated that *Trichoderma harzianum* alone as well as in combination with neem cake, oil cake, aqueous extract of *Azadirachta indica* and calixin (0.1%) provided a total control of sclerotial blight disease. Changes in the level of phenolics were also determined in tea plants (UP-3, B-157 and K 1/1) grown separately in soil ammeded with cow dung, rabbit manure and chicken manure following inoculation with *S. rolfsii*. Phenolics decreased in untreated plants of two susceptible varieties (UP-3 and B-157) following inoculation with the pathogen in relation to healthy control, whereas the resistant variety (K 1/1) responded against inoculation with the pathogen. In this case total phenol and orthodihydroxy phenol content increased in comparison with untreated healthy control

Alterations in antigenic patterns following induction of resistance in susceptible tea plants were detected using immunological assays. These antigenic changes, owing to calixin treatment, that was analysed using immunodiffusion tests, have some significance in the resistance of tea to *S. rolfsii*. Following PTA-ELISA with PAb raised against mycelia of *S. rolfsii*, it could be inferred that the absorbance (A_{405}) values were always lesser in treated root tissues in comparison to healthy untreated ones. PTA-ELISA of tea root tissues and rhizosphere soil of different treatment with pathogen and biocontrol agents, reacted with PAbs of *S. rolfsii*, *T. harzianum* and *T. viride* showed the reduction of pathogen population in rhizosphere soil and root. Reaction of various amended soil antigens with of *S. rolfsii* was also detected through dot-blot using PAb of *S. rolfsii*,. The amended soil antigens inoculated with *S. rolfsii* showed lesser colour intensity on nitrocellulose membranes than homologous antigen of *S. rolfsii*.