

# SUMMARY

1. A review of literature pertaining to 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 serological techniques for the detection of cross reactivity between host and pathogen.
2. Materials used in this investigation and experimental procedures followed have been discussed in detail.
3. Pathogenicity of *C. theae* was tested on fifteen varieties of tea ( BSS-2, BSS-3, UP-2, UP-3, UP-9, UP-26, TV-9, TV-18, TV-20, TV-22, TV-23, TV-25, TV-26, TV-29, Teenali 17/1/54) by detached leaf and cut shoot plant inoculation techniques. TV-22 and TV-23 were found to be the most susceptible and TV-20 and TV-9 and BSS-3 were found to be resistant varieties respectively.
4. 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 *C. theae* in susceptible varieties , while there was an increase in resistant varieties following inoculation.
5. Changes in two important enzymes related to phenol metabolism ie. peroxidase and phenylalanine ammonia lyase following inoculation with *C. theae* were observed in all fifteen varieties. Peroxidase and phenylalanine ammonia lyase activity increased only in resistant varieties after 48h of inoculation with *C. theae*.
6. Leaf diffusates collected from resistant varieties were more fungitoxic than those from susceptible varieties. Diffusible compound collected from the leaves of four varieties after 48h inoculation with sclerotial suspension of *C. theae* exhibited maximum absorption peak at 274nm which was not evident in case of water drops collected from the healthy leaves after 48h of incubation.

7. Antifungal compounds were extracted separately from healthy and *C.theae* inoculated tea leaves of two resistant and two susceptible varieties which inhibited mycelial growth of *C.theae* in solid medium. Two antifungal compounds ( I and II ) were detected at Rf 0.62 and 0.56 when thin layer chromatograms were developed in chloroform:methanol (9:1, v/v) and sprayed with *Curvularia lunata*.
8. Compound I from healthy leaf extract of all tested varieties showed prominent inhibition zone at Rf 0.62. This compound showed brown colour reaction when sprayed with vanillin-H<sub>2</sub>SO<sub>4</sub> . Rf value and colour reaction of this antifungal compound corresponded with catechin.
9. The compound II showed positive colour reaction of phenolics with the chromogenic sprays (Folin-Ciocalteu's reagent and diazotized p-nitroaniline) on TLC plates at Rf 0.56. This compound exhibited prominent inhibition zone on TLC plate bioassay and also inhibited spore germination of *G.cingulata*.
10. 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 in comparison to that in susceptible varieties. Concentration of this compound in healthy leaf tissue was very low .
11. Cell walls from *C.theae* were isolated, extracted and analysed on SDS-PAGE. ConA-FITC binding of the isolated cell walls showed strong fluorescence under the microscope which confirmed glycoprotein nature of mycelial wall extract.
12. The resistant varieties ( TV-9 and TV-20) showed similar disease reaction with the mycelial wall extract as that of the sclerotial suspension of *C.theae*. Mycelial wall extract of *C.theae* elicited the production of antifungal compound in tea leaves of resistant varieties as evident in Petridish bioassay with *C.theae* and spore germination bioassay with *G.cingulata*.

- ( 13 ) Polyclonal antisera were raised against antigen preparation from mycelia of *C.theae* and tea leaves. Serological cross reactivity among tea varieties and *C.theae* isolates were determined following immunodiffusion test, enzyme linked immunosorbent assay , dot blot assay and immunofluorescence.
- (14) In agar gel double diffusion tests antiserum of *C. theae* and antigens of tea varieties reacted in three different ways. Strong precipitin reactions were observed with all those varieties which exhibited susceptible reaction , while moderately susceptible varieties exhibited weak precipitin reaction . However, resistant varieties could not develop any precipitin reaction. In reciprocal cross reaction with tea leaf antisera of TV-18 and TV-26 , the tea varieties exhibited serological affinity among themselves. However, antigens prepared from isolates of *C.theae* gave positive reactions with antisera of susceptible variety (TV-18) but not with the antisera of resistant variety (TV-26).
- ( 15 ) Optimum conditions for DAC-ELISA reactions with anti-*C.theae* antiserum were determined. An antiserum dilution 1:125 and an enzyme (alkaline phosphatase) dilution of 1:10,000 were optimum. Antigen upto a concentration of 10 µg/ml were detected in homologous reaction by ELISA.
- ( 16 ) Major cross reactive antigens (CRA) shared between isolates of *C.theae* and tea varieties were determined following DAC-ELISA using antisera of *C.theae*, TV-26 and TV-18.
- ( 17 ) Detection of pathogen ( *C.theae* ) in artificially inoculated tea leaves using DAC-ELISA formats, immunofluorescence test as well as dot blot assay were also developed.
- ( 18 ) Cellular location of cross reactive antigens (CRA) in mycelia, sclerotia of *C.theae* were studied using fluorescein isothiocyanate (FITC). Cellular location of CRA in cross section of tea leaf tissues were also determined. Major CRA was concentrated on the epidermal and mesophyll tissues of tea leaves and young hyphal tips and germinated sclerotia of the pathogen.