SUMMARY

In the present investigation attempt has been made to elucidate defense strategies in tea plants against pest (*H. theivora*) and foliar fungal pathogens (*A. alternata* and *C. incisum*). Review of literature with two major objectives – biochemical defense strategies of plants against pest and pathogens and efficacy of plant extracts in plant defense response have been discussed. Methods adopted and material used in the experimental set up has been illustrated in details.

Incidence of attack by *Helopeltis theivora* on twenty-three different tea varieties was determined highest (50% to 80%) in the second and third quarters of the year. Among these varieties, UPASI was most susceptible followed by Tocklai. Darjeeling varieties showed less incidence of damage. Using Scanning Electron Microscopy (SEM) healthy as well as *H. theivora* punctured tea leaf surface was scanned. Infested leaf surface showed a number of holes due to puncture made by *Helopeltis*.

All the varieties showed changes in phenolics due to *Helopeltis* infestation. Total phenol content decreased as a result of infestation where as of orthodihydroxy phenols have increased. The accumulation of phenolics due to infestation may reflect a general increase in host metabolism. Protein content decreased following infestation in comparison to healthy ones. Changes in the protein content varied between 4-14% among the varieties tested. Maximum decrease in protein content was noticed in UPASI variety (BSS-2). In SDS-PAGE analysis higher molecular weight proteins (98.5, 97.5, 95.5, 88.4, and 77.5 kDa) of healthy leaves were mainly absent in *H. theivora* infested leaf samples.

Defense enzymes mainly phenylalanine ammonia lyase(PAL), polyphenol oxidase(PPO), peroxidase(PO), chitinase(CHT) and β-1,3-glucanase(βGLU) were studied in healthy and *H. theivora* infested tea leaves. It is interesting to note that PAL activity decreased due to *H. theivora* infestation in all the varieties tested. However UPASI varieties exhibited significant decrease in PAL activity due to infestation. However, sharp increase (2-3 folds) in PO activity was noticed in all the varieties infested with *H. theivora*. PPO activity was higher in the *H. theivora*

infested leaves in comparison to the healthy one. β-1,3 glucanase and chitinase activities increased in *H. theivora* infested leaves when compared with healthy plants. Significant increase β-1,3 glucanase activity was observed in UPASI varieties followed by Darjeeling and Tocklai varieties. When percentage increase in defense enzyme activities of twenty-three tea varieties were compared maximum increased in PPO activity were found in infested leaves followed by PAL, CHT, PO and GLU activities in comparison to healthy. Using PAb of chitinase dot immunobinding assay and Western blots analysis were done. Three bands of ca. molecular weights of 35, 59 and 65 kDa were found to be common in both healthy and *H. theivora* infested leaves, while a new band of ca. molecular weight 42 kDa was evident only in *Helopeltis* infested leaves. Results of the present study indicate the involvement of several enzymes in the insect-tea interaction process.

Antifungal phenolic (pyrocatechol) extracted from healthy and *H. theivora* infested leaf showed antimicrobial activities which were further analyzed HPLC. Catechins extracted from different developmental stages of *H. theivora* infestation were analyzed by HPLC. Four isoforms of catechins such as EGC, EC, EGCG, CG increased sharply due to *H. theivora* infestation in initial stage of puncture. In advance stage of infestation level of EGC, EGCG, GCG and ECG decreased markedly.

Application of biological resources involving metabass - a mycopesticide formulation, biocrop - a plant product and plant extracts of *Azadirachta indica*, *Catharanthus roseus* and *Diplazium esculentum* were evaluated for management of pest attack. In these varieties maximum activity of defense enzymes (PAL, TAL) was noted in infested treated one.

Two important foliar fungal diseases viz. leaf blight caused by *Alternaria alternata* and black rot caused by *Corticium invisum* which are prevalent in the Doors, have been worked out with special reference to their histopathology. Twenty three varieties of tea were scanned for resistance towards *A. alternata* and *C. invisum.*

The varieties were grouped into highly susceptible (T-17, TV-20, TV-22, UPASI-8, BSS-3 and T-78) and moderately resistant (TV-9, TV-18, T-135 and UPASI-3) moderately susceptible (TV-30, TV-25, and HV-39) towards *A. alternata*. In case of *C. invisum,* highly susceptible (TV-22, TV-23, TV-28, HV-39, UPASI-26 and BSS-1) and moderately susceptible varieties (TV-9, TV-25, UPASI-8 and BS/7A/76) were categorized. Polyclonal antibodies were prepared from mycelial antigen of *A. alternata* and *C. invisum* and packaged for ELISA format. Detection of fungal pathogens in twenty-one tea varieties following artificial inoculation with *A. alternata* and *C. invisum* were made using PTA-ELISA format, dot immunobinding assay and indirect immunofluorescence. Young mycelia gave bright fluorescence when conjugate with FITC labeled antibodies. FITC developed apple green fluorescence in infected leaves, which was distributed throughout the leaf tissue, mainly in the epidermal and mesophyll tissues.

Total phenol content decreased following inoculation with foliar fungal pathogens in the susceptible varieties. However there was an increase in the total phenol content as well as PAL, PPO, CHT, βGLU activities of resistant varieties following inoculation with *A. alternata* and *C. invisum*. Greater accumulation of antifungal compounds (pyrocatechol) in developing resistance against *A. alternata* were detected. HPLC analysis pyrocatechol extracted from healthy as well as *A. alternata* inoculated tea leaves showed a sharp peak at retention time 2.6 in both the preparation. But the peak height was higher in inoculated one. Catechins extracted from both the healthy as well as *A. alternata* inoculated tea leaves further analyzed by HPLC. Due to reaction with pathogen inoculated leaf samples exhibited more isoforms of catechin than healthy control. In *A. alternata* inoculated leaves four peaks at 15.7, 18.4, 20.8 and 22.3 retention time increased markedly in relation to healthy of which two peaks corresponding to authentic EGC and EC could be identified where as level of EGCG decreased following inoculation with *A. alternata.*

Aqueous extracts of leaf of *Azadirachta indica, Catharanthus roseus* and *Diplazium esculentum* have been applied on six varieties (TV-20, TV-22, T17/1/54, UP-3, BSS-3 and BS/7A/76) in order to alter the disease reaction against *A. alternata*. In vitro test of crude extracts against *A. alternata* spores exhibited maximum inhibition. Effect of *C. roseus* was found to be more potent in comparison to *D. esculentum* and *A. indica*. Phenolics in treated plants has considerably increased as compared to the untreated plants which further increased in the inoculated plants than in the uninoculated ones. The activity of defense enzymes (PAL, CHT, βGLU) increased in tea leaves treated with plant extracts.

In indirect immunofluorescence leaf treated with all three plants extracts developed bright apple green fluorescence when reacted with PAb-CHT which was distributed in mesophyll tissues. However, the palisade parenchymatous tissues exhibited red colouration indicating the high level of phenolics accumulated in leaf tissues following treatment with *C. roseus* and *A. indica.* This was not evident in case of *D. esculentum* treated leaves.

SA has been implicated as a component of induced resistance signaling pathway. Untreated as well as SA treated leaves were examined microscopically. Indirect immunofluorescence studies using PAb Chitinase and labeled with FITC conjugate revealed an excellent apple green fluorescence throughout the leaf tissues in SA treated leaves. Ultra-thin sections of SA treated following reaction with PAb-CHT, labeled with gold particles exhibited intense gold labeling, corresponding to CHT deposition in comparison with untreated control plants. High amount of gold labeling was found in host cytoplasm and chloroplast while lesser amount in vacuoles, mitochondria and walls. Multicomponent coordinated defense response of tea plants to pest and pathogens has been critically discussed.