CHAPTER 6: CONCLUSION

The study emphasizes on the correlation between leaf age, trichomes, and phylloplane colonization by microbes. The data provided an insight into the phylloplane colonization dynamics, and the inter-microbe and host-microbe interactions and their implication on host physiology.

Glandular and non-glandular trichomes on the phylloplane served as niches for microbial colonization. Rise in plant phenolic and flavonoids concentration and enhancement of ICP content and activities of PAL, TAL, POX, PPO were reported during host-microbe interactions. Significant ($P \leq 0.01$) increase of the parameters was reported upon treatment with microbial consortia. The study also revealed the effectiveness of human enteric pathogens colonising leaf surface. Antifungal activities of phylloplane microbiota suggested the possible bio-eliciting properties of non-pathogens; *P. koreensis*, *S. daejeonense* and *T. viride*. The results thus demonstrate the potential of phylloplane microfungi and their metabolites in influencing the physiology of the plant.

Apical leaf of 5 weeks old seedlings were tagged and sampled at weekly intervals for 15 developmental stages. Leaf imprint method on Nutrient agar and Potato dextrose agar surface was performed under aseptic conditions for isolation of bacteria and fungi respectively. Colonies were enumerated per sq.cm. of the leaf. Pure cultures were prepared and maintained on suitable media slants. The fungal species were identified microscopically and by referring to standard identification manuals. The bacteria were identified by 16S rRNA sequencing. There were specific microfungi and bacteria that repeatedly occurred at every stage of leaf development, for eg. *A. candidus, C. herbarum, P. expansum, K. pneumoniae, S. fonticola, S. daejonense, P. koreensis, P. jessenii* and *P. stutzeri*. Microbial CFU count varied as the leaves matured and the younger ones harbored significantly less no. of colonies. The microbial count increased rapidly till 9-10^{th} week and thereafter, declined considerably as the leaf senesced. Abaxial leaf surfaces supported more number of microbial populations as compared to the adaxial surface, whereas certain bacteria like *Pseudomonas jessenii* and *P. stutzeri* were found in higher number on the adaxial surface.

Leaf surface was examined under scanning electron microscope (SmartSEM V05. 04. 05. 00) to analyze the trichomes. A positive correlation was found between localization

patterns of trichomes and the colonizing microbes.. Non-glandular trichomes were dense in the mid rib and veins along with the peripheral regions of the leaf blade, however glandular trichomes were observed on the regions between consecutive veins and were present scarcely on mid rib. These trichomes were associated with *K. pneumoniae* and *P.expansum*, *P. koreensis, A. alternata* and *A. niger*. Highest number of total trichomes was recorded from eleven weeks old leaves. *A. candidus, C. herbarum, P. expansum, K. pneumoniae, S. fonticola, S. daejonense, P. koreensis, P. jessenii* and *P. stutzeri* were isolated in high numbers from non-glandular trichome rich niches.

The aseptically grown plants were grouped in five replicates. Each group was treated with microbes and metabolites either singly or in combinations. Plants treated with sterile media served as control. Total phenols and flavonoids were estimated after 24, 48, and 72 hours of treatment. A significant rise ($P \leq 0.01$) in the leaf phenolic was recorded after 48 hours of treatments with human pathogens, *K. pneumoniae* and *S.fonticola* as compared to control. *A.candidus* could induce a significant rise in total phenols when inoculated singly ($P \leq 0.01$), or in combination with *A.citri* ($P \leq 0.05$). *A. niger* significantly enhanced ($P \leq 0.01$) the total flavonoids of inoculated leaves at all sampling hours either singly or combination with *C. cladosporioides* after 48 hours. *C. lunata* and *F. oxysporum* significantly raised the total flavonoids ($P \leq 0.05$), both singly and in combination with each other**.** *T. viride* caused significant rise ($P \leq 0.05$) after 72 hours, either singly or when combined with *P. expansum*. Leaves inoculated with *A. candidus* both singly and in combination with *A.citri* could cause a significant increase ($P \leq 0.01$) after 48 hours of treatment.

Changes in Intercellular fluid protein (ICP) concentration, PAL, TAL, POX, and PPO activity were assessed. 99% increase in the ICP concentration was observed in leaves inoculated with bacterial and fungal metabolites either singly or in combinations. All possible combinations of *A.alternata, C.lunata, P.expansum, A.niger* and *A.flavus* metabolites caused significant rise ($P \leq 0.01$) in ICP concentration of treated leaves as compared to control, except those treated with *A.alternata+R.solani, A.niger+R.solani*, A.flavus+*R.solani*, P.expansum+*R.solani*, and A.flavus+*C.lunata* metabolites.

The metabolite combinations of *P. expansum, C. lunata,* and *C. herbarum* could enhance PAL activity in inoculated leaves by 99%. Metabolites applied singly had no significant

effects except for those of *T. viride*. Three and four metabolite combinations could significantly (P≤0.05) enhance PAL activity.

TAL activity significantly increased (P≤0.05) in leaves treated with combinations of microbial metabolites. Negligible change was observed in plants treated with metabolites singly. Consortia of all metabolites either with or without *P. syringae,* enhanced enzyme activity significantly (P≤0.01). The following metabolite combinations could significantly increase TAL activity by 95%: *P.koreensis+P.expansum, P.koreensis+T.viride, P.koreensis+S.fonticola, P.koreensis+A.flavus, S.fonticola+A.flavus, S.fonticola+P.expansum, S.fonticola+C.lunata, S.fonticola+C.herbarum, A.niger+T.viride, C.herbarum+R.solani*and *S.fonticola+R.solani*. *A.alternata+A.flavus+C.herbarum, A.alternata+P.expansum+C.herbarum, A.alternata+C.lunata+C.herbarum, A.alternata+ A.flavus+T.viride, A.alernata+C.lunata+T.viride* and *A.aletrnata+A.flavus+P.koreensis*.

Metabolites sprayed singly did not yield any significant increase in the PPO activity of leaves, except for those of *T.viride* (P≤0.01). All possible combinations of *A. alternata, A. flavus, P. koreensis, S. fonticola, C.lunata, R. solani,* and *T. viride*, led to significant rise in PAL, TAL, POX, and PPO activities.

The antifungal activity of the bacterial species was investigated by dual culture assays. *Pseudomonas koreensis* and *Sphingobacterium daejonense* inhibited the growth of all microfungal species tested. *Pseudomonas stutzeri* retarded the *in vitro* growth of *Curvularia lunata, Penicillium expansum,* and *Alternaria citri*, while *Klebsiella pneumoniae* did not create clear inhibition zones. It either did not suppress any fungal growth or showed negligent inhibition. Bacterial metabolites caused negligible antagonistic activity, however, physiological changes were caused by *P. koreensis* and *S. daejeonense* to *A. alternata* and *F. oxysporum* as the fungal colonies were less hairy and felty, and produced deep red and orange exudates as compared to control.

In vivo interactions were tested by treating the leaves with microbial combinations (bacteria-bacteria, bacteria-fungus, fungus-fungus) and were tested for any antagonistic activity by leaf imprint method. Leaves were sampled at 24, 48 and 72 hours of treatments. The CFU count was established to assess any inhibition. Imprints of leaves sprayed with sterile distilled water served as control. The data revealed increase in the

antimicrobial activity of *P. koreensis*, *S.daejeonense*, *S. fonticola* and *K. pneumoniae in vivo.* The human enteric pathogens could completely inhibit the growth of *C. cladosporioides*, *C. herbarum*, *T. viride*, and *F. oxysporum* and *S. daejeonense.* 99% inhibition was recorded by *P. koreensis* and *S. fonticola* against *P. stutzeri* and *P. jessenii.*

The study would help in understanding the population dynamics of phylloplane microbes with regard to variation in variety; interrelationship dynamics between fungal and bacterial colonizers; influence of leaf physiology on colonization potential of microbes not destined for phylloplane and their relationship with other phylloplane microbes. The study threw light on adaptability of human enteric pathogens to colonize plant surfaces. The data would be immensely helpful for designing bio-control strategies for combating the attacks on agriculturally important plants and crops. The bacterial and fungal isolates could also be further studied individually or as consortium for development of bio-control agents. It will prove interesting to understand the molecular dynamics of how the human pathogens provide resistance to plants against phytopathogens by acting as bio elicitors of defence response in plants. The elevated concentration of defensive metabolites, and enhancement of defence enzymes activity can prove beneficial to the plant defence dynamics and help in understanding the role of phylloplane microbiota in improvising the disease resistance mechanism of plants and crops like *Solanum lycopersicum*, and can also be further evaluated for their antimicrobial and antioxidative properties, if any.

P. koreensis and *S.daejeonense* were first isolated from soil and water samples in Korea and France respectively. Information is lacking on the plant growth promoting abilities of these bacteria. The study could not provide insight into the PGPR role if any, and the antioxidative and antimicrobial activity of phenols and flavonoids of *S .lycopersicum* against phylloplane colonising microbes, *P. koreensis*, *S.daejeonense* and human enteric pathogens couldn’t be assessed.