Chapter 6
CONCLUSION

- Disease incidence of two major foliar fungal diseases – Grey blight and leaf blight were evaluated in eight morphotypes of *Persea bombycina.* Occurrence of leaf blight disease was much frequent in the area than grey blight disease.
- Isolation of pathogen from infected leaves resulted in three isolates from leaf blight infected leaves – SOM/Cl/01, SOM/Cl/02, SOM/Cl/03 and three more isolates from grey blight infected leaves – IPL/SOM/P/01, IPL/SOM/P/02, IPL/SOM/P/03. Following completion of Koch's postulate two isolates – IPL/SOM/P/01 for *Pestalotiopsis disseminata* and SOM/Cl/02 for *Colletotrichum gloeosporioides* were further taken into consideration for molecular identification.
- Growth characters and spore morphology of these six isolates were studied. Scanning electron microscopy of the spore structures was also studied.
- Screening of resistance of eight morphotypes towards foliar fungal pathogens was studied using two techniques – detached leaf and whole plant inoculation technique. Both the techniques gave similar result where it was observed that S5 morphotype is highly susceptible to grey blight disease followed by S6, S7 and S2. On the other hand S6 morphotype was most susceptible to leaf blight disease, followed by S3, S8 and S5.
- Polyclonal antibody was raised separately against mycelial antigen of both the fungal pathogens. These antibodies were used for serological characterization of the fungal pathogens by PTA-ELISA, Dot-blot, Western blot and Indirect immunofluorescence of mycelia and spore. Western blot analyses using polyclonal antibody of *C. gloeosporioides* and *P. disseminata* revealed that the PAb could show different levels of homologous reactions with the antigens of *C. gloeosporioides* and *P. disseminata* respectively. Sharp and intense bands were produced on the nitrocellulose membrane after enzymatic reaction with NBT BCIP. Efficacy of polyclonal antibodies raised against the mycelial antigen was further tested with the help of indirect immuno fluorescence of young mycelia of *C. gloeosporioides* and *P. disseminata.* The mycelia treated with PAbs and labeled with FITC showed apple green fluorescence.

- Presence of cross reactive antigen (CRA) in host tissues was detected using PTA-ELISA. Cellular localization of these CRA in host leaf tissues were checked using indirect immunofluorescence as well as immunogold labelling .
- Immunodetection of foliar fungal pathogens in naturally infected leaf tissues was carried out using PTA-ELISA as well as Dot immunobinding assay.. Cellular localization of the pathogens in infected som leaf tissues was studied using indirect immunofluorescence with PAb-Pt and PAb-Cg followed by labelling with FITC. Transmission electron microscopy of ultrathin sections of infected leaf tissues using the polyclonal antibodies and labelling with antirabbit goat IgG (whole molecule) gold conjugates to confirm the presence of the pathogen in infected leaf samples.
- Molecular detection of foliar fungal pathogens *P. disseminata* (IPL/SOM/P/01) and *C. gloeosporioides* (SOM/Cl/02) was carried out using 18S rDNA sequencing using ITS1/ITS4 primers. The BLAST query of the 18S rDNA sequence of the isolates against GenBank database confirmed the identity of the isolate IPL/SOM/P/01 as *Pestalotiopsis* sp and SOM/Cl/02 as *Colletotrichum gloeosporioides*. The sequences have been deposited to NCBI, Genbank database under the accession no KT697994 for *Pestalotiopsis* sp and KM491736 for *C. gloeosporioides*.
- Species specific primer pair for *C. gloeosporioides* (CgINT/ITS4) was used to identify the other isolates. Electrophoresis led to the appearance of 480 bp single band only for *C. gloeosporioides* isolates separating them from the other isolates of *P. disseminata*.
- Molecular characterization of the fungal isolates was carried out using RAPD and DGGE analysis. The genetic relatedness among the different isolates of *Colletotrichum* and *Pestalotiopsis* was analyzed separately using different random primers for producing reproducible polymorphism. RAPD banding pattern revealed that the isolates of *C. gloeosporioides* and *P. disseminata* were genetically different and showed polymorphism among each other. On the other hand DGGE analysis could differentiate the isolates into two separate groups based on the migration rate of their amplified DNA. The migration of amplified 18S rDNA samples within each group was similar, suggesting that there was little intraspecific variation among the isolates.

- Activity of various defense enzymes such as PAL,POX,CHT and GLU were assayed in healthy as well as in infected leaf samples. It was recorded that the activity of these enzymes were more in infected leaves than in healthy samples. Isozymes of peroxidase was also checked using native PAGE and it was revealed that appearance of new peroxyzyme was seen in infected leaf samples.
- Changes in levels of phenolic in healthy and infected samples were also noted. The phenolic acids were checked using High Performance Liquid Chromatography and it was revealed that Catechol, Morin and Chlorogenic acid were present in both healthy and infected leaf samples, however their intensity increased in infected samples. On the other hand appearance of new peaks representing ferulic acid and salicylic acid were recorded in infected leaves. Hence it was evident that chlorogenic acid, ferulic and salicylic acids play an important role in defense mechanism of these plants against foliar fungal pathogens.
- Selected bioinoculants such as PGPR (*Bacillus pumilus* and *B. altitudinus*) and PGPF (*Trichoderma harzianum* and *T. asperellum*) were evaluated for their antagonistic effect against the fungal pathogens and it was recorded that these two bioinoculants could easily prevent the growth of the fungal pathogens *in vitro*.
- These bioinoculants were mass multiplied and applied to the som plants under nursery and field condition. Association of Arbuscular mycorrhizal fungi (AMF) with som roots was checked and these AMF were further mass multiplied for application. Vermicompost with value addition with bioinoculants was also prepared and applied accordingly. All these bioinoculants were applied alone and in combinations to determine their effects on growth promotion and biochemical changes in som plants.
- It was recorded that bioinoculants applied in different combinations enhanced the growth of som plants, irrespective of the morphotypes. Biochemical changes including defense enzymes were also seen to increase in treated plants in comparison to untreated control plants.
- Activation of defense response against *C. gloeosporioides* and *P. disseminata* in plants treated with bioinoculant was observed. Application of PGPR, PGPF and AMF in combination decreased the disease incidence of leaf blight.

Besides application of value added Vermicompost reduced the disease incidence of grey blight.

- Growth promotion of two different morphotypes (S5 and S6) of som plant was studied under field condition following application of bioinoculants. HPLC analysis of their phenolic content was also studied where it was observed that bioinoculant treated plants showed presence of more phenolic acids than control sets. On the other hand disease incidence of leaf blight decreased and levels of defense enzymes increased after artificial inoculation of these plants with *C. gloeosporioides*.
- Following these studies, cellular localization of two important defense enzymes – Glucanase and Chitinase was studied using indirect immunofluorescence and immunogold labelling. Expression of these enzymes were noted in treated leaf and root sections, confirming earlier results obtained.
- Results of the present study indicate that application of bioinoculants promotes growth and bioprimes the som plant against foliar fungal pathogens by up-regulation of defense activities. These findings could be helpful in protecting plants against fungal pathogens and improving the quality and quantity of the foliage of *P. bombycina* that would in turn provide quality yield of cocoon of muga silkworm.