Chapter 5

Summary & Conclusions
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The Sunflower (*Helianthus annuus* L.) is one of the most important oil crops globally and is grown on over 22 million hectares worldwide, with a production of 26 million tones. Among the various fungal diseases, the charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid. remains to be a challenging task in terms of management, since it is soil-borne in nature. It is distributed worldwide and is prevalent in arid, sub-tropical and tropical climate especially in the areas with low rain fall and high temperature. Various disease management methods have been implemented to combat and eradicate pathogenic fungi. These include cultural, regulatory, physical, chemical and biological methods. In recent past, it has been realized that use of chemicals in Agriculture is not as beneficial as it was visualized. Chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material. In addition to that target organism, chemical pesticides also kill various beneficial organisms, the pathogen become resistant to fungicides. Their toxic forms persist in soil and contaminate the whole environment. The use of bio-products is an alternative method for the management of plant diseases. Hence, the present study was carried out to evaluate the potential of rhizospheric microorganisms to control the charcoal rot pathogen *Macrophomina phaseolina* (Tassi.) Goid. The following aspects were carried out during the study period.

Rhizosphere soil samples of sunflower crop plant were collected from three different districts (Pudukkottai, Trichirappalli and Thanjavur) of Tamil Nadu, India. Part of the soil samples were used for the analysis of physico-chemical parameters such as pH, temperature, moisture, electric conductivity, available nitrogen, phosphorous, potassium, organic carbon, magnesium, zinc, copper, iron. Remaining soil samples were used for further analysis. Totally ten Plant Growth Promoting Rhizobacteria (PGPR) were isolated and tested for their plant growth promoting activities, such as Indole Acetic Acid (IAA) production,
Phosphates solubilization, Ammonia production, Hydrogen Cyanide production (HCN), Siderophore production, Cell wall degrading enzyme activity (Cellulase, Chitinase, Protease). Further all the isolates were tested for in vitro antagonistic activity against *M. phaseolina*. Among the ten isolates, the potential rhizobacterium CCPS1, which gave better results for all the test was identified as *Bacillus amyloliquefaciens* using 16S rDNA sequencing.

The fungal antagonist *Trichoderma* spp such as *T. viride* and *T. koningii* were isolated. Their inhibitory activity towards *M. phaseolina* was tested by dual plate assay, volatile metabolites assay and non volatile metabolites assay. It was observed that both the antagonist exhibited inhibitory activity against the test pathogen. Cell wall degrading enzymes such as cellulase and chitinase activity of these two species were measured. The highest cellulase activity of the isolate of *T. viride* and *T. koningii* observed were 3.15IU/ml and 2.75 IU/ml respectively after 72 hours of incubation. The maximum chitinase enzyme activity was 0.37IU/ml in the case of *T. viride*, whereas *T. koningii* exhibited 0.29IU/ml activity.

The root colonization and diversity of AMF from ten different agro-ecological zone were measured. A total of 4,446 spores of AMF were wet sieved from soil sample collected from ten different agro-ecosystems. The spores mainly belonged to 14 species which were identified. Diversity measurement such as Spore density (SD)/100g of soil, Species Richness (SR), Shannon-Wiener index of diversity (H’) and Sampson’s index of dominance (D) were analysed. The species richness was maximum at Vallamputhur (SR 7±0.21per/soil, SD 680±0.27/100g soil however, the spore density was minimum when compared to Meickalnaickanpatti (SR 5%, SD 704/100g soil). Spore density (SD) was positively correlated with species richness (SR). Based on relative abundance and isolation frequency, it was observed that *Glomus*
fasciculatum was most dominant (21.64±0.14% of RA) followed by Glomus mosseae (16.17± 0.17% of RA) and Acaulospora scrobiculata (13.40 ± 0.45% of RA). However Glomus mosseae contributed to greater isolation frequency (60±0.01%) and was widely distributed followed by Glomus fasciculatum and Acaulospora scrobiculata (50±0.23%). Isolation Frequency (IF) was positively correlated with Relative Abundance (RA). Correlation analysis between spore density and physico-chemical parameters were analysed and recorded.

The predominant species such as Glomus fasciculatum, Glomus mosseae and Acaulospora scrobiculata were mass multiplied by funnel culture technique and pot culture technique using onion (Allium cepa L.) as host plant. The percentage of AM colonization in the roots of onion was assessed and it was found that the colonization varied among the three different species (60%-85%). The number of spores present in 100gm of soil ranged from 150- 270. The maximum colonization and spore number were found in the roots inoculated with G. fasciculatum (85%)

These three fungi were used as single and in various combination to inoculate a most commonly cultivated variety of sunflower CO(SVF)5 under green house condition. The morphological parameters like number of leaves, root length, shoot length, fresh weights of root and shoot, dry weights of shoot, flower head diameter, and number of seeds per head and hundred seeds weight were measured at different days after inoculation (40DAI, 60DAI and 85DAI). Similarly biochemical parameters such as chlorophyll content of leaves, protein, carbohydrate, acid and alkaline phosphatase content of root tissue were measured at 40, 60, 85 DAI. Among the various combinations the combined inoculation showed better growth and yield over control.

The same sunflower crop variety was used to study the biocontrol activity of AMF fungi against the charcoal rot pathogen M. phaseolina. The pathogen was isolated from infected root tissue of sunflower plant and mass multiplied
using maize as carbon source in sand maize medium. The plants were inoculated with various combinations of AMF and pathogen, the plant without any inoculation acted as positive control and plant inoculated only with \textit{M. phaseolina} acted as negative control. Defence biochemical markers such as total phenolic content, peroxidase (POX), poly phenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and isoenzyme pattern for poly phenol oxidase (PPO) were analysed at different days after inoculation (40, 60, 85 DAI). The combined inoculation of \textit{G. mosseae} + \textit{G. fasciculatum} + \textit{A. scrobiculata} + \textit{M. phaseolina} showed maximum phenolic content of 0.882±0.006 mg/g.f.wt at 85DAI when compared to non mycorrhizal plant. The highest peroxidase activity was registered in plant treated with \textit{G. mosseae} + \textit{A. scrobiculata} + \textit{M. phaseolina} (0.686 ±0.040 ΔA_{470}/min/g.f.wt) at 85DAI. \textit{G. fasciculatum} + \textit{A. scrobiculata} + \textit{M. phaseolina} inoculation showed maximum poly phenol oxidase (0.601±0.136 ΔA_{495}/min/g.f.wt) at 85DAI. Similarly phenylalanine ammonia-lyase activity was found to be high at 85DAI in plant introduced with \textit{G. mosseae} + \textit{G. fasciculatum} + \textit{A. scrobiculata} + \textit{M. phaseolina} (4.271±0.031 nm of cinnamic acid released).

The appearance of isoform of PPO with molecular range of 45KD was observed in all the plants including positive and negative control. However new isozyme pattern above 45KD was observed in plants inoculated with \textit{A. scrobiculata} + \textit{M. phaseolina}, \textit{G. mosseae} + \textit{G. fasciculatum} + \textit{M. phaseolina} and \textit{G. mosseae} + \textit{A. scrobiculata} + \textit{M. phaseolina}. Another isoform approximately 66 KD was observed in \textit{M. phaseolina} alone, \textit{G. mosseae} + \textit{G. fasciculatum} + \textit{M. phaseolina}, \textit{G. mosseae} + \textit{A. scrobiculata} + \textit{M. phaseolina}, \textit{G. fasciculatum} + \textit{A. scrobiculata} + \textit{M. phaseolina} and \textit{G. mosseae} + \textit{G. fasciculatum} + \textit{A. scrobiculata} + \textit{M. phaseolina} inoculated plants.
The result of the present study clearly indicated that the rhizobacteria and *Trichoderma* spp effectively inhibited the charcoal rot pathogen *M. phaseolina* in *in vitro* condition. It was also observed that the combined inoculation of *G. mosseae, G. fasciculatum* and *A. scrobiculata* significantly induced morphological, biochemical and defence related compounds in sunflower crop. Thus it can be concluded that AM fungi can efficiently reduce the disease severity caused by *M. phaseolina* and their application can protect the plant against soil borne pathogens.