CHAPTER-V

SUMMARY AND CONCLUSIONS

The experiment was carried out on “Biochemical and molecular aspects of Trichoderma in groundnut (Arachis hypogaea L.) against stem rot (Sclerotium rolfsii)” is summarized and concluded in this chapter.

Groundnut is a crop which mainly cultivated under rain fed condition and hence there are more chances to attack pathogens on them. Among the diseases related to groundnut, stem rot (Sclerotium rolfsii) is one of the important disease which causes heavy loss in pod and fodder yield of groundnut. The most of the varieties of groundnut are susceptible to this disease. The disease is manifested in pre and post emergence phases. Many seed dressing fungicides are reported to be effective against stem rot of groundnut, but limited work has been done on successful exploitation of bio-control agents-Trichoderma on management of the disease. It is highly needed to keep the disease below economic threshold level without damaging the agro-ecosystem in soil.

Keeping this in view, present experiment was conducted in three parts. (1) In vitro antagonistic effect of various isolates of Trichoderma strains as bio-agents against disease causing S. rolfsii (2) Biochemical characterization of various isolates of Trichoderma strains and disease causing pathogen S. rolfsii (3) Molecular characterization of various isolates of Trichoderma strains and disease causing pathogen S. rolfsii

Antagonistic effect of 6 species of Trichoderma (T. harzianum NBAII Th 1; T. hamatum NBAII Tha-1; T. virens NBAII Tvs 12; T. viride NBAII Tv 23 T.koningii 796 and T.pseudokoningii 2048) against stem rot disease causing fungus S. rolfsii was studied in vitro and observed that T. viride NBAII Tv 23 inhibited maximum (61%) growth of test fungus followed by T. harzianum NBAII Th-1 (55%). The specific activity of pathogenesis related enzymes chitinase, β-1,3-glucanase and protease were examined maximum in the culture medium of antagonists who inhibited the growth of test fungi maximum. However, Cell wall degrading enzymes cellulase and PG were found maximum in control S. rolfsii containing culture medium.

A significant positive correlation (p=0.01) between per cent growth inhibition of test fungus and biochemical parameters chitinase, β-1,3-glucanase and protease in
the culture medium of antagonist treatment established a relationship to inhibit growth of fungal pathogen by increasing the levels of this lytic enzymes. However, cell wall degrading enzymes cellulase and PG were significantly negatively correlated with percent growth inhibitions of *S. rolfsii*.

Thus, enzymes related to *Trichoderma*- chitinase, β-1, 3 glucanase, and protease were released during antagonism and inhibited the growth of fungal pathogen. Among the 6 bio-control agents of *Trichoderma*, *T. viride* NBAII Tv 23 was the best agent to inhibit the growth of fungal *S. rolfsii* on PDA media. Thus, *T. viride* NBAII Tv 23 is a suitable strain to be used in biological control of plant pathogen *S. rolfsii*.

The results of SDS-PAGE revealed a total number of 41 bands with molecular weights (MW) ranging from about 156.97 to 1113.16 KDa and Rf values ranging from 0.788 to 0.220 were detected at 4 DAI in different treatments. Treatment with *T. viride* gave more no of bands 10 and *T. harzianum* 6 followed by *T. koningii*, *T. hamatum*, *T. pseudokoningi*, *T. virens* a no of total band 6, 6, 5, and 3 bands were detected respectively. Data showed no common bands (monomorphic), while the remaining all 41 bands were polymorphic. Polymorphism at 4 DAI was due to various *Trichoderma* species efficiency utilize cell wall.

Protein band of 157 KDa observed only in T₆ antagonist (*T. viride* x *S. rolfsii*) which may be considered as marker for disease resistance against stem rot in groundnut. Maximum polymorphic bands (ten) appeared in *T.viride* and minimum (3) protein bands appeared in *T.virens*. In protein profile *T. viride* NBAII Tv 23 *T. harzianum* NBAII Th-1 gave more number of bands then that of other at 4 DAI. Number of polymorphic bands was increased because of resistance induction by *Trichoderma viride* against *S. rolfsii* which are causing stem rot disease in groundnut. The extracellular proteins from *T. viride* NBAII Tv 23 synthetic media for 96 hrs underwent 2-DE analysis, which were revealed significant result shows 59.95 to 131.83 KDa of protein spots. The Rf value of proteins ranged from 0.702 to 0.914.

Two major clusters were observed among six *Trichoderma* species and *S. rolfsii*. Cluster analysis was carried out by the UPGMA method using Jaccard’s similarity coefficient. The dendrogram revealed that 6 *Trichoderma* species and pathogen *S. rolfsii* segregated into two main clusters-A and B with the similarity of 36% (Fig. 4.8). Cluster-A is divided into two sub groups A₁ consisting *T. harzianum*...
and *T. viride* and *S. rolfsii* in sub group A₂ consist of *T. virens* with 38% similarity. Cluster-B is divided into two Sub group B₁ consisting of *T. hamatum, T. koningii* and in sub group B₂ consisting *T. pseudokoningii* with 54% similarity.

Amplified products were observed when the genomic DNA of microbes (*Trichoderma* species and *S. rolfsii*) was subjected to RAPD analysis using random decamers of fungal primers. The RAPD profiles showed presence of high level of polymorphism between the isolates of *Trichoderma* and pathogen *S. rolfsii*. A total 305 bands were produced by 21 RAPD primers with an average frequency of 14 bands per primer. The calculated PIC values for RAPD markers ranged from 0 to 0.911.

Dendrogram was constructed using the NTSYS 2.2 software. The similarity coefficient ranged from 0.27 to 0.50. The highest growth inhibition of pathogen *S. rolfsii* was 61% and 63.4% by *T. viride* and 55% and 56.4% by *T. harzianum*, respectively, during *in vitro* study which were also in same cluster and shared 43.5% similarity. Primer OPC-05 generated unique band of *T. koningii* (500 bp) and *T. viride* (900 bp) and OPA-16 generated unique band of *T. harzianum* (220 bp) and *S. rolfsii* (500 bp) which will be purified and used to develop SCAR marker for particular species. However, primer OPA-17 generated unique band of *T. koningii* (900 bp). Primer OPA-16 generated 431.73 and 514.89 bp RAPD markers from DNA of *S. rolfsii* Similarly, OPB-08 associated with 419.91, 507.39, 805.82 and 1059.82 bp marker for *S. rolfsii*.

Among 21 RAPD primers only two primers were selected for designing the SCAR primers. OPC-05 gave a unique fragment of size 900 bp in *T. viride* isolate and gave no amplification in other five species. OPA-16 gave a polymorphing fragments of size 220 bp in *T. harzianum*. The unique fragment of each species was not present in other species. The 900 bp fragment produced by OPC-05 random primer was sent for sequencing. The unique fragments produced by OPA-16 random primer in *T. harzianum* were directly sent as purified PCR product for sequencing. The sequences of these species were subjected for BLAST search at NCBI, but none of the sequence showed similarity with data base sequences of *Trichoderema*. SCAR primers were designed by using these sequences and sent for synthesis.

The designed putatively species-specific SCAR primer pairs for two species of *Trichoderma* (*T. harzianum* and *T. viride*) were used to amplify the genomic DNA of
other 5 species. A single and sharp 900 bp band corresponding to the original RAPD fragment was obtained in *T. viride* with VIR-900 SCAR primer and no amplification was observed in the other six species. Single and bright 220bp band corresponding to the original RAPD fragment was obtained in *Trichoderma harzianum* with HAR-220 SCAR primer and amplification was not observed in the other six species.

**THRUST AREAS OF FUTURE WORK**

Future consideration with this research is isolation of genes from best *Trichoderma* strain (*T. viride* NBAII Tv 23) which encoding either enzymes (chitinase and β-1,3-glucanase) or structural or regulatory proteins, or components of signaling pathways, and manipulating such genes into host plant (groundnut) to develop resistant lines against stem rot disease as so far no resistant varieties developed. These tools will allow more efficient formulations to control fungal pathogens in pre and post emergence period.