CHAPTER - VI

SUMMARY
Chilli (*Capsicum* sp.) is considered as one of the most important commercial crops worldwide, especially in tropical and subtropical regions and is a widely used universal spice, called as wonder spice. Chilli suffers from several foliar fungal diseases amongst which cercospora leaf spot disease (CLSD) has shown to cause significant crop losses in various parts of the world including India. The only effective control measure for CLSD is to use the chemical fungicides. However, in view of the concerns raised about the economic and ecological consequences of chemical fungicides, more environmentally-benevolent control measures have been explored. Arbuscular mycorrhizal fungi have long been known to improve vegetative growth, yield and overall health of the plants. Role of organic amendments in plant disease management has also been investigated by various researchers. However, scientific investigations concerning the combined role of AM fungi and organic amendments such as vermiwash in suppression of cercospora leaf spot disease of chilli are meagre. In view of this, the current study entitled “Exploring the role of arbuscular mycorrhizal fungi and organic amendments in suppression of the cercospora leaf spot disease of *Capsicum* sp.” was conducted. Various experiments, their results and salient findings pertaining to the current study are summarized as under:-

- Sixteen arable fields representing all six agro-climatic zones of Assam were surveyed and rhizospheric soil was collected, and subjected to physico-chemical characterization and DGGE-based AM fungal community analysis. Results revealed that the soil of the studied sites was mostly acidic and low in available phosphorus having maximum recorded value of phosphorus 12 Kg/ha for hill zone thereby suggesting potential of AMF
application in crop improvement strategies. Enzyme assays revealed that phosphatase activity was the highest in hill zone followed by North Brahmaputra valley zone. Total DNA from all rhizospheric soils was extracted. AM fungal DNA fragments of ITS2 and LSU-D2 regions were amplified through PCR using specific primers. Amplicons belonging to ITS2 and LSU-D2 region were then subjected to denatured gradient gel electrophoresis (DGGE). Resultant DGGE banding pattern was photographed and analysed using Gel Compar II ver 6.5 software. Further, prominent DGGE bands were sequenced. Cluster analysis of banding pattern was carried out using unweighted pair group mean average (UPGMA). Results revealed that ITS2 and LSU-D2 region-based AM fungal diversity pattern was quite matching. Based on the prevalancy in various agro-climatic zones, *Rhizophagus irregular* and *Glomus mosseae* were selected for further experimentation.

In order to screen out the most appropriate substrate and earthworm species for vermiwash preparation, a comparative evaluation of two different earthworms (*Eisenia fetida* and *Perionyx excavatus*) with four different substrates (vegetable waste, paddy straw, water hyacinth and mixed waste) was carried out. These wastes were collected and mixed with the ratio of 15:85 (cow dung: waste) after drying. Each substrate was heaped above the ground as a vermicomposting bed for treatments by two different earthworms (*E. fetida* and *P. excavatus*). Results suggested that *P. excavatus* performed consistently in mineralization of all the substrates at 60th DOS when compared with that with *E. fetida*. Based on the
comparative performance, *P. excavatus* with mixed waste substrate was further selected for the vermiwash preparation.

- In order to identify and describe the fungal phytopathogen causing leaf spot disease of *Capsicum assamicum* (Naga chilli), detailed micromorphological characteristics using light microscopy and scanning electron microscopy (SEM) were investigated. Morphological characterization revealed several peculiar and hitherto unreported features of the fungal isolate. In addition, multi-locus sequence typing (MLST) approach using four loci namely *ACT*, *CAL*, *HIST* 3 and *TEF*-1a in combination was adopted. In MLST, the sequences of the amplified regions of these four loci were aligned, appended and subjected to likelihood (Bayesian inferences and maximum likelihood) and parsimony analysis. Phylogeny analysis suggested that the fungal isolate demonstrated closeness with *Cercospora* sp. (CBS132619) of the *Musa* host and *C. rodmani* (5H-GTOX) of the *Eichhornia* host. Further, single nucleotide polymorphisms (SNPs) analysis of sequences of closely related *Cercospora* sp. along with the test isolate was carried out. Results of SNP analysis also revealed that the sequences of the test isolate had significant number of polymorphic sites. Overall, based on the morphological and molecular characterization, the test isolate was described as a novel species of *Cercospora*, for which the name *C. tezpuresis* is provided. Detailed description of the novel isolate has been submitted to international database Mycobank under reference number MB 803969. Further, the fungal culture has been deposited to National Fungal Culture Collection of
India (NFCCI), Pune under accession number NFCCI-3067.

- In order to evaluate the comparative response of Capsicum towards the treatments with AM fungi and vermiwash, a pot experiment was conducted with foliar spray of vermiwash as an organic amendment strategy on Ca. assamicum exposed to two different arbuscular mycorrhizal fungi (AMF: Rhizophagus irregularis and G. mosseae) in acidic soil under naturally ventilated greenhouse conditions. Plant growth parameters, mycorrhizal colonization and its spore count in soil along with mycorrhizal dependency and, physico-chemical properties of soil and vermiwash were recorded. Results revealed that the VW spray significantly influenced the growth of plants receiving the dual treatment of AMF+VW. Plant growth was more prominent in the G. mosseae plus vermiwash treatment group than that in the R. irregularis plus vermiwash treatment group. The plant-AMF interactions in relation to growth and nutrient requirements were also significantly influenced by the application of VW. Interestingly, the VW treatment appeared to contribute more N to plants than did the AMF treatment, which led to changes in the C:N:P stoichiometry in plant shoots.

- Vermiwash was evaluated in vitro for its possible role in suppression of cercosporin toxin biosynthesis by C. tezpurenensis. The fungal culture was grown on PDA with 20% vermiwash (aqueous) and toxin was extracted from the culture in acetone as solvent and subjected to quantification by HPLC. Results revealed that cercosporin quantity was 93% less in the culture grown on PDA amended with 20% vermiwash as compared to
Based on the comparative response of *Capsicum* towards inoculation with two different AM fungi and VW, the role of *G. mosseae* (GM) and vermiwash (VW) in suppressing cercospora leaf spot disease of *Capsicum* under growth pouches with *C. tezpuresis* was investigated. In this study, expression analysis of 31 genes and physiological attributes belonging to pathogenesis-related (PR) gene family, antioxidant groups, genes related to cell death, carotenoid pathway, salicylic acid dependent pathway, systemic acquired resistance, secondary metabolite pathway and cercosporin toxin biosynthesis (CTB) was carried out. Results suggested that the test plant appeared to adopt antioxidant pathway for disease suppression in presence of VW treatment. However when treated with GM, the test plant seemed to follow a relatively broad-spectrum strategy for disease suppression as was evident by significantly expressed genes related to different activities such as cell death, carotenoid, salicylic acid and systemic acquired resistance.