Chapter 7
CONCLUSION

Agricultural land in the district of Jalpaiguri in West Bengal was the main area of the following study. A total number of 17 actinomycetes isolates were obtained from rhizospheric soil of potato fields. All the isolates were characterized by their morphological, biochemical and physiological attributes. The actinomycetes isolates were identified initially as belonging to the group Streptomycetes .

Further study of the isolates with both normal bright field microscopy and Scanning Electron microscopy revealed about their spore chain morphology and identified the isolates as *Streptomyces* spp.

In vitro screening of the isolates for phosphate solubilisation activity was done. Out of 17 isolates 13 isolates were found to be phosphate solubilizer. *Streptomyces tricolor* (ARHS/PO/26) was found to be the most efficient phosphate solubilizer among the isolates. This isolate was able to solubilise both tricalcium phosphate and rock phosphate efficiently.

The isolates were also tested for their growth promoting attribute i.e. production of Indole Acetic Acid (IAA). All the isolates were found to produce IAA but the level of the hormone was highest in the isolate ARHS/PO/26, ARHS/PO27 and ARHS/PO/15 respectively. In the other isolates the amount of IAA was not very high.

In vitro screening of the isolates for antifungal activity was evaluated with two fungal root pathogens *Fusarium solani* and *Sclerotium rolfsii*. The fungal pathogens *Fusarium solani* and *Sclerotium rolfsii* cause root rot disease of *Phaseolus vulgaris* and *Vigna radiata* respectively. From the *in vitro* study it was found that ARHS/PO/26 showed antagonist activity against *Sclerotium rolfsii* whereas ARHS/PO/27 was highly antagonist against *Fusarium solani*.

Selected isolates were identified successfully by their 16S rDNA sequences. Three isolates were identified as being *Streptomyces griseus* (ARHS/PO/15), *Streptomyces tricolor* (ARHS/PO/26) and *Streptomyces flavogriseus* (ARHS/PO/27). Genetic relatedness of the three isolates was also measured. The sequences have been

deposited to NCBI GenBank and Accession numbers of the same were obtained. The accession numbers for the isolates are *Streptomyces griseus* ARHS/PO/15 (KX894282), *Streptomyces tricolor* ARHS/PO/26 (KX894280) and *Streptomyces flavogriseus* ARHS/PO/27 (KX894281).

Growth promoting attributes of the three isolates *Streptomyces griseus* (KX894282), *Streptomyces tricolor* (KX894180) and *Streptomyces flavogriseus* (KX894281) were tested in both pot and open field conditions. Though two of the isolates *Streptomyces griseus* (KX894282) and *Streptomyces flavogriseus* (KX894281) could not solubilise phosphate *in vitro* they successfully contributed in plant growth *in vivo* condition. The effect of the isolates on nodulation of the leguminous plants were also studied. Though the nodulation frequency increased in the treated plants in comparison to the untreated plants the nodulation index variation was not that much.

Disease symptoms were observed in plants artificially inoculated with the test pathogens. The histo-pathological study of the infected root tissue was done. Plants were treated with *Streptomyces griseus* (KX894282), *Streptomyces tricolor* (KX894280) and *Streptomyces flavogriseus* (KX894281) in open field and pots, and activities of various defence enzyme activities, PAL, POX, Glucanase, Chitinase were assayed in both untreated and treated plant samples. Increase in the level of defence enzymes were observed in the treated plants in comparison to the untreated control plants. Application of the fungal pathogen by artificial inoculation method to both untreated and treated plants increased the level of defence enzymes furthermore.

In *Phaseolus vulgaris* after the pathogen inoculation it was found that levels of defence enzymes was highest in the treated plants after pathogen inoculation followed by the treated plants, inoculated plants and untreated control plants. The defence enzyme level was highest in the plants treated with the isolate *Streptomyces flavogriseus* (KX894281). Levels of all the defence enzymes were higher in the leaves than the roots. Total phenol content of the root and leaves tissue were also assayed. Root tissues showed higher phenol activity. Total protein content of the plants were also assayed which was much higher in the leaf tissues than the root tissue.

In *Vigna radiata* also the level of defence enzyme increased in treated inoculated plant samples. Here also the lowest levels of enzymes were found in untreated control plant samples. The enzyme level was highest in plants treated with

Streptomyces tricolor (KX894280) and inoculated with the pathogen *Sclerotium rolfsii*. Total phenol content of the root and leaves tissue were also assayed. Root tissues showed higher phenol content. Total protein content of the plants were also assayed which was much higher in the leaf tissues than the root tissue.

Cellular localization of the two defence enzymes Chitinase and Glucanase were also studied by indirect immunofluorescence. Expressions of these enzymes were observed in treated leaf sections, in accordance to the earlier results obtained.

The study undertaken is indicative of the positive role of actinomycetes in crop cultivation and very much supports the aim of the study, that they play a significant role towards plant growth promotion and selected strains of locally isolated groups can also act as antagonist against fungal plant pathogens. The findings of the study can be exploited to explore the potential application of actinomycetes formulation, for dual application, as plant protector and growth enhancer of plant, not just for the pulse crop like *Phaseous* or *Vigna*, the crop plants identified for the present study but also for plantation crops like tea and Large cardamom in the near future.