Summary

A review of literature has been presented to focus the microbial resources available in the rhizosphere of various plants with special references to the colonization of roots with Arbuscular Mycorrhizal fungi (AMF), as well as involvement of plant growth promoting Rhizobacteria (PGPR) for the improvement of plant health status. Materials used and methods followed have been presented in Materials and Methods.

Initially survey was conducted in Darjeeling Hills to record prevalent diseases of mandarin (*Citrus reticulata*) caused by pest and pathogens and an inventory was outlined. Among the root diseases caused by fungal pathogens, *Macrophomina phaseolina*, *Fusarium solani* and *Fusarium oxysporum* were mostly recorded in various mandarin orchards. One of the predominant root rot disease caused by *Macrophomina phaseolina* was selected for its management using bioinoculants available in the rhizosphere of mandarin plants.

Charcoal root rot pathogen (*Macrophomina phaseolina*) isolated from mandarin orchards of Darjeeling hill was used for present study after completion of Koch's postulate. Development of root rot disease of *Citrus reticulata* was studied using mandarin seedlings from eight different locations, viz. Rangali Rangliot, Bijanbari, Sukhia Pokhari, Kurseong, Mirik, Kalimpong Block I, Kalimpong Block II and Gorubahan. The root rot index as well as percentage loss in dry weight of roots were found very low at the initial stage of infection which increased significantly with time in compatible interaction. Mandarin seedlings of three locations (Mirik, Kalmpong Block-I and Sukia Pokhri) were found to be highly susceptible. Cultural conditions affecting growth of the pathogen were studied *in vitro*.

The soil samples collected from eight different locations were analysed before the isolation of microorganisms. Moisture content, pH, soil type, soil texture, carbon and nitrogen ratio, available K and P were determined. Rhizosphere microflora of mandarin plants were studied with special reference to their growth and sporulation behaviour. It was found that most of the fungal isolates belonged to the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Sporotrichum*, *Rhizopus*, *Macrophomina*, *Emenicella* and *Trichoderma*. Bacterial isolated were characterized based on morphological and biochemical studies following Bergey's manual of Systematic Bacteriology. Isolates were characterized for H_2S production, phosphate solubilization, starch hydrolysis, casein hydrolysis, chitin degrading, siderophore production, catalase production, protease production, urase production, cellulase production

and indole production. Overall, *Bacillus pumilus, Bacillus cereus, Bacillus sp.*, and *Pseudomonas sp.*, were found to be more abundant. Among these *Bacillus pumilus* was found to be phosphate solubilizer and also antagonistic against root pathogens (*M. phaseolina, F. oxysporum, F. solani, F. graminearum* and *Rhizoctonia solani*)

Screening of arbuscular mycorrhizal fungi (AMF) from rhizosphere of mandarin plants grown in Darjeeling hill yielded *Glomus mosseae, G. fasciculatum, G. intraradices, G. versiforme, Gigaspora margarita, G. rosea, G. gigantea, Acaulospora spinosa, A. bireticulata, Scutellospora* sp. Some of the characteristic features were considered for identification of all those isolates which were found as consistent association and maximum colonization with mandarin roots. Scanning electron microscopic observation of three important genera *Glomus, Gigaspora* and *Acaulospora* were made. *Glomus mosseae* and *G. fasciculatum* were found to be dominant. However, based on maximum percent root colonization of mandarin seedlings *Glomus mossese* was selected for mass multiplication in sorghum and maize plants.

Polyclonal antibodies (PAbs) were raised against mycelial antigens of *M. phaseolina* . IgG were purified and further packaged into immunological formats using PTA-ELISA, dot blot, western blot and immunofluorescence for quick and accurate detection of pathogens from soil and mandarin root tissue. Indirect staining of mycelia and sclerotia of *M. phaseolina* with homologous PAb and labeling with goat antirabbit IgG conjugated with FITC developed strong fluorescence in young hyphal tips, conidia and sclerotia of pathogens. Major cross reactive antigens shared by root and the pathogen was detected using PTA-ELISA format. Cellular location of CRA in mandarin root tissue was confirmed using PAb of the pathogen and FITC conjugates.

Besides, PCR based molecular detection of pathogens have also been developed. Genomic DNA from *M. phaseolina, F. solani, F. oxysporum and F. graminearum* (among root pathogens), biocontrol agent (*Trichoderma asperellum*) and a selective PGPR (*Bacillus pumilus*) were prepared, purified. PCR amplifications of 18S rDNA were done for the root pathogens and BCA isolate using ITS specific primer pairs. The product size was approximately 570 bp for *Fusarium*, 620 bp for *Macrophomina* and 600 bp for *Trichoderma* with the size variation across the isolates. RAPD profile was also obtained for fungal and bacterial isolates using random decamer primers from Operon technology kit. All

reproducible polymorphic bands were scored and analysed following UPGMA cluster analysis protocol and computed into similarity matrix using NTSYS computer programme. A dendogram was made with similarity coefficient ranged from 0.67 to 0.95.

In vitro screening of *Trichoderma asperellum* was made for evaluation of their antagonistic activity against *M. phaseolina* and *F. solani* and further evaluated *in vivo* on the development of root rot disease of mandarin under green house as well as field conditions. Based on the screening of association of arbuscular mycorrhizal fungi (AMF) with mandarin roots in four locations of Darjeeling hills, percentage colonization behavior established by artificial inoculation of mandarin seedlings, predominant AMF *Glomus mosseae* and *Glomus fasciculatum* were selected for application in mandarin plants. Total phosphate content of soil was determined after application of *G. mosseae* and *G. fasciculatum* singly or jointly. Results revealed that soil P content had decreased due to application of AMF indicating that the plant could uptake phosphorus which had been solubilized by AMF. Application of *G. mosseae* in the mandarin saplings exhibited marked increase in growth of the plants. Application of *G. mosseae* and *T. asperellum* singly or jointly suppressed root rot of mandarin caused by *F. solani* was suppressed to certain extent by *G. mosseae*. Reduction of root rot in *Citrus reticulata* following application of bioinoculants (*G. mosseae*, *T. asperellum* and *B. pumilus*) were evident both singly or jointly. However, joint inoculation with both AMF (*G. mosseae*) and PGPR (*B. pumilus*) as well as AMF (*G. mosseae*) and BCA (*T. asperellum*) reduced disease markedly.

Defense responses of mandarin plants against root rot pathogen (*M. phaseolina*) were demonstrated during early stages of root colonization by *G. mosseae* and *T. asperellum* following application of *B. pumilus.* Activities of β 1,3 - glucanase, chitinase and peroxidase were assayed in roots and leaves and of mandarin seedlings subjected to various treatments- ie., *G. mosseae*, *B. pumilus*, *T. asperellum*, *M. phaseolina*, *B. pumilus* + *G. mosseae*, *B. pumilus* + *M. phaseolina*, *G. mosseae* +*M. phaseolina*, *T. asperellum*+ *G. mosseae*, *B. pumilus* + *G. mosseae* +*M. phaseolina*,*T. asperellum*+ *G. mosseae*+*M. phaseolina* . Activities of all 3 enzymes, in both leaves and roots, were significantly enhanced which was also confirmed by immunological assays. Cellular location of Chitinase in root and leaf tissues of *Citrus reticulata* following induction of resistance was also demonstrated using PAb of Chitinase and FITC conjugates.