CONCLUSION

Soil microflora plays the most important role in the soil region of the higher plants. The variable microfloras changes the soil fertility conditions of a specific plant and are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation; decomposition of organic matter; toxin removal; and the cycling of carbon, nitrogen, phosphorus, and sulphur. In addition, microorganisms play key roles in suppressing soil borne plant diseases, in promoting plant growth, and changes in vegetation. To this end the present study was carried out to analyze soil microbial diversity of Darjeeling Hills which included forests, agricultural rhizosphere soil and riverine soils followed by evaluation of potential plant growth promoting fungus (PGPF), Biocontrol agents (BCA) and Plant growth promoting Rhizobacteria (PGPR) for utilization for improvement of different legumes, cereals, vegetable crops as well as chief plantation crop-tea grown in this region. The overall findings of this study have been concluded as under:

- Microbial population in soils ranged between $4x10^3$- $6x\ 10^4$ cfu in case of fungi and $5x10^6$cfu-$6x10^6$cfu in case of bacteria. A total of 637 fungal isolates were obtained from the major forest, agricultural fields and river basins of Darjeeling hills. Out of the total collection, 205 isolates were obtained from forest, 373 from agricultural and 59 isolates were obtained from river basins. Similarly, a total of 135 bacterial isolates were obtained from various sources. Among them 39 were obtained from forest soil, 73 from rhizosphere of agricultural crops and 23 from riverine soil.
- Dominant fungal isolates belonged to the genera *Absidia, Acremonium, Alternaria, Aspergillus, Byasiochlamus, Colletotrichum, Drechslera, Emericella, Fusarium, Curvularia, Gonronella, Macrophomina, Noesertoria, Paecilomyces, Penicellium, Pseudoeutatum, Rhizoctonia, Rhizopus, Sclerotianum, Sporotrichum, Syncephalastrum, Talaromyces, Thanetophorus and Trichoderma.*

- The most common and abundant bacterial species were *Bacillus sp., Micrococcus sp., Coryneform sp. Staphylococcus sp. Serretia sp. Paenibacillus sp., Pseudomonus sp., Enterobacter sp.* well as *Bukholderia sp.*
- Isolates of *Aspergillus niger* (FS/L-04, RS/P-14, FS/L-40, FS/C-140), four isolates of *A. melleus* (RHS/R 12, FS/L 13, FS/L 17, FS/L 18), three isolates of *A. clavatus* (RHS/P 38, RHS/P-114, RHS/T-99) and four isolates of *Talaromuces flavus* (RHS/P 50, RHS/P 51, RHS/P 54, RHS/P 120 were found to solubilize rock phosphate and tricalcium phosphate more efficiently than rest of the others and were designated as potential plant growth promoting fungus (PGPF)
- One of the interesting findings of the present study was isolation of one potential funlgal isolate *Talaromyces flavus* RHS/P-51/ NAIMCC-F-01948, which is reported as a potential phosphate solubilizers for the first time in this study. This fungal isolate not only solubilized phosphate efficiently *in vitro* but also inhibited a number of root pathogens to a greater extent.
- Isolate *B. pumilus*, BRHS/C-1, *Bacillus altitudinis* BRHS/S-73 & BRHS/P-22 , *Enterobacter cloacae*, BRHS/R-71, *Paenibacillus polymyxa* BRHS/R-72, *B. methylotrophicus* BRHS/P-91, *Burkholderia symbiont* BRHS/P-92 and *B. aerophilus* BRHS/B-104 were found to possess all the tested plant growth promoting traits like phosphate solubilization, IAA, siderophore, HCN and ACC deaminase production. Apart from this these potential bacterial isolates could inhibit root pathogens upto 80%. They were designated as potential plant growth promoting rhizpbacteria (PGPR) isolates.
- Among the PGPR isolates *B. altitudinis*, *B. aerophillus*, *Burkholderia* spp. and *Enterobcter cloacae* though there are reports from the other parts of the world to be potential PGPRs, they have been isolated and characterized for the first time from soils of Sub-Himalayan regions of Darjeeling hills.
- A total of 26 isolates of *Trichoderma harzianum* 10 isolates of *T. viride*, 13 isolates of *T. asperellum* and 6 isolates of *T. erinaceium* were obtained from various sources and were tested for their ability to produce Chitinase *in vitro*. *T. harzianum* RHS/S-559/ /NAIMCC-F-01968 and RHS/S-560//NAIMCC-F-01967, *T. viride* isolates, isolate RHS/G 251, *T. asperellum* and *T. erinaceum* RHS/Rd-551 showed maximum endo and exo chitinase activities. All these potential *Trichoderma* isolates were designated as BCA isolates. Potential PSF

and BCA as well as commonly occurring fungal isolates have been deposited to National Agriculturally Important Culture Collection (NAIMCC) of NBAIM.

- ❖ Diversity among all the PGPF, PGPR and BCA isolates were carried out using RAPD markers and DGGE formats. The analysis of genetic relatedness using random decamer primers revealed a significant amount of genetic variation among the tested organisms. Similarly DGGE analysis of the conserved sequences could detect genetic variation even among closely related isolates. This technique was useful in detecting similarities among the functionally similar group of isolates as well as to find out the similarities between unidentified organisms with reference to a known one.
- ❖ ITS-PCR technique was successfully used to confirm the identities of all the potential PGPF, PBCA and PGPR isolates using universal primers. The sequences have been deposited to NCBI Genbank database and accession number for each isolate has been provided. The potential isolates and their NCBI Acc. numbers are: *T. flavus* RHS/P-51 (GU324073), *Trichoderma erinaceum* (HM107419, GU187915, GU191829, HM117841), *T. harzianum* (HQ334995, HQ334997), *T. asperellum* (HQ334996), *B. pumilus* BRHS/C-1 (JF836847), *B. altitudinis* BRHS/P-22 & BRHS/S-73 (HQ849482 & JF899300) *Enterobacter cloacae* BRHS/R-71 (KC703974), *Paenibacillus polymyxa* BRHS/R-72 (KC703775)**,** *Bacillus methylotrophicus*-BRHS/P-91 (JQ765577), *Burkholderia spp.* BRHS/P-92 (JQ765578) and *B. aerophilus* BRHS/B-104 (KC603894)
- ❖ All the PGPF, BCA and PGPR isolates were tested for their effect on enhancement of growth and resistance against root diseases of few important legumes (*Vigna radiata*, *Cicer arietinum*, *Glycine max*, *Pisum sativum* and *Phaseolus vulgaris*) Cereals (*Triticum aestivum* and *Oriza sativum*) vegetable crops (*Lycopersicon esculentum* and *Brassica juncea*) and plantation crop (*Camellia sinensis*). Among the PGPF isolates *T. flavus* was found to enhance the growth of tested crops more efficiently than the others and was accompanied by high soil phosphatase activities. Among the PGPR isolates *B. pumilus* and *B. altitudinis* were most effective in enhancing growth in the field conditions. Both BCA and PGPR isolates successfully reduced Sclerotial blight and root rot incidence of the test crops. which was accompanied with enhanced activities of key defense enzymes like β-1,3 Glucanase, Chitinase, Phenylalanine ammonia lyase and Peroxidase and enhanced levels of phenolics. Bio-priming of the

seeds and seedlings prior to sowing and after germination proved to be effective in growth enhancement and to induce resistance against fungal root pathogens. Reduction of root diseases by both BCA and PGPR was associated with all the elements commonly known to be involved in the induced systemic resistance which were found to have been enhanced.