

CONCLUSION

Jute is a natural long, soft, shiny vegetable fibre that can be spun into coarse, strong threads. Jute is one of the cheapest natural fibres and is second only to cotton in amount produced and variety of uses. Cultivation of jute also suffers from attacks by various pathogens during different stages of growth, the most important being *Macrophomina phaseolina* causing stem rot. Moreover, *M. phaseolina* infects more than three hundred plants. Use of chemical fungicide for last few decades exploited the natural microflora of the soil. In spite of being cultivated in a large area of Bangladesh and West Bengal (India) very little work has been done on jute rhizosphere. Keeping this in mind present study has been undertaken. The principle moto of this study was to screen jute rhizosphere for potent plant growth promoting rhizobacteria and evaluation of their efficacy for growth promotion and disease management. The major conclusions drawn from this study have been enumerated below:

- ❖ A total seventy six isolates were obtained from rhizosphere of jute plants from 32 soil samples, collected from different areas of north Bengal. Among the isolates about 50% of was IAA producer, 45% could solubilize inorganic phosphate and only 5% showed antagonistic activity against *M. phaseolina*.
- ❖ On the basis of preliminary screening five isolates were selected and further characterised morphologically, biochemically. The identity of all five isolates were confirmed by 16S rDNA sequencing and BLAST analysis. The sequences were deposited in NCBI, GeneBank databse under the accession nos. KT192627.1 (*B. amyloliquefaciens*), KT266821.1 (*B. subtilis* B-3), KT031388.1 (*P. agglomerans*), KX618651.1 (*Stenotrophomonas* sp.) and KP247498.1 (*R. pickettii*).
- ❖ On the basis of *in vitro* phosphate solubilization activity and antagonistic activity two strains, *P. agglomerans* (KT031388.1) and *B. amyloliquefaciens* (KT192627.1) were finally selected, and further characterized by scanning electron microscopy, carbon source utilization and protein pattern analysis by SDS-PAGE.

- ❖ The growth of *B. amyloliquefaciens* and *P. agglomerans* was found to be best at 48 h. After that growth of both bacteria declined. At 48 h (log phase) log cfu values of *B. amyloliquefaciens* and *P. agglomerans* were found to be highest- 16.5 log cfu/ml broth and 14.4 log cfu/ml broth respectively. Both the bacteria grew best at 35°C and optimum pH for the growth was found to be 6.0.
- ❖ The optimum cultural conditions for the growth of the pathogen *Macrophomina phaseolina* was also determined. *M. phaseolina* showed growth in all the media used in the study but highest growth was observed in PDA. The effect of incubation period on mycelial dry weight was determined and found that after 12 days of incubation the isolate showed highest mycelial dry weight. *Macrophomina phaseolina* showed maximum growth at pH 6.5, in presence of dextrose and peptone as carbon and nitrogen source respectively.
- ❖ Both the bacterial isolates were tested for their *in vitro* plant growth promoting activities. Both the bacterial isolates were able to produce IAA, siderophores, ammonia and could solubilize phosphate. *B. amyloliquefaciens* produced HCN, chitinase, protease. Whereas *P. agglomerans* was found to be non-chitinolytic, non-cyanogenic and produced amylase and protease in less quantity.
- ❖ Chitinase and protease production by *B. amyloliquefaciens* was optimized. The isolate could produce optimum chitinase after 72 hours of incubation and optimum temperature was 35°C for the same. When effect of pH and nitrogen sources was studied it was observed that, the optimum pH for chitinase production was around pH 8.0 and peptone served the best as nitrogen source.
- ❖ Optimization studies related to protease production revealed that, the strain *B. amyloliquefaciens* showed optimum protease production after 36 hours of incubation. The optimum pH of medium for protease production was found to be 8.5 and optimum substrate for production of protease from *B. amyloliquefaciens* strain was fructose as carbon source and yeast extract as nitrogen source. In presence of metal ions such as calcium, magnesium and manganese chloride the production protease increased. Decrease in protease activity was observed in case of Zn⁺².

- ❖ The antagonistic activity of *B. amyloliquefaciens* was tested against known phytopathogens- *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. semitectum*, *Alternaria alternata*. The percentage of inhibition varied between 61.5-86.8%. Scanning electron microscopic study confirmed the antagonistic activity of the isolate *B. amyloliquefaciens*. Abnormalities, attachment, lysis and disintegration, in hyphal structure of *M. phaseolina* was observed.
- ❖ Partial characterization of cell free culture filtrate of *B. amyloliquefaciens* was done. The ethyl acetate fraction was characterized by GC/MS analysis. The analysis revealed presence of several compounds. Most abundant was 10-Octadecenoic acid, methyl ester, which had a retention time 18.88 min.
- ❖ In order to determine *in vivo* plant growth promoting activities in four different jute varieties (JRC 212, JRC 321, JRO 524 & JRO 8432) in potted condition. Two isolates *P. agglomerans* and *B. amyloliquefaciens* were applied to the rhizosphere by drench application to evaluate the effect on growth parameters of jute plants. The bacteria were applied singly or in dual combination. In all the cases plant growth was increase significantly in comparison to control.
- ❖ The effect of both the isolates on biochemical parameters were determined. The studies showed that, application of *P. agglomerans* and *B. amyloliquefaciens* significantly increased the total chlorophyll, total and reducing sugar, protein as well as total phenolics content of the jute plants.
- ❖ In order to evaluate the efficacy of these two isolate in disease suppression several combinations treatments with rhizobacteria and pathogens was tried. Single as well as dual application of *P. agglomerans* and *B. amyloliquefaciens* effectively reduced the disease incidence. Lowest disease incidence of 16.50% (79.11% reduction over control) was recorded in *P. agglomerans* +*B. amyloliquefaciens*+*M. phaseolina* treatment followed by 20.5% of *Bacillus amyloliquefaciens* + *M. phaseolina* (74.05% reduction) and 23.5% of *Pantoea agglomerans*+ *M. phaseolina* (70.25% reduction). Highest disease incidence of 79.0% was recorded in untreated plants (*M. phaseolina*). More disease severity was observed in only pathogen treated plants without PGPR, followed by *Pantoea agglomerans*+ *M.*

phaseolina, *Bacillus amyloliquefaciens* + *M. phaseolina* and *P. agglomerans* + *B. amyloliquefaciens* + *M. phaseolina*.

- ❖ In addition to this, time course accumulation of defense enzymes such as chitinase, Phenyl alanine ammonia lyase, Peroxidase and β -1, 3 Glucanase was determined following the inoculation with both pathogen and *P. agglomerans*, *B. amyloliquefaciens*. Higher activities of CHT, PO, PAL, β -1, 3 GLU and accumulation of higher phenolic compounds were observed in jute varieties following the application of *P. agglomerans*, *B. amyloliquefaciens*.
- ❖ Statistical analyses- ANOVA, LSD were performed.