6. SUMMARY

Twenty eight mangrove sediment and rhizosphere soil samples were collected from various locations of mangrove forests in Tamil Nadu viz., South Pichavaram, Pichavaram, Kodiymphalayam, Kavarapattu, Muzhukuthurai, Parangipettai and Muthupet. The samples were designated based on the locations and type of sample. The samples were collected to analyze the total microbial population, exopolysaccharide producing bacteria, *Azotobacter* and *Pseudomonas* sp. population.

The enumeration of microorganisms in the mangrove sediment and rhizosphere soil samples revealed that the rhizosphere soil samples contained higher microbial populations compared to mangrove sediments. The Pichavaram rhizospheric soil showed the highest microbial population (6.12 x 10^6 cfu/g , for bacteria, 4.04 x 10^4 cfu/g for fungi) followed by South Pichavaram (6.08 x 10^6 cfu/g for bacteria, 3.64 x 10^4 cfu/g for fungi) and Muthupet (6.00 x 10^6 cfu/g for bacteria, 3.88 x 10^4 cfu/g for fungi). In mangrove sediment Pichavaram showed the highest microbial population (5.86 x 10^6 cfu/g, for bacteria, 3.68 x 10^4 cfu/g for fungi). The least microbial population was observed in Muzhukuthurai (5.02 x 10^6 cfu/g for bacteria, 3.02 x 10^4 cfu/g for fungi).

The maximum populations were 5.8 x 10^6 cfu g^-1 for *Azotobacter* and 4.4 x 10^5 cfu g^-1 for *Pseudomonas* obtained from the rhizospheric soils of Pichavaram followed by 5.3 x 10^5 cfu g^-1 for *Azotobacter* and 4.4 x 10^5 cfu g^-1 for *Pseudomonas* from Muthupet mangrove rhizospheric soil. One isolate from each mangrove rhizosphere soil and sediment sample was designated same as the sample and randomly selected for further studies.

All the selected isolates were screened for exopolysaccharide production and which revealed a variation in the ability of the twenty eight isolates to produce
exopolysaccharide under *in vitro* conditions. The highest exopolysaccharide production was recorded by PR-1 isolate (2.84 g/L) followed by MPR-2 (2.83 g/L). The lowest exopolysaccharide production of 1.20 g/L was recorded by MR-1 isolate. Based on this all the isolates were graded under three categories. Four isolates, namely, SPR-2, PR-1, PR-2 and MPR-2 were ranked in the first category and constituted 14.30 percent of the total isolates. PR-1 and MPR-2 were used for further studies.

PR-1 and MPR-2 were characterized by biochemical tests, compared with reference strains and 16S rRNA sequencing and were identified as *Azotobacter vinelandii* and *Pseudomonas aeruginosa* respectively. The isolates PR-1 and MPR-2 recorded a higher survival population 4.50 log 10 CFU/ml, 4.00 log 10 CFU/ml, compared with the reference strain MTCC- 2459 (4.08 log 10 CFU/ml), MTCC- 2492 (3.76 log 10 CFU/ml), when subjected to osmotic shock experiments. When subjected to osmotic pressure experiments the isolates PR-1 and MPR-2, recorded the number of viable cells 4.02 log 10 CFU/ml, 3.80 log 10 CFU/ml, respectively. It is interesting to note that these selected isolate PR-1 and MPR-2 here performed better than the reference strain.

The desiccation resistances of PR-1 and MPR-2 isolates were studied. The isolates PR-1 and MPR-2 recorded the highest survival population 4.18 log 10 CFU/ml, and 4.08 log 10 CFU/ml, after one week, while reference strain MTCC- 2492 recorded the least resistance 4.01 log 10 CFU/ml. The isolates PR-1 and MPR-2 exhibited the highest thermal resistance, which recorded a survival population of 3.78 log 10 CFU/ml, 3.40 log 10 CFU/ml, after heat treatment at 50°C.
The alginate production efficiency was carried out and the product formation related to dry cell biomass was 0.98 g/L at 12 h, for *A. vinelandii* PR-1 and 0.88 g/L at 12 h for *P. aeruginosa* MPR-2. The alginate produced by *Azotobacter vinelandii* PR-1 was further studied by FT-IR and NMR. The important adsorption bands located at 3480.2, 2910.8, 1750.4 and 1120.6 cm⁻¹ indicated that all of them have chemical structure identical those of β-D mannuronic acid and α-L guluronic acid. The important adsorption bands located at 0 ppm to 3.5 ppm indicated chemical structures identical to β-D mannuronic acid and α-L guluronic acid, which are composed of polymer chains.

The alginate production by *Azotobacter vinelandii* PR-1 was optimized using various carbon, nitrogen sources, different pH, temperature, Agitation speed, Inoculum load and various culture media. It was found that the maximum alginate production and dry cell biomass was produced with sucrose as carbon source (2.77 g/L, 4.57 g/l dry cell biomass), peptone as nitrogen source (2.58 g/l with dry cell biomass of 2.48 g/l) at pH 7 (2.76 g/l, 4.42 g/l dry cell biomass), 30°C (2.72 g/l, 4.52 g/l dry cell biomass), at 120 rpm (2.65 g/l, 4.83 g/l dry cell biomass) with 2% inoculum load (2.56 g/l) in Modified Burk’s medium (2.78 g/l, 4.56 g/l dry cell biomass). Selected isolate, *A. vinelandii* PR-1 producing alginate recorded 24 per cent for mannuronic, 76 per cent for guluronic, 52.5 per cent for MM, 22.5 per cent for MG, 0.4 per cent for GG blocks. PR-1 produced alginate recorded higher molecular weight 986.2 K Da.

Three agriculturally beneficial microorganisms were selected and incorporated in the prepared alginate beads. SEM photographs of the blank beads compared with inoculums loaded beads showed a difference in surface morphology. Smoothness increased when inoculum was loaded in the beads. The size of the bead
was found to be 500μm. The surviving populations were $76.06 \times 10^8$ cfu g$^{-1}$ for *Bacillus megaterium* (BM), $77.88 \times 10^8$ cfu g$^{-1}$ for *Pseudomonas fluorescens* PF, $76.44 \times 10^8$ cfu g$^{-1}$ for *Azospirillum lipoferum* (AZP) in single inoculants preparation after one month storage compared to other carriers (Lignite, vermiculite and pressmud). The surviving population of consortium (BM+PF+ AZP) were $78.66 \times 10^8$ cfu g$^{-1}$ after one month storage. However it was found that the required inoculants cell load was maintained up to six months.

The selected beneficial plant growth promoting bacteria immobilized in alginate beads were used to evaluate the growth and development in rice ADT 36 under *in vitro* conditions, pot and field trials.

The application effect of different formulations of *Azospirillum*, *Pseudomonas* and *Bacillus* cells on the enhancement of adhesion to rice roots was studied under *in vitro* condition. The highest rice root adhesion ($340.26 \times 10^4\text{g of dry weight /h}$), considerable increase in the root length (16.96 cm) and shoot length (24.23 cm) was with T$_8$ (*A. lipoferum* - AZP + *P. fluorescens* - PF + *B. megaterium* - BM). Highest germination percentage of 93.33 and maximum vigour index (2224.05) was also observed with T$_8$ treatment.

The treatment T$_8$ (*A. lipoferum* - AZP + *P. fluorescens* - PF + *B. megaterium* - BM) directly influenced the starch content in rice var. ADT-36. In control, the starch content was the least with 32.80 mg at the end of the sampling period. Starch content gradually increased up to 14th day of sampling and then slightly decreased at 21st day in all the treatments. The maximum amino nitrogen content of 7.69 mg was recorded in healthy control at initial sampling. The minimum content of 3.87 mg was observed in T$_8$ (*A. lipoferum* - AZP + *P. fluorescens* - PF + *B. megaterium* - BM) at 21st day.
The maximum level of protein was observed in healthy control with 52.00 mg at the initial sampling. Minimum amount of 50.06 mg was recorded in T₈ at the initial sampling where as at the final sampling it was reduced 40.07 mg. The treatment T₈ (A. lipoferum - AZP + P. fluorescens - PF + B. megaterium - BM) showed a high chlorophyll content of 0.957 mg when compared to control (0.738 mg) at 21st day.

In pot culture, the biometrics of rice showed that treatment T₈ (A. lipoferum - AZP + P. fluorescens - PF + B. megaterium - BM) at 30 DAT increased plant height (76.23 cm), panicle length (18.37 cm), Number of tillers (15.33), Number of productive tillers per clump (13.67), thousand grain weight (19.20g).Percentage of filled grain (84.14 %), grain yield (45.62 g/pot) and straw yield (58.48 g/pot) was high when compared to other treatments. The treatment T₁ (Control) was found have least effect on increasing the growth and yield parameters of rice. Hence, it is concluded that the treatment T₈ (A. lipoferum - AZP + P. fluorescens - PF + B. megaterium - BM) was found to be the most effective in improving the various biometrics and yield parameters.

In field trial, the maximum plant height (75.93 cm) was observed in T₈ (A. lipoferum - AZP + P. fluorescens - PF + B. megaterium - BM) and control recorded the least plant height (76.20 cm). The maximum number of tillers / clump recorded (18.67) followed by T₅ (17.33). Control recorded the lesser number of productive tillers / clump (11.67). The maximum panicle length (20.17 cm) was observed with T₈(A. lipoferum - AZP + P. fluorescens - PF + B. megaterium - BM), maximum filled grain percentage (85.76 %) and maximum thousand grain weight of 19.22 g, maximum grain yield of 6.53 t/ha and straw yield of 8.66 t/ha during the year 2013.
FUTURE PERSPECTIVES OF THE STUDY

The results of the present study clearly confirmed the ability of the selected efficient mangrove rhizosphere isolate *Azotobacter vinelandii* PR-1 to produce bacterial alginate and incorporate it in the agricultural field for carrier development for rice. This is an important first step in the production of alginate from bacteria. The subject needs further detailed research at biochemical, physiological and molecular levels to exploit the exact mechanism of alginate production and enhancement for various industrial level applications.