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Management of Agro forestry nurseries by polybioinoculant for Sustainable tree plantations

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ABSTRACT
Agroforestry nursery has considerable potential in afforestation. Management of healthy saplings in nurseries and a high survival rate after plantations is a challenging task. AM fungi and PGPR is highly host specific, adoption centric in nature and forest geography plays a major role in acclimatization. *Bacillus* sp.(PSB), *Rhizobium sp.LT669953 a*nd *Gigaspora roses*(PUAM-9) were formulated as a polybioinoculant from Kottagudem forest soil for *Sesbania grandiflora*, *Pongamia pinnata* and *Albizzia lebbeck.* A tremendous enhancement in plant growth inferred this novel approach can be employed for reclamation of barren lands, polluted soils and coal mines for sustainable tree plantations across the country.
Keywords: Agroforestry tree species, polybioinoculant, reclamation, AMF pure culture, *Rhizobium sp* and *Bacillus* sp.(PSB)

INTRODUCTION

Agroforestry has considerable potential, not as the only way to improve agricultural production, but as one important way to enhance and maintain overall productivity of the small upland farm, the agricultural unit that is becoming more prevalent in many parts of the world. A large number of biotic and abiotic factors can contribute to the inability of an inoculant strain to nodulate under field conditions. Currently establishment of nitrogen fixing bacteria (NFB) in the mycorrhizosphere and manipulation of these microbial associations as a biotechnological tool to enhance plant growth are two important topics of research. Nitrogen fixing tree species (NFT) possess all the virtues of multipurpose tree species (MPT) and are given top priority in agroforestry. Sustainable crop production depends much on good soil health which is in turn imparted by a number of useful soil microorganisms (Jaya Shankar Singh et.al. 2011). Repeated use of chemical fertilizers destroys soil biota. Their utility can be enhanced with human intervention by selecting efficient organisms, culturing and adding them to soils directly or through seeds.

Plant growth rhizobacteria (PGPR), a group of beneficial plant bacteria is potentially useful for stimulating plant growth and increasing crop yields. PGPR are root-colonizing bacteria which may promote plant growth directly or indirectly or synergistically (Joseph et.al. 2007, Yasmin et.al. 2007). The bacterial inoculation has a much better stimulatory effect on plant growth in nutrient deficient soil than in nutrient rich soil (Tipre et al., 2015; Egamberdiyeva 2007).

Though late, the importance of AM fungi in nursery management and in revegetation efforts of various types of lands has been realized and of late, ithas become an integral part of all stages of afforestation programmes. A rapid production of tree seedlings in the nurseries and a high survival rate after planting is important for reversing the current degradation of natural forests, woodlands and shrub lands in the tropics. An efficient production of seedlings of exotic tree species would permit the allocation of more resources to the establishment of indigenous tree species.

1.1 Plant Growth Promoting Rhizo organisms include:

1.1.1. Rhizobium:

When rhizobia colonize the roots from non-legume plant in a non specific relationship the strains from this genus may behave as PGPR. Inoculations of Rhizobium sp. causes a greater increase in growth and yield and the number of nodules per root system is significantly higher in plants inoculated with Rhizobium sp. compared to plants without Rhizobium sp. under field condition (Akhter et.al. 2009). The P-solubilising strains and the N2fixing bacterial strains have great potential in being formulated and used as biofertilizers (Cakmakc et.al. 2007). The IAA production is studied in Rhizobium strains associated only with a few legume hosts (Basu and Ghosh 2001, Roy and Basu 2004). Sridevi and Mallaiah (2007) showed that all the strains of Rhizobium isolated from root nodules of Sesbania sesban (L) Merr, produces IAA.

1.1.2. Bacillus:

Species of Bacillus are common inhabitants among the resident microflora of inner tissues of various species of plants, including cotton, grape, peas, spruce, and sweet corn, where they play an important role in plant protection and growth promotion (Berg et al. 2005; Shishido et al. 1999; Bell et al. 1995). Reva et al. (2002) studied the diversity of endophytic AEFB in the inner tissues of healthy cotton plants (Gossypium sp. Dushanbe, Tajikistan). Enhancement of plant growth by root colonizing species of Bacillus and Paenibacillus is well known (Idris et al. 2007; Kloepper et al. 2004). It is also very likely that growth promoting effects of various PGPRs are due to bacterial production of plant growth regulators such as indole-3-acetic acid (IAA), gibberellins, and cytokinins (Tipre et al., 2015; Pindi et al., 2016; Bottini et al. 2004; Bloemberg and Lugtenberg 2001).

1.1.3. Mycorrhizal fungi (AM fungi):

Mycorrhizal fungi are one of the soil organisms that create a direct connection between the soil mass and plant root systems (Tasleem et al., 2014; Alizah 2010). Mycorrhizal associations formed between the roots of higher plants and Zygomycete fungi (Smith and Read 2008) belonging to the order Glomales. Fungi play a central role in many microbiological and

ecological processes, influencing soil fertility (Gosling et.al. 2006, Cordoso and Thomas 2006) decomposition, cycling of minerals and organic matter, as well as plant health and nutrition (Smith and Andrew, 2011). In addition to improving plant uptake of mineral nutrients (Khosro et al. 2011), many mycorrhizal fungi may play a significant role in mobilizing nutrients from organic substrates.

Telangana is the semiarid region of India with recurring meteorological drought and worsened by overexploitation of meager ground water resources. Isolation of novel PGPR from the same geographical area was innovative method will improve the efficiency of use of fertilizers, reducing the total input costs and improving the nursery plant growth survival thereby incrasing the afforestation process leading to sustainable Harita haram in Telangana. In the paper, *Bacillus sp.* (PSB), *Rhizobium sp.LT669953 a*nd *Gigaspora roses*(PUAM-9) were formulated as a polybioinoculant from Kottagudem forest soil for *Sesbania grandiflora, Pongamia pinnata* and *Albizzia lebbeck.* A tremendous enhancement in plant growth inferred this novel approach can be employed for reclamation of barren lands, polluted soils and coal mines for sustainable Haritha Haram across the country.

2. Material and methods:

The Experiment was carried out in the Department of Microbiology, Palamuru University, Mahabubnagar, Telangana State, India.

2.1. Location of the sample collected:

12 different soil samples were collected from forest areas of Khammam, Warangal, Mahabubnagar and Karimnagar. The geographical conditions of these areas are similar to each other (temperature, pH, moisture).

Table: Collection of soil samples from different locations of same Geographical areas

District Forest area	GPS Location	Latitude	Longitude	Soil type
Khammam	Kottagudem	80.64689	17.54458	Red soil
	Bhadrachalam	80.89359	17.66879	Red soil clay
	Yellandu	80.32410	17.58796	Red soil
Warangal	Eturnagarm	18.3384786	80.42698	Red soil
	Mulugu	18.1920214	79.9457973	Deep black soil
	Pakhal	17.95	79.983333	Red clay
	Srisailam	78.86873	16.07327	Red soil

Mahabubnagar	Achampet	16.3989567	78.6329538	Red soil
	Amrabad	78.16072	18.69627	Red shallow ground clay
Karimnagar	Mahadevpuram	11.0185193	76.9743756	Red clay
	Manthani	79.66815	18.65099	Red clay
	Palmala	79.1500	18.4300	Deep black

2.2. Nature of soil:

Undisturbed forest rhizosphere soils were target. These soils were not having any previous history of chemical fertilizers, so there was no chance of growth inhibition of natural bioinoculants by the action of chemical fertilizers. Plants of these areas were healthy and green; it might be due to the presence of PGPR in the rhizosphere region.

2.3. Physico-chemical analysis of soil:

Soil available nitrogen was estimated by alkaline potassium permanganate method (Subbiah and Asija 1956), and available phosphorous was determined after Bray and Kurtz (1945) Potassium determined by flame photo metrically (Jackson 1973).

2.4. Preliminary Experiments:

Preliminary the soils from 12 forest places were brought to the laboratory and were taken in the pots (Triplicates) and sandwiched between two layers of normal nursey soil and it was sown with the seeds of agroforestry tree species viz., *Sesbania grandiflora*, *Albizzia lebbeck* and *Pongamia pinnata*. Best plant growth supporting forest soil was selected for the screening of PGPR and AM fungi. *Rhizobium* and PSB were isolated from soil collected from Kothagudem forest on different culture media by serial dilution method. For the isolation of Rhizobium YEMA medium, and for PSB, Tripticase soya agar was used.

2.5. Preparation of standard inoculums of each novel species:

The standard inoculum was prepared by inoculating log phase cultures of all species of Rhizobium and PSB in nutrient broth. One µl of each sample was added over the seeds of *S.grandiflora*, *Albizzia lebbeck* and *Pongamia pinnata* at the time of sowing in sterilized soil for a period of 90 days.

2.6. Mycorrhizal spore isolation:

Mycorrhizal infection was studied by Trypan blue method (Phillips and Hayman 1970). Spore extraction from the soil was carried out using wet sieving and decanting technique (Gerdemann and Nicolson, 1963), the spores were identified according to the manual of Schenck and Perez (1990) and maintained in pure culture through funnel experiment.

2.7. Genomic DNA Extraction:

The genomic DNA from the bacterial cells was obtained using a modification of the method described by Sadam et al. (2017). DNA isolation The bacterial cells from pure culture were harvested by centrifugation (12,000rpm) for 2min, and the cell pellets mixed with 600µl of lysis buffer (10mm tris –HCl, 1mM edta [pH 75], 0.5% SDS 100/g/ml proteinase c) and incubated at 37°c for 1h after the addition of 100 µl 5 M NaCl, and 80µl CTAB NaCl. Samples were incubated at 65°c for 10min. The sample were cooled to room temperature, followed by extraction of the aqueous phase with an equal volume of chloroform: isoamyl alcohol [24:11, v/v] and then with an equal volume of phenol: chloroform: isoamylalcohol (25:24:1, v/v_ which was centrifuged at 12,000rpm & 4°c for 10 min. Isopropanol (0.6x) and mixed with the aqueous phase, and centrifuged at 12,000rpm and 4°c for 10 min. The DNA pellets were dried under vacuum, and then dissolved in TS Buffer (10mM Tris-HCl, and 1Mm EDTA [pH.75]).

2.8. PCR Analysis

The small subunit rRNA gene of each sample's culture DNA was amplified using 16S rRNA Universal primers. The PCR amplification reaction mixture of 50µl contained 4µl bacterial DNA (nearly 200ng), 1µl Taq-DNA polymerase, 5µl of Taq buffer, 5µl of 2mM dNTP mix, 5 µl of forward primer (10 pM/µl) and 5 µl of reverse primer (10 pM/µl). Amplification was carried out in a Bio-Rad thermo cycler run for 30 cycles. In each cycle denaturation was done for 94ºC for 20s, annealing at 48ºC for 20s and extension was done at 72ºC for 40s and a final extension was carried out for 5min at 72ºC at the end of all 30 cycles. The amplified DNA fragment of approximately 1542 bp was separated on a 1% agarose gel and purified by Quiagen spin columns. (Mullis 1990 and Barlett and Stirling 2003). The desired DNA band from the agarose gel was cut weighed and then transferred to a sterile microfuge tube and add QE buffer thrice the volume of weighed excised band. Place it on a thermomixer at 65ºC for 10min and the contents were then transferred to a Quiagen column and spun at 8000 xg for 2min. Then it was washed with 750 µl of PE buffer and eluted with small quantity (30-40µl) of sterile water. The purified PCR product was then used for sequencing.

2.9. 16S rRNA gene Sequencing:

The purified 1542bp PCR product was sequenced using universal primers. The resultant almost complete sequence of the 16S rRNA gene sequence of the isolate was subjected to BLAST sequence similarity search and Ez Taxon to identify the nearest taxa. The entire related 16S rRNA gene sequence were downloaded from the database (http://www.nbi.nlm.nih-gov) aligned using the clustal –program.

3.0. Collection of soil samples from problematic sites:

Soil samples from problematic sites such as Coal Agriculture soil, Polluted soils and Barren lands were collected in order to check the efficiency and potentiality of novel isolates. Soil samples were collected from Jadcherla, Kothur and Maddur.

3.1 Preparation of Standard Inoculum of Bioinoculants:

The standard inoculum was prepared by inoculating log phase cultures of *Rhizobium sp.LT669953* and *Bacillus* sp. (PSB) in nutrient broth which were isolated from Kothagudem soil & maintained as pure cultures in laboratory of Microbiology, Palamuru University.

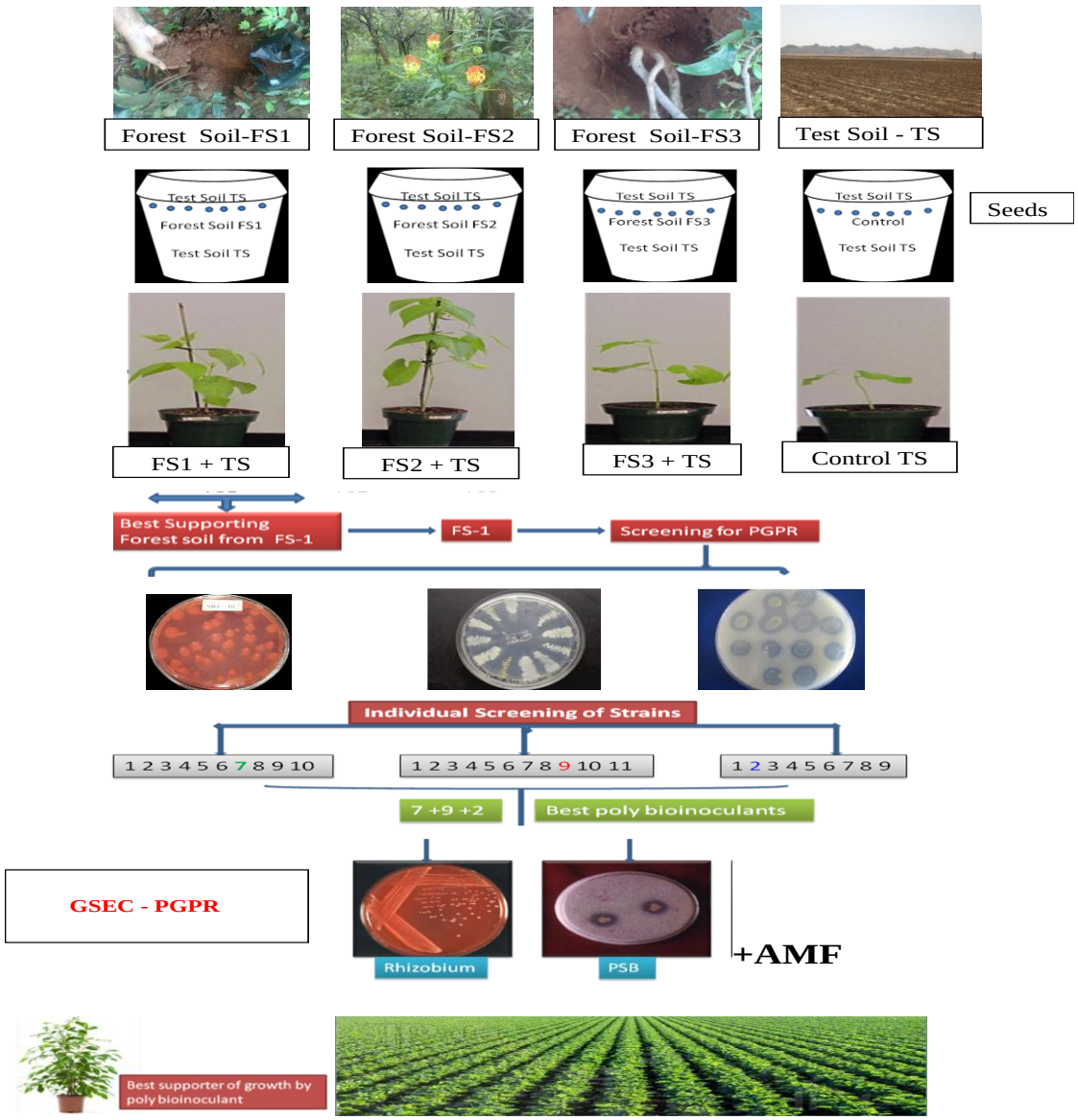

Figure : Methodology for formulation and application of polybioinoculants

3. Results and Discussion:

The present investigation is a novel and innovative method to isolate the potential, efficient and novel PGPR from same geographical area after the preliminary screening with *Sebania grandifloa*, *Albizzia lebbeck* and *Pongamia pinnata* which have reduced time, and cost of the investigation. Among 12 forest soil samples, Kottagudem soil sample has shown maximum

growth of *Sesbania grandiflora*, *Albizzia lebbeck* and *Pongamia pinnata* plants in terms of plant height and dry weight after 90 and 180 days of growth (Table 1-3 and Figure 1-3).

Table - 1: Variation in mycorrhizal colonization, nodulation and growth of *Sesbania grandiflora* in twelve social forestry nurseries (Age of the plant 90 days)

Nursery		**Mycorrhizal colonization (%)**	**No. of spores/ 10 g. soil**	**Nodulation**			**Height of the plant (cm)**		**Plant dry weight (gm)**		**N content (%)**	**P content mg/g**
				No.	**Size (cm)**	**Dry weight (gm)**	Shoot	Root	Shoot	Root		
1	K.U. Campus	39.6	11	28	0.5	0.32	30.8	20.1	0.93	0.51	1.1	2.20
2	Kothagudem	83.9	31	68	0.7	0.61	62.3	38.6	2.87	1.07	3.4	4.80
3	Elukathurthy	50.8	17	41	0.5	0.38	38.6	25.1	1.41	0.62	2.2	3.49
4	Eturunagaram	62.5	22	39	0.6	0.38	47.2	30.6	1.72	0.77	2.6	4.06
5	Forest Office	53.2	19	36	0.5	0.34	40.9	28.2	1.41	0.65	2.2	3.66
6	Godhavari khani	41.5	11	20	0.4	0.28	31.3	20.8	0.95	0.48	1.4	2.36
7	Huzurabad	47.2	16	25	0.5	0.31	35.6	24.9	1.32	0.59	2.0	3.29
8	Bhadrachalam	71.4	26	49	0.6	0.46	52.3	35.3	2.27	0.97	3.1	4.40
9	Jangaon	43.7	13	29	0.6	0.32	32.8	22.6	1.03	0.52	1.6	2.55
10	Kaleshwaram	54.8	19	31	0.6	0.33	41.3	29.2	1.34	0.67	2.3	3.75
11	Mulugu	67.9	23	55	0.5	0.47	49.2	33.8	1.97	0.88	2.8	4.15
12	Rampur	46.5	15	40	0.5	0.35	35.1	23.4	1.26	0.57	1.8	2.75

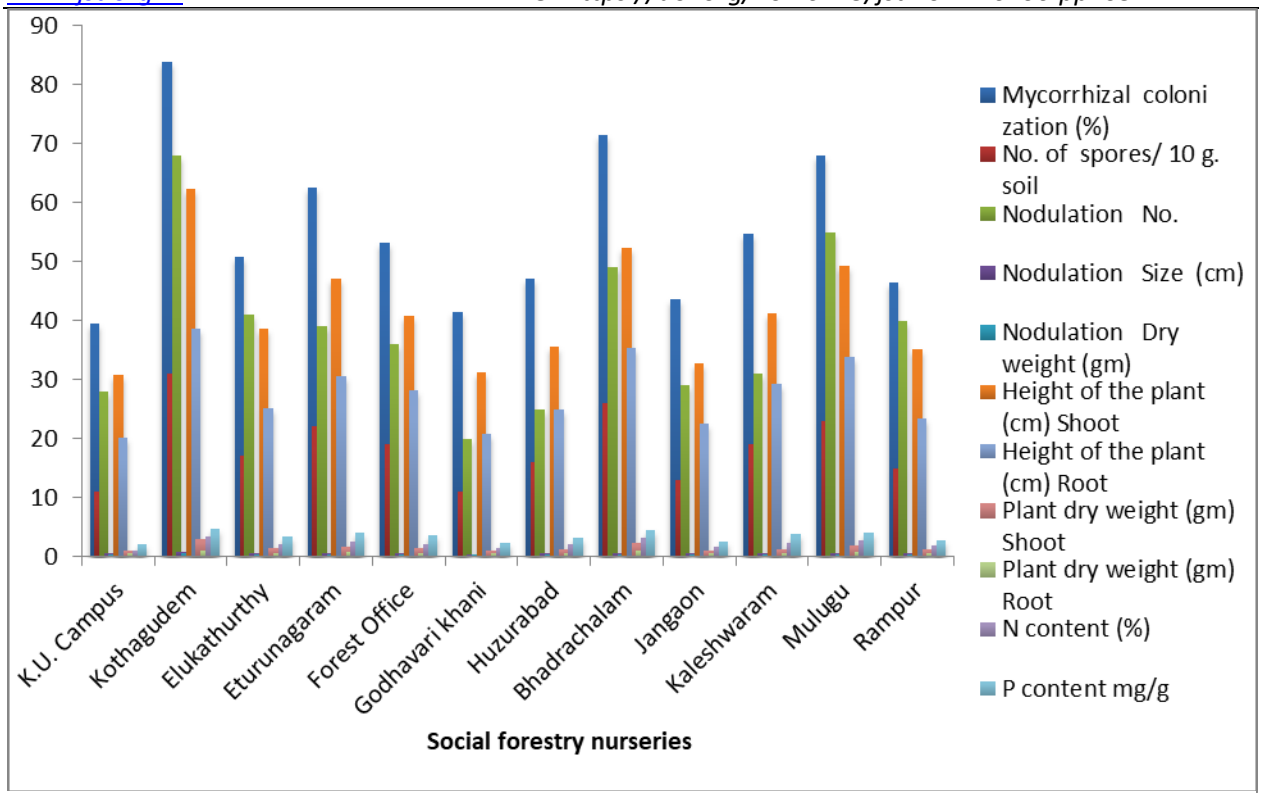

Figure 1: Variation in Mycorrhizal colonization, nodulation and growth of *Sesbania grandiflora* in twelve social forestry nurseries

Table 2: Variation in mycorrhizal colonization, nodulation and growth of *Albizzia lebbeck* in twelve social forestry nurseries (Age of the plant 90 days)

Nursery		Mycorrhizal colonization (%)	No. of spores/ 10 g soil	Nodulation			Height of the plant (cm)		Plant dry weight (gm)		N content (%)	P content (mg/g)
				No.	Size (cm)	Dry weight (gm)	Shoot	Root	Shoot	Root		
1	K.U. Campus	34.8	10	17	0.2	0.13	23.2	18.1	0.68	0.35	1.3	1.81
2	Kothagudem	65.8	22	34	0.4	0.31	51.4	40.6	1.24	0.73	2.9	4.01
3	Elukathurthy	46.7	15	16	0.3	0.13	36.2	27.1	0.90	0.62	2.0	2.37
4	Eturunagaram	53.8	18	25	0.3	0.22	39.4	29.2	0.99	0.66	2.5	3.15
5	Forest Office	48.1	15	21	0.2	0.17	38.1	27.4	0.91	0.63	2.1	2.56
6	Godhavarikhani	41.3	11	23	0.4	0.22	26.1	19.9	0.79	0.46	1.5	1.91
7	Huzurabad	45.6	14	17	0.2	0.15	34.3	26.8	0.89	0.59	1.8	2.27
8	Bhadrachalam	61.3	20	26	0.3	0.22	45.8	38.2	1.12	0.71	2.7	3.68
9	Jangaon	42.9	12	21	0.3	0.19	28.2	21.3	0.81	0.51	1.5	2.07
10	Kaleshwaram	48.9	15	30	0.3	0.29	38.3	28.4	0.92	0.64	2.2	2.64
11	Mulugu	57.6	19	17	0.3	0.15	44.3	35.1	1.06	0.69	2.6	3.57
12	Rampur	45.2	14	19	0.3	0.16	31.8	25.8	0.85	0.55	1.7	2.15

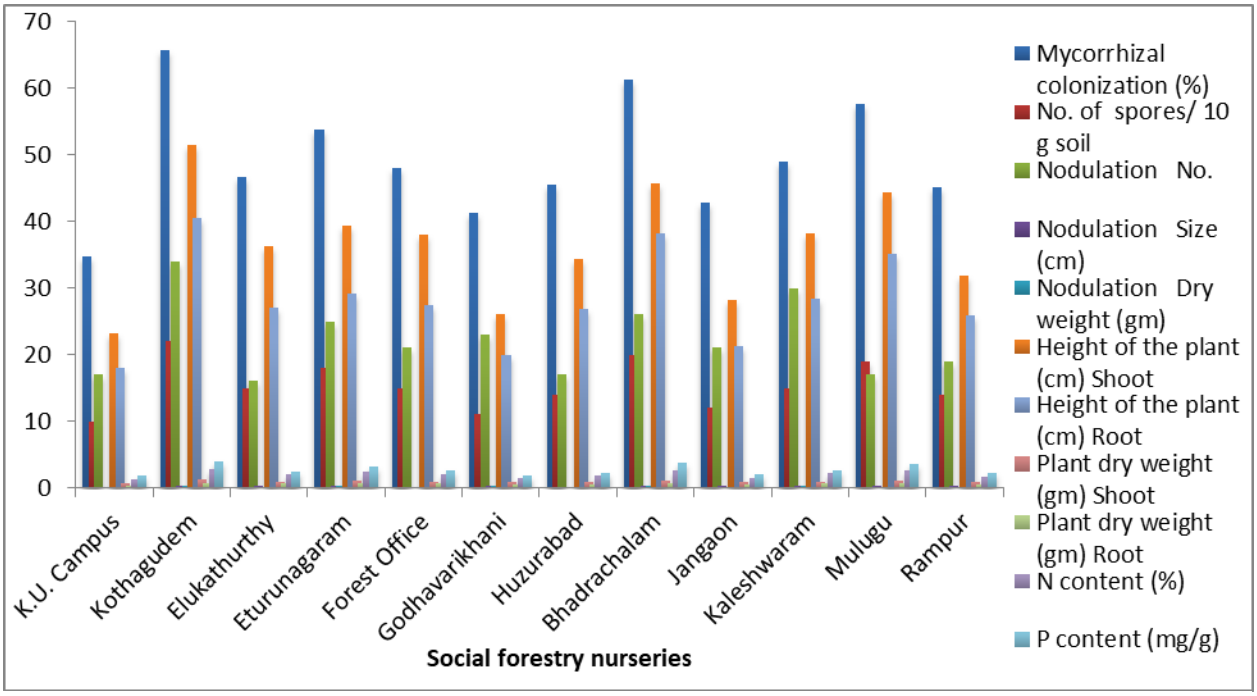

Figure 2: Variation in Mycorrhizal colonization, nodulation and growth of *Albizzia lebbeck* in twelve social forestry nurseries

Table 3: Variation in Mycorrhizal colonization, nodulation and growth of *Pongamia pinnata* in twelve social forestry nurseries (Age of the plant 90 days)

Nursery		Mycorrhizal colonization (%)	No. of spores/ 10 g. soil	Nodulation			Height of the plant (cm)		Plant dry weight (gm)		N content (%)	P content (mg/g)
				No.	Size (cm)	Dry weight (gm)	Shoot	Root	Shoot	Root		
1	K.U. Campus	37.2	9	15	0.5	0.18	21.8	17.1	0.82	0.48	1.4	2.03
2	Kothagudem	74.3	25	36	0.7	0.56	54.4	34.3	2.17	1.37	3.3	4.63
3	Elukathurthy	50.3	13	21	0.6	0.36	29.1	20.7	1.40	0.83	2.0	3.19
4	Eturunagaram	61.7	20	24	0.8	0.47	34.8	23.1	1.76	1.02	2.5	3.82
5	Forest Office	53.1	15	22	0.6	0.42	31.4	23.8	1.55	0.94	2.2	3.42
6	Godhavarikhani	39.7	10	12	0.5	0.16	23.4	18.9	0.96	0.61	1.5	2.18
7	Huzurabad	48.9	12	18	0.6	0.31	27.7	19.6	1.29	0.81	1.8	2.90
8	Bhadrachalam	68.9	22	30	0.6	0.51	39.7	29.4	1.95	1.13	3.0	4.36
9	Jangaon	43.6	11	33	0.8	0.55	25.7	19.1	1.04	0.74	1.6	2.40
10	Kaleshwaram	57.6	17	23	0.7	0.42	33.2	24.5	1.68	0.98	2.3	3.53
11	Mulugu	65.3	21	27	0.7	0.49	36.4	25.2	1.81	1.06	2.9	4.02
12	Rampur	45.3	11	34	0.6	0.55	26.1	19.4	1.16	0.82	1.6	2.68

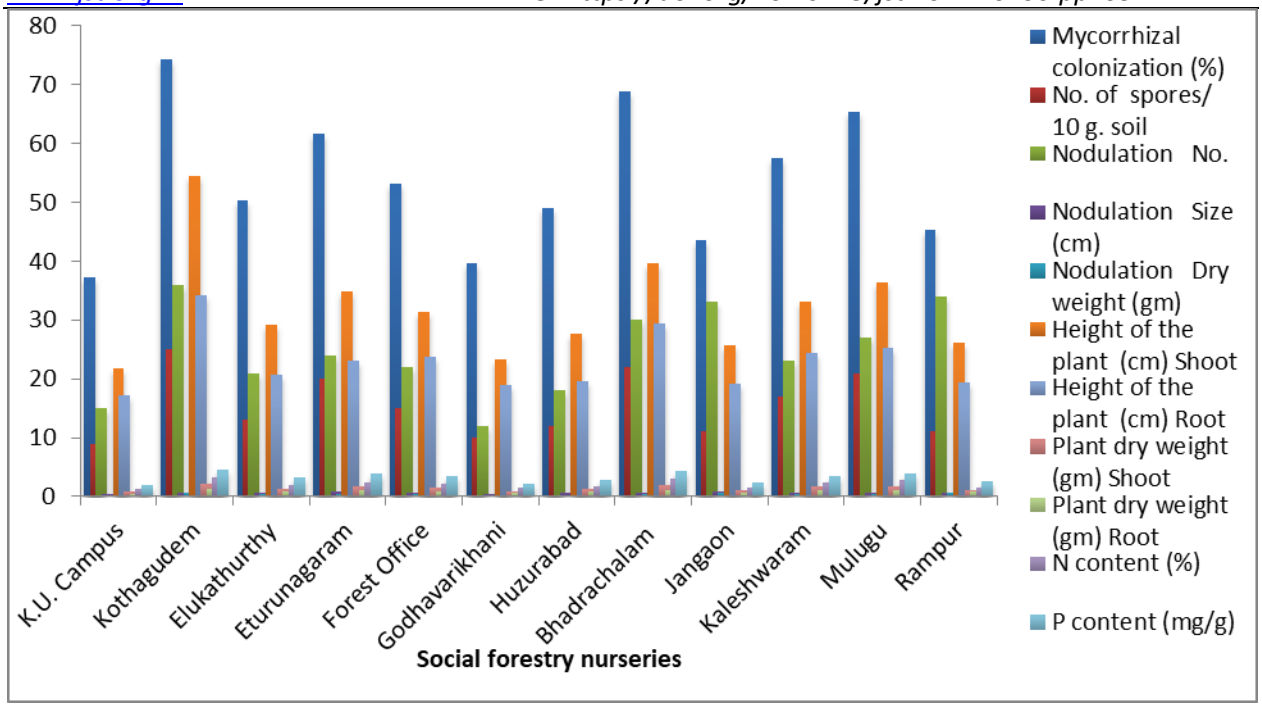

Figure 3: Variation in Mycorrhizal colonization, nodulation and growth of *Pongamia pinnata* in twelve social forestry nurseries

So there might be some Plant Growth Promoting Rhizomicroorganisms (PGPR) and Mycorrhizal spores (Jefwa et.al 2012, Paul gosling et. al. 2010) which were responsible for the maximum plant growth, because NPK ratio of all 12 samples is almost similar

Kottagudem soil sample was subjected to PGPR analysis and for mycorrhizal spore isolation. Different species of Rhizobium and Bacillus (PSB) were isolated on specific media and identified based on 16S rRNA gene sequence. Each new species of Rhizobium and Bacillus were found which were showing less than 97% homology (Figure 4 and 5) with already existing once and were named as *Rhizobium* RHPU-7 and *Bacillus* sp. (PSB). Also, Different AM fungal spores were also isolated from Kottagudem sample. All species were belonging to the four genera were developed as pure cultures by funnel experiment (Tasleem sultana and Pavan kumar 2012). The efficiency of each individual species was tested by examine the root colonization after 90 days growth of *S.grandiflora* plants; among them Gigaspora species were dominant interms of plant height. Among the Gigaspora genus the best supporting species Gigaspora rosea was selected for bioformulation.

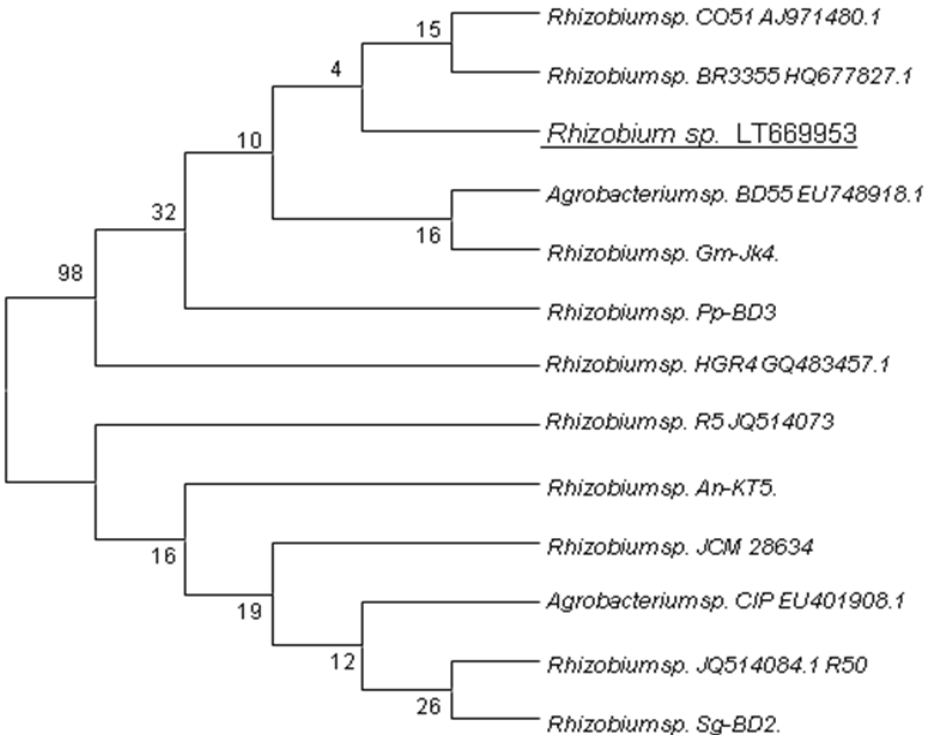

Figure 4: Phylogenetic tree for *Rhizobium sp.LT669953*

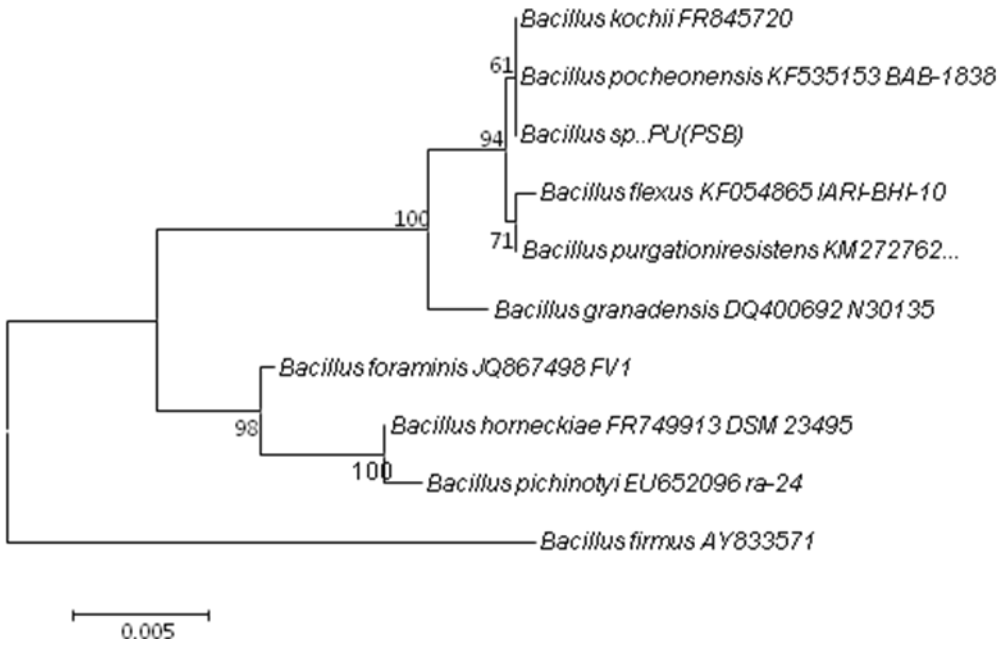

Figure 5: Phylogenetic tree for *Bacillus* sp.(PSB)

3.1. Efficiency of isolates and *Gigaspora rosea*:

Potentiality of the isolates along with *Gigaspora rosea* was examined in soils of nurseries. Normal soils were sown with seeds of *Sesbania grandiflora, Albizzia lebbeck* and *Pongamia pinnata* with bioformulations (*Rhizobium* sp RHPU-7, *Bacillus* sp. (PSB) and *Gigaspora rosea*). Soils with bioformulations have shown good growth of plants of *S.grandiflora, A. lebbeck* and *P. pinnata* and when compared with the normal soil of nurseries without the application of the bioinoculants proving them to be efficient in promoting plant growth and maintaining the nursery plants (Figure 6-8 and Table 4-6).

Table 4: Influence of bioinoculants (AMF, rhizobia and PSB) on the growth of *Sesbania grandiflora* seedlings under nursery conditions

Inoculant		Age of the plant (DAYS)	Mycorrhizal colonization (%)	No. of spores/ 10 g. soil	Nodulation			Height of the plant (cm)		Plant dry weight (gm)		N content (%)	P content mg/g
					No.	Size (cm)	Dry weight (gm)	Shoot	Root	Shoot	Root		
1	SS-Control (sterilized soil)	90	--	--	--	--	--	28.3	17.1	0.71	0.41	0.7	1.59
		180	--	--	--	--	--	43.1	27.8	2.01	0.98	1.1	1.90
2	NS-Normal soil (un-sterilized)	90	43.1	10	33	0.7	0.36	39.8	21.0	0.99	0.54	1.1	2.31
		180	43.6	41	60	0.8	0.67	61.2	32.1	4.60	1.89	2.3	2.78
3	NS+AMF-Gigospora	90	70.1	19	--	--	--	58.6	31.2	2.09	1.02	2.3	3.31
		180	76.9	28	--	--	--	116.1	58.1	6.52	2.12	2.9	4.74
4	NS + AMF+ *Rhizobium*	90	83.9	24	106	0.9	0.36	72.3	39.1	3.01	1.60	2.8	3.81
		180	92.9	35	193	1.1	2.47	143.2	69.8	13.36	4.19	3.8	5.34
5	NS + AMF + PSB	90	80.1	22	--	--	--	69.3	31.0	2.60	1.30	3.0	4.41
		180	87.2	31	--	--	--	120.1	61.1	9.51	3.68	3.2	6.21
6	NS + AMF+ Rhizobium+ PSB	90	84.1	25	--	--	--	73.1	40.5	3.11	1.71	2.9	4.64
		180	93.5	36	--	--	--	149.2	71.9	14.60	4.21	4.1	7.23

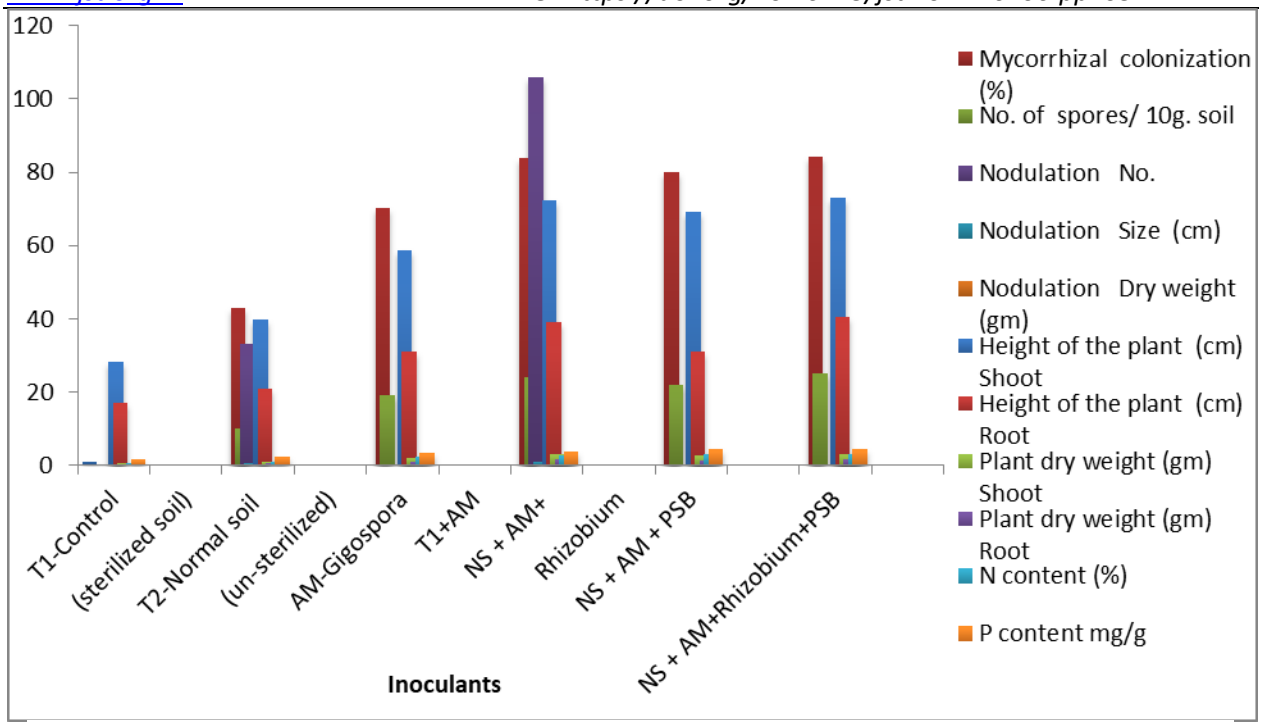

Figure 6: Influence of bioinoculants (AMF, rhizobia and PSB) on the growth of *Sesbania grandiflora* seedlings under nursery conditions

Table 5: Influence of bioinoculants (AMF, rhizobia and PSB) on the growth of *Albizzia lebbeck* seedlings under nursery conditions

Inoculant		Age of the plant (DAYS)	Mycorrhizal colonization (%)	No. of spores/ 10 g. soil	Nodulation			Height of the plant (cm)		Plant dry weight (gm)		N content (%)	P content mg/g
					No	Size (cm)	Dry weight (gm)	Shoot	Root	Shoot	Root		
1	SS-Control (sterilized soil)	90	--	--	--	--	--	16.3	10.4	0.39	0.16	0.8	1.21
		180	--	--	--	--	--	29.1	18.6	2.01	0.71	1.1	1.89
2	NS-Normal soil (un-sterilized)	90	39.1	9	14	0.3	0.13	26.1	17.0	0.62	0.29	1.2	1.69
		180	43.5	11	21	0.4	0.16	41.0	29.9	2.91	1.01	1.5	2.20
3	NS+AMF-Gigospora	90	63.7	17	--	--	--	35.9	25.0	0.71	0.39	1.8	2.78
		180	69.7	20	--	--	--	65.1	40.6	4.01	1.71	2.5	3.36
4	NS + AMF+ *Rhizobium*	90	79.1	20	33	0.4	0.32	47.1	29.9	0.84	0.46	2.6	3.21
		180	84.7	26	45	0.6	0.41	81.0	57.1	5.17	2.31	3.2	3.74
5	NS + AMF + PSB	90	71.6	19	--	--	--	42.9	27.2	0.81	0.51	2.2	3.51
		180	81.2	24	--	--	--	74.0	48.9	5.01	2.10	2.8	3.92
6	NS+AMF+	90	80.1	20	--	--	--	48.2	30.1	0.92	0.52	2.8	3.61

	Rhizobium+ PSB	180	85.3	27	--	--	--	82.1	58.6	5.23	2.42	3.9	4.12

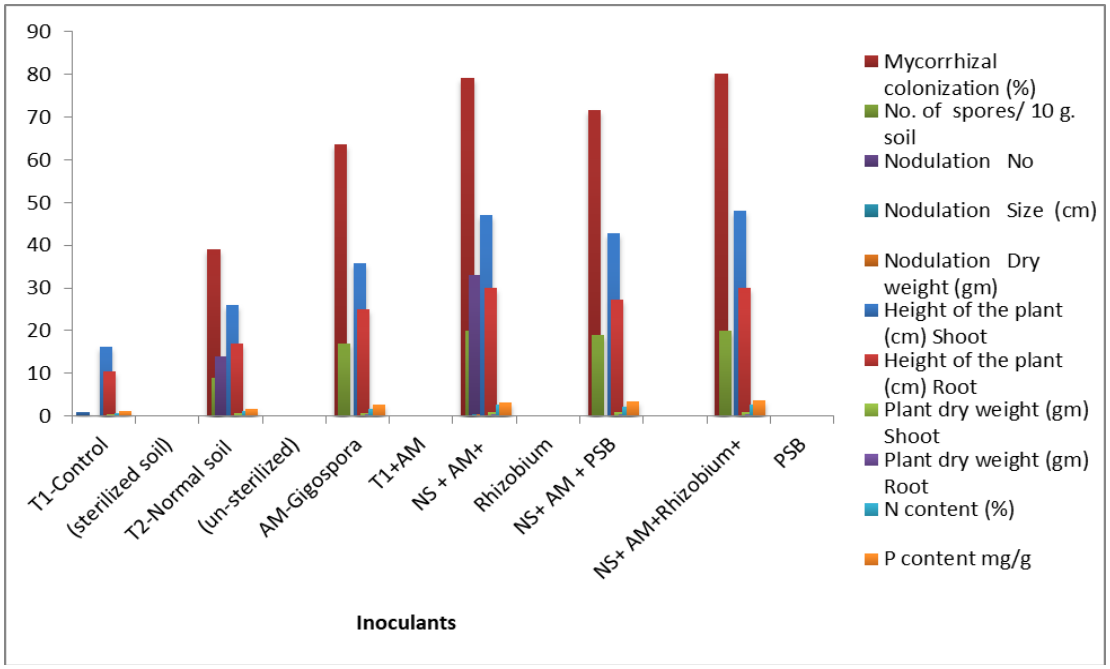

Figure 7: Influence of bioinoculants (AMF, rhizobia and PSB) on the growth of *Albizzia lebbeck* seedlings under nursery conditions

Table 6: Influence of bioinoculants (AMF, rhizobia and PSB) on the growth of *Pongamia pinnata* seedlings under nursery conditions

Inoculant		Age of the plant (DAYS)	Mycorrhizal colonization (%)	No. of spores/ 10 g. soil	Nodulation			Height of the plant (cm)		Plant dry weight (gm)		N content (%)	P content mg/g
					No	Size (cm)	Dry weight (gm)	Shoot	Root	Shoot	Root		
1	SS-Control (sterilized soil)	90	--	--	--	--	--	14.3	12.8	0.56	0.34	0.9	1.33
		180	--	--	--	--	--	19.8	17.6	4.01	2.91	1.3	2.10
2	NS-Normal soil (un-sterilized)	90	42.3	10	13	0.6	0.19	21.8	16.9	0.81	0.51	1.3	2.00
		180	45.9	12	17	0.8	0.27	31.0	26.1	4.98	3.21	1.9	2.68
3	NS+AMF-Gigospora	90	59.6	17	--	--	--	31.5	22.6	1.56	1.19	2.3	2.81
		180	64.8	19	--	--	--	47.8	36.9	6.98	5.01	2.7	3.79
4	NS + AMF+ *Rhizobium*	90	69	22	40	0.8	0.41	37.8	25.8	2.25	1.50	2.6	3.51
		180	78.4	26	47	1.0	0.62	60.4	42.0	8.27	6.22	3.6	4.51
5	NS + AMF +	90	62.0	20	--	--	--	35.1	23.4	2.21	1.41	2.4	3.71

	PSB	180	70.4	23	--	--	--	55.2	40.2	7.91	5.65	3.1	5.01
6	NS + AMF+ Rhizobium+ PSB	90	71.0	22	--	--	--	38.2	26.1	2.30	1.61	2.7	3.82
		180	79.2	27	--	--	--	61.2	43.1	8.34	6.32	3.7	5.23

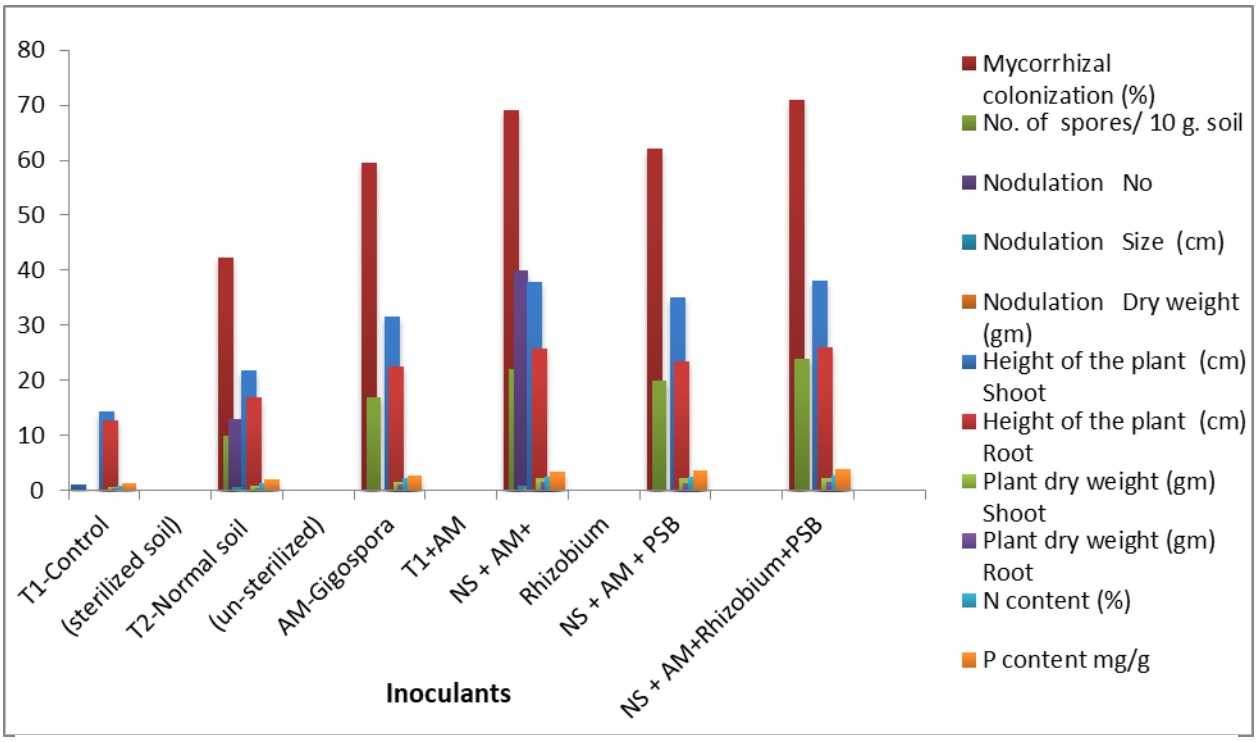

Figure 8: Influence of bioinoculants (AMF, rhizobia and PSB) on the growth of *Pongamia pinnata* seedlings under nursery conditions

Studies on influence of different bioinoculants (AMF fungi, *Rhizobium*,, PSB) on the growth of nursery seedlings of three agroforestry tree species revealed that bioinoculants gave varied responses. Maximum growth response was observed in the mixed inoculations of AMF+PSB+ Rhizobium followed by dual inoculations of AM fungi and *Rhizobium.* Next to the this treatment, co-inoculations of mycorrhizae, PSB, and mycorrhizae favoured the better growth. . Among the individual fungi, *Gigaspora rosea* was found to be better inoculant than the other species.

The results (Table 7) revealed that, all the agroforestry tree species saplings, without any exception, showed the mycorrhizal association. However, the percentage of colonization, type of association and the number of resting spores in the rhizosphere varied from species to species.

Mycorrhizae could contribute substantially to achieve better results in revegetation programmes. Inoculations of forestry nursery saplings with AM fungi are being recognised (Huang et al., 1985). The beneficial effects of mycorrhizae are not only confined to the nursery but also carried to the field. Nursery management practices vary from place to place and affect the mycorrhizal colonization.

Table 7: Mycorrhizal dependency of some agroforestry tree species saplings

S.No	Plant species	Family	% of colonization		P content (%)				Mycorrhizal Dependency %
					Mycorrhizal		Non-mycorrhizal		
			Mycorrhizal	Non-mycorrhizal	Shoot	Root	Shoot	Root	
1	*Acacia nilotica*	Mimosoideae	67	--	0.20	0.30	0.12	0.20	170
2	*Acacia melanoxylon*	Mimosoideae	71	--	0.40	0.40	0.20	0.26	183
3	*Albizzia lebbeck*	Mimosoideae	88	--	0.80	0.90	0.60	0.70	240
4	*Annona squamosa*	Annonaceae	59	--	0.12	0.12	0.10	0.11	125
5	*Azadirachta indica*	Meliaceae	90	--	0.90	0.90	0.71	0.80	253
6	*Cassia siamea*	Caesalpinioideae	77	--	0.50	0.70	0.30	0.38	211
7	*Citrus* spp.	Rutaceae	58	--	0.11	0.14	0.09	0.10	117
8	*Dalbergia sissoo*	Fabaceae	59	--	0.13	0.20	0.07	0.09	126
9	*Dendrocalamus strictus*	Poaceae	58	--	0.10	0.10	0.03	0.05	107
10	*Diospyros melanoxylon*	Ebenaceae	42	--	0.01	0.07	0.01	0.02	95
11	*Emblica Officinalis*	Euphorbiaceae	59	--	0.12	0.20	0.06	0.08	136
12	*Eucalyptus tereticornis*	Myrtaceae	62	--	0.14	0.30	0.10	0.10	150
13	*Gliricidia maculata*	Fabaceae	80	--	0.70	0.80	0.30	0.40	215
14	*Leucaena leucocephala*	Mimosoideae	78	--	0.60	0.80	0.31	0.70	213
15	*Mangifera indica*	Anacardiaceae	66	--	0.20	0.30	0.16	0.20	167
16	*Murraya koenigii*	Rutaceae	58	--	0.10	0.10	0.70	0.11	105
17	*Peltophorum pterocarpus*	Caesalpinoideae	70	--	0.30	0.40	0.13	0.21	175
18	*Polyalthia longifolia*	Annonaceae	52	--	0.05	0.10	0.01	0.04	97
19	*Pongamia pinnata*	Fabaceae	76	--	0.40	0.70	0.40	0.61	195
20	*Psidium guava*	Myrtaceae	48	--	0.04	0.03	0.03	0.09	93
21	*Punica granatum*	Myrtaceae	55	--	0.10	0.10	0.03	0.11	105
22	*Sapindus emerginatus*	Sapindaceae	68	--	0.30	0.40	0.21	0.30	171
23	*Saraka indica*	Caesalpinoideae	44	--	0.03	0.03	0.01	0.08	93
24	*Sesbania grandiflora*	Fabaceae	71	--	0.40	0.50	0.32	0.47	188
25	*Syzygium cuminii*	Myrtaceae	65	--	0.15	0.30	0.10	0.18	158
26	*Tamarindus indica*	Caesalpinoideae	54	--	0.10	0.10	0.09	0.12	104
27	*Tectona grandis*	Verbenaceae	78	--	0.60	0.80	0.70	0.83	212
28	*Terminalia catappa*	Combretaceae	40	--	0.02	0.01	0.01	0.01	92
29	*Zizyphus mauritiana*	Rhamnaceae	50	--	0.04	0.03	0.02	0.05	93

Table 8 reveals growth parameters of S.*grandiflora*, *Albizzia lebbeck* and *Pongamia pinnata* (agroforestry trees) in barren lands, polluted soil and coal dumped area soil sinferring that he application of these bioinoculant formulation, there is a significant increase in the growth of plants by showing significant increase in nodulation properties and growth parameters even in barren, coal mine dumped soils and polluted soils from the tabulated forms (Table 8). These results support field trials with S. grandiflora, *Albizzia lebbeck* and *Pongamia pinnata* in nursery at campus premises of Palamuru University that demonstrated similar results and yield increases.

Hence, these novel isolates along with *Gigaspora rosea* can be used as new bioformulations for Sesbania *grandiflora*, *Albizzia lebbeck* and *Pongamia pinnata* (any agroforestry trees) in any part of the country with same geographical and climatic conditions.

Table 8: Effect of polybioinoculants on growth of the agroforestry tree species in agricultural, barren and polluted soils

Name of the plant	Type of Soil where bioinoculants were tested	Nodulation			Height of the plant (cm)		Dry weight of the plant (gm)	Root	N Content (%)	P Content (%)
		No	Size (mm)	Dry weight (gm)	Shoot	Root				
SG	Coal mine soil	50	0.6	0.52	50.1	32.1	1.98	0.84	2.9	4.23
	Polluted soil	41	0.5	0.51	50.0	32.0	1.99	0.78	2.8	4.12
	Barren soil	40	0.4	0.56	51.1	32.4	2.01	0.88	2.9	4.26
AL	Coal mine soil	30	0.4	0.27	38.5	29.1	1.01	0.70	2.12	2.54
	Polluted soil	24	0.4	0.24	38.1	29.3	1.05	0.71	2.18	2.55
	Barren soil	23	0.5	0.29	39.2	29.9	1.1	0.74	2.21	2.64
PP	Coal mine soil	24	0.7	0.42	33.2	24.5	1.68	0.98	2.3	3.53
	Polluted soil	23	0.6	0.38	33.0	24.4	1.57	0.95	2.12	3.50
	Barren soil	32	0.8	0.47	33.8	25.1	1.73	1.01	2.38	3.59

*AL→ *Albizzia lebbeck*; SG→ *Sesbania grandiflora*, PP→ *Pingamia pinnata*

4. Conclusions

From the results of present investigation it can be concluded that, the isolated organisms serves as potential bioinoculants for sustainable agriculture. Selections of combinations of microbes for highest efficiency, simultaneously biofertilising and biocontrolling activities are a key in future research in this technology. Achieving such as "novel thought" will still be extremely challenging but the designed innovative method is proved to be reliable for "ever green revolution". This novel and innovative method can be applied for the reclamation of problematic sites like polluted, coal dumped areas and barren lands. From the present investigations, it can be

concluded that mycorrhizae seems to play an important role in the seedling growth and perhaps in the establishment of saplings in new habitats after transplantation. It also envisages the importance of meticulous nursery management practices that encourage the mycorrhizal colonization.

Also, a successful nursery operation depends on many factors: selection and development of a suitable site; efficient supervision and administration; adequate planning; forecasting and control procedures; orderly timing of operations and use of appropriate cultural methods; and protection from pests, diseases and other damages.

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6. Conflict of Interest:

The authors declare that they do not have any conflict of interest.

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