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Microbial Technology for Biomass Production in Barren Land

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Abstract : *The effect of seed bacterization with eight isolates of Azotobacter chroococcum, and three isolates of Rhizobium sp. on seed germination of Leucaena leucocephala and Prosopis juliflora was tested in laboratory; and on plant growth and soil properties in field nursery. Leucacena leucocephala the test tree, was registered remarkable in improving the fertility status of wasteland with maximum biomass production.* ***(Key words :*** *Azotobacter and Rhizobium sp., seed bacterization, nursery stocks, total biomass, barren land).*

The constant rise in population and subsequent increase in demand for fuel, food and fodder, has led to massive deforestation resulting in land degradation such as loss of fertile soils, increasing salinity and alkalinity, and desertification etc. and thus causing an alarming expansion in barren land. It has been estimated that India has about 175 M hectares of wastelands (Sardar 1991). Owing to disturbed soil ecology and other associated conditions, these soil have been robbed of organic matter and thus acquire poor potential for supplying essential plant nutrients and thus becoming inhospitable to plant growth. It is therefore, imperative to evolve a biotechnological package which can improve the fertility of such barren land. In view of this, the present investigation was undertaken to screen the most efficient strains of N-fixing bacteria and to evaluate their effect on improving the soil fertility of barren land.

MATERIAL AND METHODS

Laboratory and Nursery Experiments : Seed germination of *Leucaena leucocephala* and *Prosopis juliflora* was tested in laboratory and nursery by dormancy breaking methods, and by seed inoculation with eight isolates of *A. chroococcum* (A5, MA5, M7, SM1, SM2, S2, SM3 & SM4) and three isolates of *Rhizobium sp.* (LM7, LM8 & LM9). The effect of these microbial isolates on overall plant growth and soil properties was also tested in the nursery to screen the most efficient strains that produce healthy plantation.

Field Experiments : Experimental site is located adjacent to the PG. Department of Microbiology, Nagpur University, Nagpur, (Maharashtra, India) which is situated at 321 m above MSL in subhumid agroecological zone. A total rainfall of 1046 mm (from July to September) was recorded during the year of field experiments. The field site was undulating and stony. Soil was loamy-skeletal, extremely shallow and excessively drained.

Leucaena leucocephala and *Prosopis juliflora* inoculated with *Rhizobium sp.* LM7 and LM9 respectively, were put under field testing for one year (July 1992 - July 1993) in randomised block design replicated thrice with treatments : Control; FYM 10 t ha^{-1}; N (Urea) 25 kg ha^{-1}; P (single superphosphate) 50 kg ha^{-1}; *Rhizobium* isolates (5ml /pit); mycorrhizae (11.25 kg ha^{-1}). The details of each treatment is given in Table 3. The plot size was 2m x 2m. The test seedlings were planted with the spacing of 1m x 1m. The composite soil samples from 0-20 cm depth were collected and analysed for texture and important physico-chemical properties; and bacterial, fungal, *Rhizobium, Azotobacter* and actinomycetes counts (dilution plate technique).

Three random plants from each treatment were tagged for recording the observation on height every month. *Rhizosphere* soil samples were collected from each tagged tree at 30 days interval for recording population of bacteria, fungi, actinomycetes, *Azotobacter* and *Rhizobium.*

Tagged trees were cut, after one year of growth, from the ground level and weighed immediately for fresh total biomass. The leaf area was recorded using automatic leaf area recorder. Nitrogen content in the plants was determined by using microKjeldahl method (Jackson 1958). The rhizosphere soil samples were drawn and analysed for pH, EC, organic carbon, total N, available K and P.

The data collected was analysed statistically using 'Analysis of Variance' technique (Fischer 1958).

RESULTS AND DISCUSSION

Nursery Experiments : The results revealed that the isolates of *Rhizobium* and *Azotobacter* improved the overall plant growth as reflected from the dry matter, plant height, leaf area and root volume (Table 1 and 2).

In case of *Leucaena leucocephala* the isolate LM7 was found to be superior to other isolates increasing the dry matter from 1.35 g to 5.85 g in the control treatment. This isolate was also most effective in increasing the N content in plant (2.41%) and soil (0.076%) as compared to the control (Table 1). In case of *Prosopis juliflora* the *Rhizobium* isolate LM9 improved the dry matter by 1.55 g as compared to control. This particular isolate also recorded the highest nitrogen content in plant (2.0%) and soil (0.063%) (Table 2.) Rhizobium and *Azotobacter* population in the rhizosphere soil of these two test trees inoculated with LM7 and LM9 was found to be increased significantly when compared with the data recorded at '0' day (i.e. Azotobacter = $3x10^3$ cells/g and *Rhizobium* = $7x10^3$ cells/g of soil). Thus, it is clear that *Rhizobium* inoculation can boost up the growth of *Leucaena leucocephala* and *Prosopis juliflora* due to better fixation of atmospheric nitrogen through effective nodulation, and suppression of pathogens as is also reported by Prasad *et al.* (1984); Bhatnagar *et al.* (1986); Wattal *et al.* (1992).

Thus, on the basis of their impact on the nursery stocks the *Rhizobium* isolates LM7 and LM9 were selected and tested under field conditions.

Field Experiments : In case of *Leucaena leucocephala*, maximum population of *Rhizobium* was observed after 60 days of inoculation in the treatment of *Rhizobium* LM7 + mycorrhizae + FYM + N+P ($45x10^3$ cells/g) followed by the treatment *Rhizobium* LM7 + mycorrhizae + N +P ($42x\ 10^3$ cells/g); *Rhizobium* -LM7+ *mycorrhizae* ($40x\ 10^3$ cells/g) and *Rhizobium* LM7 + mycorrhizae+ FYM ($36x10^3$ cells/g). However, the native *Rhizobium* counts in the treatments of control ($4x\ 10^3$ cells/g), FYM ($16x10^3$ cells/g), N+P ($23x10^3$ cells/g) and FYM +N+P ($19x10^3$) have shown comparatively less number.

Similarly, increased trend of *Rhizobium* population was observed in case of *Prosopis juliflora* with *Rhizobium* inoculated treatments when compared to the treatments FYM ($7x10^3$ cells/g), N+P ($15x10^3$ cells /g), FYM+ N+P ($17x10^3$ cells/g) and control ($8x10^3$ cells/g). Overall *Rhizobium* LM9 + mycorrhizae + FYM +N+P was superior to other treatments raising the population to the highest count of $40x10^3$ cells/g of soil after 60 days of inoculation. In this particular treatment of both the test trees i.e. *Prosopis juliflora* and *Leucaena leucocephala*, high population of *Azotobacter* (16-22 x 10^3 cells/g), actinomycetes (26-30 x 10^3 cells/g), bacteria ($45-61x10^3$ cells/g) and fungi ($9-12x10^5$ cells/g) was also noticed respectively.

These results indicate that the *Rhizobium* inoculation not only increases the *Rhizobium* population as compared to the control (Fig.1) in the rhizosphere but, also proliferation of other beneficial microorganisms such as *Azotobacter* (Fig. 2) which along with the inoculated organism stimulates the plant growth due to suppression of pathogens, by increasing the availability of plant nutrients and providing growth promoting substances (Cooper 1959; Mishustin & Naumova 1962; Shende *et al.* 1957; Brown 1974). It has been suggested that, the steady release of carbohydrate rich organic material

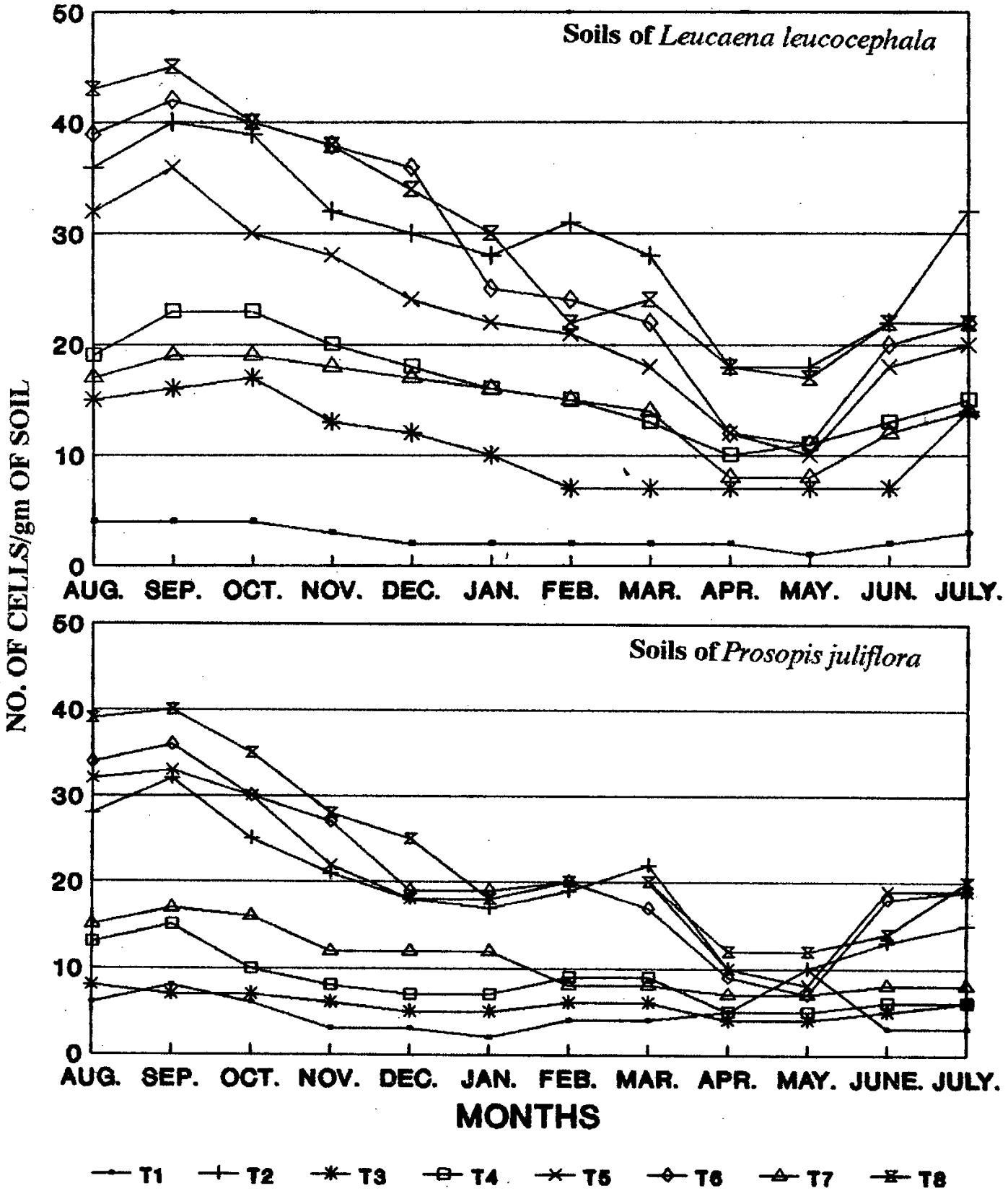

Figure 1. Effect of the various treatments on *Rhizobium* population (No. of cells x 10^3) in the rhizosphere during the period of plantation

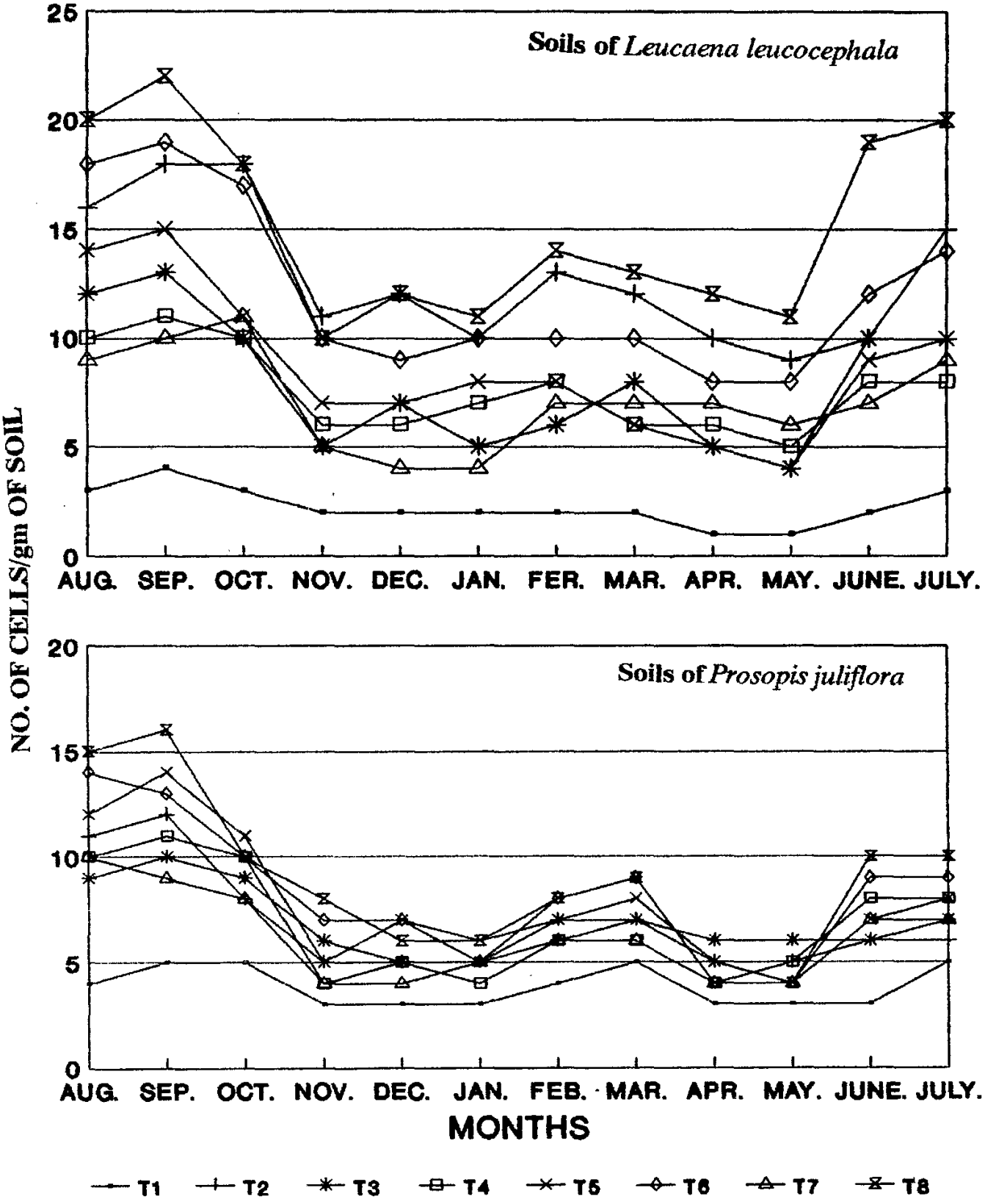

Figure 2. Effect of various treatments on *Azotobacter* population (No. of cells x 10^3) in the rhizosphere during the period of plantation

TABLE 1. Influence of various treatments on nursery stocks of *Leucaena leucocephala* after 150 days (Average of five replications)

Treatment	Rhizobium population	Azotobacter population	Length of plant (cm)	Leaf area (cm^2)	Root volume (ml)	No. of nodules	Plant dry wt. (g)	Nodules dry wt. (mg)	Plant 'N' (%)	Soil 'N' (%)
Control	24.00	13.50	11.15	6.18	1.25	1.25	1.35	4.56	1.61	0.047
Azotobacter SM_4	24.50	50.50	12.88	10.57	3.13	20.00	2.80	27.66	1.94	0.050
Azotobacter S_2	26.25	60.50	17.35	8.27	6.25	31.00	5.20	145.79	1.98	0.018
Azotobacter SM_3	41.25	52.75	17.15	9.30	6.26	34.00	5.22	128.81	2.16	0.046
Rhizobium LM_7 (without pre-treatment)	58.50	40.75	18.00	9.50	7.50	39.00	5.85	201.64	2.41	0.076
SE (m)±	3.05	3.47	1.45	0.91	1.08	5.33	0.78	35.99	0.087	0.005
CD at 5%	9.39	10.69	4.46	2.80	3.33	16.42	2.40	110.88	0.268	0.015
CD at 1%	13.17	14.98	6.26	3.93	4.67	23.02	3.36	155.46	0.375	0.021

Note : i) Seeds treated with pre-treatment of conc. H_2SO_4 for 30 sec.
ii) Rhizobium and Azotobacter (cells x 10^3 per gram of rhizosphere soil) on oven dry basis.

TABLE 2. Influence of various treatments on nursery stocks of *Prosopis juiflora* after 150 days (Average of five replications)

Treatment	Rhizobium population	Azoto-bacter population	Length of plant (cm)	Leaf area (cm^2)	Root volume (ml)	No. of nodules	Dry wt. of plant (g)	'N' content of plant (%)	'N' content of soil (%)
Control	9.00	31.25	20.6	1.9	1.0	4.0	2.05	1.48	0.043
AztobacterSM4	51.50	29.25	28.2	2.3	2.0	2.0	2.95	1.81	0.050
RhizobiumLM9	20.50	71.00	27.7	2.0	2.2	15.0	3.60	2.00	0.063
SE(m)±	5.77	6.90	1.43	0.16	0.11	2.73	0.26	0.016	0.002
CD at 5%	17.77	21.25	4.40	NS	0.33	8.41	0.80	0.049	0.006
CD at 1%	24.92	29.80	6.17	NS	0.47	NS	1.12	0.069	0.009

Note i) Rhizobium and Azotobacter (cells x 10^3 per gram of rhizosphere soil) on oven dry basis.
ii) NS = Non-significant.

from actively growing roots would represent an energy input into soil ecosystem capable of supporting a sustainable microbial population (Martin 1977). In case of both the test trees, the overall microbial population was highest in the rhizosphere soil of treatment consisting *Rhizobium* + mycorrhizae + FYM+N+P which indicates that a small amount of nitrogenous fertilizers added to the soil along with FYM stimulates the multiplication of *Rhizobium* and other beneficial microorganisms. After completion of one year of plantation it was observed that, this particular treatment also significantly influenced the plant height of both the test trees increasing to 418.22 cm over the treatment of control (200.77 cm), FYM (311.56 cm), N+P (328.44 cm) and FYM + N+P (335.11 cm) in case of *Leucaena leucocephala* (Fig.3). Similar observation was recorded in case of *Prosopis juliflora* with maximum height of 220.66 cm in the treatment of *Rhizobium* LM9 + mycorrhizae + FYM+N+P. Whereas, the control treatment recorded a height of 98.00 cm only (Fig.3).

Further, the *Leucaena leucocephala* responded significantly to the treatment consisting of *Rhizobium* LM7 + mycorrhizae + FYM + N+P with total biomass of 74.4 t/ha followed by the treatment

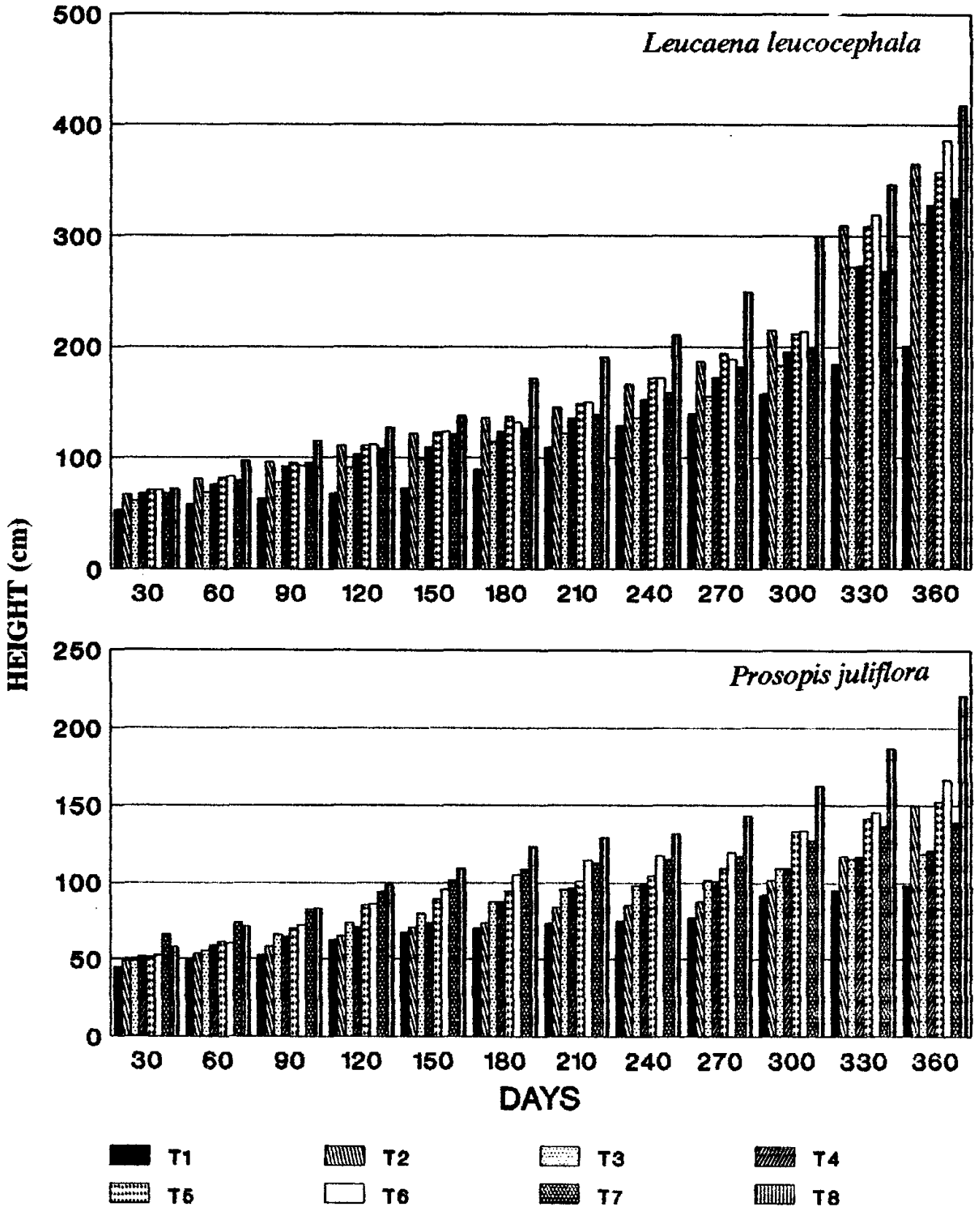

Figure 3. Influence of various treatments on the height (cm) of the test trees during the period of plantation

TABLE 3. Effect of bacterial inoculation on biomass production of *Leucaena leucocephala Prosopis juliflora*

Treatments	Leucaena leucocephala Biomass (t ha^{-1})	Leucaena leucocephala Leaf area (cm^2)	Prosopis juliflora Biomass (t ha^{-1})	Prosopis juliflora Leaf area (cm^2)
T1. Control	23.2	74.6	1.8	12.7
T2. Rhizobium + mycorrhizae	51.5	230.6	7.0	24.4
T3. FYM		42.7	105.6	6.1
T4. N+P	33.9	115.0	6.9	12.8
T5. Rhizobium + mycorrahizae+ FYM	47.2	222.3	8.9	21.5
T6. Rhizobium+ mycorrahizae N+P	53.9	189.0	10.2	18.3
T7. FYM +N+P	30.9	126.3	7.0	12.4
T8. Rhizobium + mycorrhizae FYM+N+P	74.4	252.3	16.6	22.9
'F' test	Sig	Sig.	Sig.	NS
SE (m)±	8.90	22.43	0.24	4.03
CD 5%	26.99	68.05	0.73	-

Rhizobium LM7 + mycorrhizae +N+P (53.9 t/ha). Similarly, in case of *Prosopis juliflora*, these two treatments i.e. *Rhizobium* LM9 + mycorrhizae + FYM + N+P and *Rhizobium* LM9 + mycorrhizae +N+P proved to be most superior yielding a total biomass of 16.6 t/ha and 10.2 t/ha respectively (Table 3).

These results indicate that the performance of Leucaena leucocephala and *Prosopis juliflora* is by and large superior only in inoculated treatments than the uninoculated ones. This increase in the total biomass and plant height is attributed to beneficial action of both *Rhizobium* and mycorrhizae which ensure better nitrogen fixation, mobilization and uptake of essential nutrients and other elements thereby promoting vigorous growth (Subbarao *et al.* 1986; Dinesh Kumar 1987; Jagpal *et al.* 1988; Manjunath *et al.* 1989; Young 1990). However, the addition of FYM is found to be most congenial.

It is also evident from Table 3 that, microbial inoculations were found to be highly influential over the leaf area of *Leucaena leucocephala* with combined treatment of FYM and N+P (252.3 cm^3). However, none of the treatments could influence the leaf area of *Prosopis juliflora*.

TABLE 4. Soil properties as influenced by different microflora under *Leucaena leucocephala and Prosopis juliflora*.

Treatments	pH	EC dSm^{-1}	OC (%)	N (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Total N> (%)
Leucaena leucocephala							
T1	6.8	0.20	0.47	0.047	44	313	1.44
T2	7.0	0.20	0.82	0.082	54	413	3.80
T3	7.1	0.19	0.54	0.054	44	314	2.46
T4	7.1	0.21	0.62	0.062	49	319	2.65
T5	6.8	0.16	0.87	0.087	53	323	3.95
T6	7.1	0.20	0.92	0.092	59	384	4.16
T7	6.8	0.20	0.68	0.068	53	313	3.30
T8	7.1	0.22	0.98	0.098	79	391	4.30
'F' test	-	-	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	-	0.079	0.0079	5.43	2.43	0.014
CD 5%	-	-	0.2	0.024	16.46	7.37	0.042
Prosopis juliflora							
T1	6.8	0.18	0.49	0.049	41	342	1.58
T2	6.7	0.18	0.70	0.070	49	370	3.31
T3	6.7	0.20	0.53	0.053	54	353	2.89
T4	6.7	0.21	0.66	0.066	62	388	2.95
T5	6.5	0.16	0.79	0.079	56	364	3.22
T6	6.5	0.15	0.83	0.083	53	434	3.48
T7	7.2	0.18	0.69	0.069	52	346	3.25
T8	6.6	0.18	0.90	0.090	61	453	3.69
'F' test	-	-	Sig.	Sig.	NS	NS	Sig.
SE (m) ±	-	-	0.03	0.003	7.05	62.61	0.0001
CD 5%	-	-	0.09	0.009	-	-	0.0003

Analysis of wasteland at 'O' day before biomass production

pH	EC dSm^2	OC (%)	N (%)	P2O5 (kg ha^{-1})	K2O (kg ha^{-1})
8.0	0.22	0.54	0.054	15.16	393

The total N% of the plants in case of both the test tree inoculated with *Rhizobium* + mycorrhizae + FYM +N+P was found to be most superior i.e., 4.30 and 3.69% respectively (Table 4). The results of the experiments on the physicochemical soil properties

indicated that the *Rhizobium* and mycorrhizae inoculation can play a significant role in accumulation of C, N & P in the rhizosphere of *Leucaena leucocephala* as compared to control and data recorded at '0' day. Whereas, in case of *Prosopis juliflora* through there was significant increase in C & N content, however, the P & K content was found to be non-significant statistically. Thus, the soil fertility levels are always found to be directly correlated with microbial activity of soil (Joshi & Joshi 1953).

Overall, the application of microbial inoculums with the amendments of FYM and small doses of N + P proved to be superior than other treatments.

Thus, an integrated biotechnological approach involving use of biofertilizers and vesicular arbuscular mycorrhizae seems to be the most appropriate viable technology for bioreclamation and development of barren/wasteland.

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