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Impact of biofertilizers on morpho-physiological attributes in pungam (*Pongamia pinnata* (L.) Pierre) seedlings

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ABSTRACT

Investigations were conducted to determine the efficacy of biofertilizer inoculations with *Rhizobium*, phosphobacteria and Vesicular-Arbuscular Mycorrhiza (VAM), individually and in combination on the growth, biomass, biochemical parameters and nutrient yield of pungam (*Pongamia pinnata* (L.) Pierre) seedlings under nursery conditions. Six months old seedlings were transplanted to polybags containing different inoculum combinations arranged in a completely randomized design with three replications. Uninoculated treatment formed the control. The results showed enhanced shoot and root length, total dry matter and nutrient uptake due to triple inoculation with *Rhizobium*, phosphobacteria and VAM. All dual inoculations proved as good as the triple inoculation with regard to P yield. For K yield, the dual inoculation with *Rhizobium* + VAM was comparable with triple inoculation. With regard to total chlorophyll, soluble protein and nitrate reductase activity also, the triple inoculation performed best. Thus, inoculation with a combination of *Rhizobium,* Phosphobacteria and VAM would improve the nursery performance of pungam.

Key words: biofertilizer, phosphobacteria, *Pongamia pinnata, Rhizobium*; VAM.

INTRODUCTION

Pungam is extensively planted for afforestation of watersheds in the drier parts of India (Anon. 1969). The amount and distribution of rainfall play an important role in deciding the success or failure of an afforestation programme. The rainfall received is not uniform over the years. Besides rainfall, the establishment and survival of the planting stock in the field mainly depend on the production of good quality planting stock through seed and seedling management technologies. One such management technology is developing suitable biofertilizer for tree species. The biofertilizers containing live cells of nitrogen fixing micro-organisms or phosphorus solubilizers have helped in minimising the dependence on inorganic fertilizers. It also helps to reduce environmental pollution.

The role of biofertilizers has already been proved extensively in annual crops, but its exploitation in forest species is scanty in India. In forestry, few research reports are available to demonstrate that biofertilizers stimulate the growth (Sudhir *et al.* 1994; Mukerji *et al.* 1996), biomass (Sekar *et al.* 1995; Reddy *et al.* 1996), biochemical parameters (Niranjan *et al.* 1990) and uptake of N (Mathur and Vyas 1996), P (Dela Cruz *et al.* 1988), K (Merina Prem Kumari 1991) and other micronutrients. Ultimately, the survival rate of planted seedling is enhanced.

Hence, a study on the effect of biofertilizers on seedling growth, biomass, physiological parameters and nutrient yield of pungam was initiated.

MATERIALS AND METHODS

Six- month-old seedlings of *Pongamia pinnata* (L.) Pierre of uniform size were chosen for biofertilizer inoculation at Forest College and Research Institute, Mettupalayam, Tamil Nadu, India. The seedlings were transplanted in 30 x 45 cm polybags filled with nursery mixture of red soil, sand and farm yard manure (FYM) (2:1:1). The experiment was set up in a completely randomized design and replicated three times. The nursery soil mixture of the polybags was inoculated with biofertilizers. The biofertilizer

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Abbreviations: FYM, Farm Yard Manure; NR, Nitrate Reductase; VAM, Vesicular-Arbuscular Mycorrhiza treatments were (i) *Rhizobium* (*Rhizobium* spp. ALM-2), (ii) Phosphobacteria (*Pseudomonas striata* PB-2), (iii)Vesicular-Arbuscular Mycorrhizae (VAM) (*Glomus fasciculatum*), (iv) *Rhizobium* + Phosphobacteria, (v) Phosphobacteria + VAM, (vi) *Rhizobium* + VAM, (vii) *Rhizobium* +Phosphobacteria +VAM and (viii) Uninoculated control. The biofertilizers were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Biofertilizer inoculation was prepared with a base of peat soil. Two hundred grams of *Rhizobium* (10^8 cells g^{-1}) and Phosphobacteria (10^9 cells g^{-1}) were weighed and mixed with 3 kg of well decomposed and powdered FYM, separately. Fifty grams of this each inoculum mixture and twenty grams of VAM (4 spores g^{-1}) inoculum were applied to each polybag at 5 cm depth near the root zone.

Six seedlings in each treatment were selected at random and observed initially, and 2 and 4 months after inoculation for shoot and root length, total dry matter, biochemical parameters such as total chlorophyll content (Yoshida *et al.* 1971), soluble protein (Lowry *et al.* 1951), nitrate reductase (Nicholas *et al.* 1976), and yield of nitrogen, phosphorus and potassium (Jackson 1973). The results were subjected to analysis of variance and tested for significant differences (P=0.05) according to Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The seedlings inoculated jointly with *Rhizobium*, phosphobacteria and VAM registered the highest shoot and root length than the other biofertilizer treatments or the control (Table 1). The increase was more than 30 per cent over the control (Table 4). The increase in plant growth attributes might be due to increased uptake of nutrients in mycorrhiza-associated plants and the synergistic effect with other inoculants. Increase in root and shoot length due to biofertilizer inoculation was earlier reported by Subba Rao and Barrueco (1993). The increase in shoot length due to combined inoculation of biofertilizers has been reported in various Shola species (Rangarajan and Narayanan 1990; Sekar *et al.* 1995).

The total dry matter was also higher in the seedlings inoculated with *Rhizobium* + phosphobacteria + VAM (Table 1). Compared to control the increase was 54.9 per cent (Table 4).

Table 1. Effect of biofertilizers on shoot length, root length and total dry weight of pungam seedlings.

Bio Fertilizers (B)	Shoot length, cm				Root length, cm				Total dry weight, g plant^{-1}			
	Months after inoculation (M)				Months after inoculation				Months after inoculation			
	0	2	4	Mean	0	2	4	Mean	0	2	4	Mean
Rhizobium (R)	42.43	57.57	69.30	56.43	52.40	64.47	69.43	62.10	9.14	27.56	36.13	24.27
Phospho bacteria (P)	42.87	62.63	64.60	56.70	52.40	57.40	68.40	59.40	9.45	32.35	41.01	27.60
VAM	42.27	53.73	83.37	59.79	51.53	58.67	70.67	60.29	9.08	27.61	39.80	25.50
R + P	43.53	57.90	67.13	56.19	52.57	64.73	70.83	62.71	9.25	31.15	40.12	26.84
P + VAM	41.80	62.77	69.60	58.06	52.70	56.67	63.97	57.78	9.44	29.88	40.60	26.64
R + VAM	41.83	61.77	83.37	62.32	52.27	57.53	78.5	62.77	9.11	32.22	42.39	27.91
R + P + VAM	42.70	73.37	88.40	68.16	52.70	77.47	90.97	73.71	9.01	43.18	46.94	33.04
Control	42.83	52.67	58.00	51.17	51.60	55.43	60.93	55.99	9.11	23.15	31.88	21.38
Mean	42.53	60.30	72.97		52.27	61.55	71.71		9.20	30.89	39.86	

	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
B	2.180	4.385	2.449	4.925	0.839	1.686
M	1.335	2.685	1.500	3.016	0.514	1.033
B x M	3.777	7.595	4.242	8.530	1.453	2.921

SEd - Standard error deviation
CD - Critical difference

Similar increases in biomass production due to VAM inoculation were reported in *Leucaena leucocephala* (Michelson and Rosendahl 1990), *Acacia* spp. (Reena and Bagyaraj 1990) and in *Albizia* spp. (Purohit *et al.* 1995). In the present investigation individual inoculation of VAM has also increased the biomass to some extent, relative to the control. The increase in seedling biomass production may be strongly correlated with increased accumulation of N due to *Rhizobium* (Jamaluddin *et al.* 1995) or mycorrhizal fungi and phosphobacteria (Durga and Gupta 1995; Reddy *et al.* 1996).

A significant enhancement in biochemical parameters like total chlorophyll content, soluble protein and NRase activity was brought about with the combined inoculation of *Rhizobium*, Phosphobacteria and VAM (Table 2). The increase relative to the control was 10.7, 48.5 and 43.6 per

Table 2. Effect of biofertilizers on total chlorophyll, soluble protein and nitrate reductase activity of pungam seedlings.

Bio fertilizers (B)	Total chlorophyll, mg g^{-1}				Soluble protein, mg g^{-1}				Nitrate reductase activity, µg g^{-1} hr^{-1}			
	Months after inoculation (M)				Months after inoculation				Months after inoculation			
	0	2	4	Mean	0	2	4	Mean	0	2	4	Mean
Rhizobium (R)	2.39	2.51	2.63	2.51	1.70	2.22	2.65	2.19	0.230	0.337	0.417	0.328
Phospho bacteria (P)	2.31	2.53	2.66	2.50	1.73	2.22	2.63	2.19	0.232	0.312	0.384	0.309
VAM	2.39	2.55	2.66	2.54	1.75	2.57	2.77	2.36	0.234	0.344	0.431	0.337
R + P	2.37	2.59	2.74	2.57	1.72	2.68	2.92	2.44	0.232	0.356	0.452	0.347
P + VAM	2.31	2.64	2.79	2.58	1.78	3.08	3.25	2.70	0.232	0.384	0.479	0.365
R + VAM	2.32	2.68	2.81	2.60	1.85	3.07	3.45	2.39	0.227	0.417	0.488	0.377
R + P + VAM	2.37	2.76	2.93	2.68	1.73	3.20	3.98	2.97	0.231	0.473	0.532	0.412
Control	2.41	2.38	2.48	2.42	1.72	2.00	2.27	2.00	0.227	0.284	0.350	0.287
Mean	2.36	2.58	2.71		1.75	2.63	2.99		0.231	0.363	0.442	

	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
B	0.033	0.067	0.055	0.110	0.0086	0.0172
M	0.020	0.041	0.033	0.066	0.0052	0.0105
B x M	0.058	0.117	0.095	0.190	0.0148	0.0298

SEd - Standard error deviation
CD - Critical difference

Table 3. Effect of biofertilizers on N, P and K yield of pungam seedlings.

Bio fertilizers (B)	N yield (g $plant^{-1}$)				P yield (g $plant^{-1}$)				K yield (g $plant^{-1}$)			
	Months after inoculation (M)				Months after inoculation				Months after inoculation			
	0	2	4	Mean	0	2	4	Mean	0	2	4	Mean
Rhizobium (R)	18.6	78.2	155.7	84.17	4.7	17.6	26.4	16.23	2.3	13.0	22.4	12.57
Phospho bacteria (P)	19.2	81.8	171.0	90.67	4.9	22.0	31.6	19.50	2.4	14.6	26.2	14.4
VAM	18.3	70.4	169.9	86.20	4.6	18.8	30.6	18.00	2.1	13.0	25.5	13.53
R + P	18.8	97.2	176.9	97.63	4.8	22.4	32.5	19.90	2.0	16.5	26.5	15.00
P + VAM	19.1	86.3	175.7	93.70	4.9	22.7	34.1	20.57	2.2	15.8	26.8	14.93
R + VAM	18.4	104.0	187.3	103.23	4.6	23.5	34.3	20.80	2.1	17.4	28.8	16.10
R + P + VAM	18.2	146.3	214.5	126.33	4.6	34.1	40.8	26.5	2.1	25.9	33.3	20.43
Control	18.5	48.3	91.4	52.73	4.7	13.4	19.8	12.63	2.1	8.6	15.6	8.77
Mean	18.64	89.06	167.8	-	4.7	21.81	31.26	-	2.16	15.6	25.64	-

	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
B	0.038	0.153	0.017	0.067	0.016	0.064
M	0.014	0.058	0.006	0.026	0.006	0.024
B x M	0.115	0.462	0.050	0.204	0.048	0.193

SEd - Standard error deviation
CD - Critical difference

Table 4. Percent increase over control by different biofertilizer treatments

Bio fertilizers	Shoot length	Root length	Total dry weight	Total chloro-phyll	Soluble protein	Nitrate reductase activity	N yield	P yield	K yield
Rhizobium (R)	9.47	11.2	13.6	3.7	9.5	14.3	56.6	28.5	43.3
Phospho bacteria (P)	10.0	6.3	29.4	3.3	9.5	7.7	71.4	54.4	64.2
VAM	16.0	7.9	19.6	5.0	18.0	17.4	62.9	42.6	54.2
R + P	9.0	12.3	25.9	6.2	22.0	20.9	81.6	57.6	71.0
P + VAM	12.6	3.4	24.9	6.6	35.0	27.2	77.1	62.9	70.0
R + VAM	20.9	12.4	31.0	7.4	19.5	31.4	95.1	64.7	83.5
R + P + VAM	32.2	31.9	54.9	10.7	48.5	43.6	138.8	109.9	132.9

cent, respectively. This is in agreement with several reports. Thus an increase in chlorophyll content and soluble protein was observed in *Ziziphus mauritiana* when inoculated with VAM (Mathur and Vyas 1996). Similar effects were also reported in *Dalbergia sissoo* inoculated with *Rhizobium* and Mycorrhizae (Niranjan *et al.* 1990) and in Shola species inoculated with *Azospirillum* + Phosphobacteria and VAM (Sekar *et al.* 1995).

High N yield was observed in the seedlings inoculated with *Rhizobium* + Phosphobacteria + VAM (Table 3). Increased N content in the plant samples of various tree seedlings, jointly inoculated with different biofertilizers has been reported (Merina Prem Kumari 1991; Sekar *et al.* 1992). The increased N yield in the present investigation may be due to increase in NRase activity, which has been observed in the seedlings inoculated with *Rhizobium* + Phosphobacteria + VAM. The increased P yield in the seedlings inoculated with VAM has also been reported by various workers (Narayana Bhat 1991; Durga and Gupta 1995; Mathur and Vyas 1996). Improved P content in the plant inoculated with *Rhizobium* + VAM has also been reported (Dixon *et al.* 1993). The increase of P yield in the present investigation may be due to symbiosis exhibited by various biofertilizers which ultimately help to increase the availability of nutrients (Varma and Schuepp 1995) at the root zone. The above reason may also be attributed to higher K yield in the microbial inoculated seedlings. Increased K uptake was also reported in *Acacia nilotica* (Suresh 1994) by *Rhizobium* inoculated with VAM. In general, there seems to be a trend of significant higher values for biofertilizers vs control but often less variation within biofertilizers. This may be attributable to inoculum carriers and also would explain the best results obtained when all three inoculants were added. From this study, it is concluded that combined inoculation with *Rhizobium* + Phosphobacteria + VAM will improve the plant growth, biomass and nutrient uptake of the seedlings and thereby help to improve the survival rate of planted seedlings.

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