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STUDIES ON THE EFFECT OF BIOFERTILIZERS ON THE GERMINATION OF ACACIA NILOTICA LINN. SEEDS

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Introduction

Forests protect and enrich the soil mantle by reducing soil erosion and nutrient loss and by facilitating nutrient recycling and microbiological activities. They act as 'sink' for atmospheric CO_2 and release large amounts of O_2. Forests also serve as a unique storehouse of plant and animal genetic resources and contribute significantly to the biological diversity of the country which inturn serves as an insurance against food crisis and as an assurance for health care. The forest zones of India occupy about 22% of geographical area and recent aerial survey has indicated that only about 12% is under functional forest area. The satellite data of National Remote Sensing Agency (NRSA) has revealed that India keeps losing its natural forests at the rate of 1.3 million hectares every year.

The forestry sector is an important contributor to the economic and social well being of a country. Today earths green shield is under threat of extinction along with its natural resources. This is due to over exploitation by man. The only solution to overcome this problem is large scale afforestation. A successful afforestation programme is completely depended upon the availability and supplyof high quality seeds of different tree species, since most of them are propagated by seeds.

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The good quality planting stock material can be produced by applying biofertilizers, biocides and nutrients to the seeds and seedlings at the nursery level. Biofertilizers have attracted greater attention particularly in developing countries like India as a substitute for costly chemical fertilizers. India's production of nitrogen ad phosphate fertilizers is well below their provisional consumption and part of the deficit is met through importation. Biofertilizers can obviate the need of importation of fertilizers and at the same time be cost effective and compatible with ecology.

Biofertilizer or microbial inoculants can be generally defined as preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms used for application of seed, soil or composting areas with the objective of increasing the numbers of such microorganisms and accelerate certain microbial processes to augment the extent of the availability of nutrients in a form which can be easily assimilated by plants. Biofertilizers which improve soil quality are ecofriendly and provide yield increments which greatly benefit farmer with only very small input cost.

Keeping this in view, a study was conducted to find out the effect of biofertilizers on the germination of *Acacia nilotica* seeds.

Materials and Methods

Acacia nilolica Linn is an armed tree with thorns. Wood is brown, hard, strong and used for agricultural and many other purposes. It belongs to the family Mimosaceae. Fresh seeds of *Acacia nilotica* (Karuvelam) were procured from Forest college and Research Institute, TamilNadu Agricultural University (TNAU), Mettupalayam, Coimbatore district and used for pot culture and laboratory experiments.

Pot culture experiments in red loamy soil using biofertilizers singly and in combination

The pot culture experiments were carried out at Bharathiar University, Coimbatore, using completely Randomized Block Design (RBD) with four replications. The biofertilizers were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore.

The pots were filled with 9 kg of red loamy soil and inoculated with biofertilizers like *Rhizobium*, phosphobacteria, and vesicular arbuscular mycorrhiza (VAM). The various treatments employed are detailed below.

T_1 - *Rhizobium* @ 18 g/pot
T_2 - Phosphobacteria @ 18g/pot
T_3 - VAM @ 45 g/pot
T_4 - *Rhizobium* + phosphobacteria @ 9 + 9 g/pot
T_5 - *Rhizobium* + VAM @ 9 + 22.5 g/pot
T_6 - Phosphobacteria + VAM @ 9 + 22.5 g/pot
T_7 - *Rhizobium* + phosphobacteria + VAM @ 6+6+15 g/pot
T_8 - Uninoculated with none of the above served as control.

All the samples both inoculated as well as uninoculated consisted of 50 seeds. They were sown separately in each pot. The recommended nursery management practices were followed for the production of elite seedlings.

Germination percentage was recorded after emergence of plumule (seventh day) for all the four selected tree species. The growth parameters like root length, shoot length, seedling fresh weight and seedling dry weight were recorded after 60, 90 and 120 days after sowing (DAS).

Besides this, the seedlings were analysed for the biochemical parameters such as the contents of chlorophyll a, chlorophyll b, total chlorophyll, total soluble carbohydrates, reducing sugars, total free amino acids, total proteins and total free phenolics.

The analysed minerals include nitrogen, phosphorus, potassium, calcium and magnesium. In each replication, measurements were taken for five seedlings and mean was worked out.

Total chlorophyll content was measured by using the method of Arnon (1949), total soluble carbohydrates by the method of Clegg (1956), reducing sugars by the method of Nelson (1944), total free amino acids by the method of Moore *et al* (1948), total proteins by the method of Lowry *et al* (1951) and total free phenolics by the method of Maxon and Rooney (1972) for extraction and Sadasivam and Manickam (1992) for estimation.

The seedlings were oven-dried at 80°C for 24 hours and subjected to the analysis of selected minerals. Nitrogen content was calculated by microkjeldahl method (Humphries, 1956), phosphorus content by the method of Jackson (1973), potassium content by flame photometric method (Jackson, 1973). Calcium and magnesium contents were measured by versenate method (Cheng and Bray, 1951). Date obtained were subjected to statistical analysis as per the procedure outlined by Panse and Sukhatme (1967). The results are presented in Tables 1 to 7.

Table 1. Effect of biofertilizer treatment on germination, root length and shoot length of *Acacia nilotica*

Biofertilizer		Germination (%)	Root length (cm)			Mean	Shoot length (cm)			Mean
		Mean	Stage				Stage			
			60	90	120		60	90	120	
Rhizobium	(T_1)	96.00	23.20	25.78	28.60	25.86	57.86	58.14	69.84	61.94
Phosphobacteria	(T_2)	96.00	28.10	30.26	30.56	29.64	56.62	63.56	81.44	67.20
VA mycorrhiza	(T_3)	80.00	27.50	24.40	29.85	27.25	62.86	65.92	65.52	64.76
Rhizobium + phosphobacteria	(T_4)	88.00	26.70	27.62	29.90	28.07	53.36	68.92	73.10	65.12
Rhizobium + VAM	(T_5)	84.00	25.60	29.40	29.80	28.26	58.62	72.82	81.26	70.90
Phosphobacteria + VAM	(T_6)	92.00	20.60	28.90	31.76	27.08	61.00	71.42	79.10	70.50
Rhizobium + phosphobacteria + VAM	(T_7)	80.00	19.90	30.22	31.74	27.28	53.50	64.54	75.22	64.42
Uninoculated control	(T_8)	68.00	15.80	20.52	29.62	21.98	32.74	43.44	63.60	46.59
Stage mean		85.50	23.42	27.13	30.22		54.57	63.59	73.63	
			T	S	T x S		T	S	T x S	
Sed		0.76	1.161	0.711	2.011		1.10	0.67	1.90	
CD (P=0.05)		1.57	2.304	1.411	3.992		2.18	1.33	3.78	

Table 2. Effect of biofertilizer treatment on fresh weight and dry weight of *Acacia nilotica*

Biofertilizer		Fresh weight (g/seedling) Stage 60	Stage 90	Stage 120	Mean	Dry weight (g/seedling) Stage 60	Stage 90	Stage 120	Mean
Rhizobium	(T_1)	6.59	7.47	10.19	8.08	1.72	1.97	2.63	2.11
Phosphobacteria	(T_2)	5.51	8.23	11.21	8.32	1.45	2.14	2.95	2.18
VA mycorrhiza	(T_3)	8.81	9.31	9.89	9.33	2.27	2.42	2.58	2.42
Rhizobium + phosphobacteria	(T_4)	4.12	10.03	10.37	8.17	1.09	2.58	2.67	2.11
Rhizobium + VAM	(T_5)	7.33	10.71	10.87	9.63	1.91	2.79	2.85	2.52
Phosphobacteria + VAM	(T_6)	8.35	10.19	10.71	9.75	2.20	2.64	2.80	2.54
Rhizobium + phosphobacteria + VAM	(T_7)	4.95	8.53	10.50	8.00	1.29	2.23	2.74	2.08
Uninoculated control	(T_8)	2.11	3.16	4.81	3.36	0.54	0.83	1.28	0.88
Stage mean		5.97	8.45	9.82		1.56	2.20	2.56	
		T	S	T x S		T	S	T x S	
Sed		0.018	0.011	0.031		0.002	0.001	0.003	
CD (P=0.05)		0.036	0.022	0.063		0.004	0.002	0.007	

Table 3. Effect of biofertilizer treatment on chlorophyll 'a', chlorophyll 'b' and total chlorophyll of *Acacia nilotica*

Biofertilizer		Chlorophyll `a' (mg/g)				Chlorophyll `b' (mg/g)				Total chlorophyll (mg/g)			
		Stage				Stage				Stage			
		60	90	120	Mean	60	90	120	Mean	60	90	120	Mean
Rhizobium	(T_1)	0.21	0.31	0.35	0.29	1.19	2.05	2.65	1.96	1.40	2.36	3.00	2.25
Phosphobacteria	(T_2)	0.22	0.29	0.48	0.33	1.25	1.70	3.36	2.10	1.47	1.99	3.84	2.43
VA mycorrhiza	(T_3)	0.38	0.46	0.84	0.56	2.16	2.70	5.84	3.56	2.54	3.16	6.68	4.12
Rhizobium + phosphobacteria	(T_4)	0.30	0.50	0.54	0.44	1.73	3.15	3.50	2.79	2.03	3.65	4.04	3.24
Rhizobium + VAM	(T_5)	0.17	0.38	0.45	0.33	0.96	2.26	3.13	2.11	1.13	2.64	3.58	2.45
Phosphobacteria + VAM	(T_6)	0.25	0.35	0.43	0.34	1.44	2.52	3.00	2.32	1.69	2.87	3.43	2.66
Rhizobium + phosphobacteria + VAM	(T_7)	0.27	0.42	0.43	0.37	1.52	2.43	3.03	2.32	1.79	2.85	3.46	2.70
Uninoculated control	(T_8)	0.13	0.14	0.19	0.15	0.77	0.85	1.79	1.13	0.90	0.99	1.98	1.29
Stage mean		0.24	0.37	0.44		1.38	2.20	3.28		1.62	2.56	3.75	

	T	S	T x S	T	S	T x S	T	S	T x S
Sed	0.002	0.001	0.003	0.004	0.002	0.007	0.014	0.008	0.024
CD (P=0.05)	0.003	0.002	0.005	0.009	0.005	0.015	0.028	0.017	0.049

Table 4. Effect of biofertilizer treatment on total soluble carbohydrates and reducing sugars of *Acacia nilotica*

Biofertilizer		Total free amino acids (mg/g)				Total proteins (mg/g)				Total free phenolics (mg/g)			
		Stage				Stage				Stage			
		60	90	120	Mean	60	90	120	Mean	60	90	120	Mean
Rhizobium	(T_1)	0.09	0.69	2.93	1.24	17.85	30.71	54.68	34.41	0.12	0.13	1.14	0.46
Phosphobacteria	(T_2)	0.47	1.80	2.88	1.71	16.10	31.32	62.13	36.52	0.10	0.14	1.34	0.53
VA mycorrhiza	(T_3)	0.31	1.02	1.34	0.89	15.50	32.87	53.35	33.91	0.08	0.15	1.25	0.49
Rhizobium + phosphobacteria	(T_4)	0.64	0.97	1.36	0.99	19.61	32.89	55.90	36.13	0.15	0.15	1.22	0.50
Rhizobium + VAM	(T_5)	0.51	1.26	1.76	1.18	19.31	31.12	47.42	32.61	0.12	0.16	1.14	0.47
Phosphobacteria + VAM	(T_6)	0.48	1.89	1.92	1.43	12.40	31.38	56.76	33.51	0.13	0.14	1.33	0.53
Rhizobium + phosphobacteria + VAM	(T_7)	0.57	1.14	1.55	1.08	10.37	31.05	52.36	31.26	0.10	0.15	1.26	0.50
Uninoculated control	(T_8)	0.05	0.09	1.08	0.40	9.30	25.79	40.16	25.08	0.07	0.10	0.94	0.37
Stage mean		0.39	1.11	1.85		15.05	30.89	52.84		0.11	0.14	1.20	
		T	S	T x S		T	S	T x S		T	S	T x S	
Sed		0.013	0.008	0.022		0.018	0.011	0.031		0.008	0.004	0.014	
CD (P=0.05)		0.026	0.016	0.045		0.036	0.022	0.062		0.016	0.009	0.028	

Table 5. Effect of biofertilizer treatment on total free amino acids, total proteins and total free phenolics of *Acacia nilotica*

Biofertilizer		Total free amino acids (mg/g)				Total proteins (mg/g)				Total free phenolics (mg/g)			
		Stage				Stage				Stage			
		60	90	120	Mean	60	90	120	Mean	60	90	120	Mean
Rhizobium	(T_1)	0.09	0.69	2.93	1.24	17.85	30.71	54.68	34.41	0.12	0.13	1.14	0.46
Phosphobacteria	(T_2)	0.47	1.80	2.88	1.71	16.10	31.32	62.13	36.52	0.10	0.14	1.34	0.53
VA mycorrhiza	(T_3)	0.31	1.02	1.34	0.89	15.50	32.87	53.35	33.91	0.08	0.15	1.25	0.49
Rhizobium + phosphobacteria	(T_4)	0.64	0.97	1.36	0.99	19.61	32.89	55.90	36.13	0.15	0.15	1.22	0.50
Rhizobium + VAM	(T_5)	0.51	1.26	1.76	1.18	19.31	31.12	47.42	32.61	0.12	0.16	1.14	0.47
Phosphobacteria + VAM	(T_6)	0.48	1.89	1.92	1.43	12.40	31.38	56.76	33.51	0.13	0.14	1.33	0.53
Rhizobium + phosphobacteria + VAM	(T_7)	0.57	1.14	1.55	1.08	10.37	31.05	52.36	31.26	0.10	0.15	1.26	0.50
Uninoculated control	(T_8)	0.05	0.09	1.08	0.40	9.30	25.79	40.16	25.08	0.07	0.10	0.94	0.37
Stage mean		0.39	1.11	1.85		15.05	30.89	52.84		0.11	0.14	1.20	
		T	S	T x S		T	S	T x S		T	S	T x S	
Sed		0.013	0.008	0.022		0.018	0.011	0.031		0.008	0.004	0.014	
CD (P=0.05)		0.026	0.016	0.045		0.036	0.022	0.062		0.016	0.009	0.028	

Table 6. Effect of biofertilizer treatment on nitrogen, phosphorus and potassium of *Acacia nilotica*

Biofertilizer		Nitrogen (%)				Phosphorus (%)				Potassium (%)			
		Stage				Stage				Stage			
		60	90	120	Mean	60	90	120	Mean	60	90	120	Mean
Rhizobium	(T_1)	1.45	2.16	2.28	1.96	0.26	0.27	0.27	0.27	0.70	0.87	1.44	1.00
Phosphobacteria	(T_2)	0.85	2.32	2.38	1.85	0.26	0.28	0.31	0.28	1.15	1.20	1.57	1.30
VA mycorrhiza	(T_3)	0.67	1.82	2.42	1.64	0.27	0.29	0.32	0.29	0.78	0.92	1.48	1.06
Rhizobium + phosphobacteria	(T_4)	0.91	1.72	2.48	1.70	0.20	0.27	0.28	0.25	0.47	1.08	1.49	1.01
Rhizobium + VAM	(T_5)	1.05	1.45	2.32	1.61	0.21	0.26	0.27	0.25	0.77	0.78	1.40	0.98
Phosphobacteria + VAM	(T_6)	1.33	1.90	2.36	1.86	0.24	0.28	0.28	0.27	0.54	0.63	1.50	0.89
Rhizobium + phosphobacteria + VAM	(T_7)	1.27	2.33	2.49	2.03	0.23	0.27	0.29	0.26	0.84	1.31	1.53	1.23
Uninoculated control	(T_8)	0.62	1.41	2.06	1.36	0.15	0.24	0.24	0.21	0.35	0.49	1.23	0.69
Stage mean		1.02	1.89	2.35		0.23	0.27	0.28		0.70	0.91	1.46	

	T	S	T x S	T	S	T x S	T	S	T x S
Sed	0.013	0.008	0.023	0.002	0.001	0.003	0.008	0.005	0.014
CD (P=0.05)	0.026	0.016	0.045	0.004	0.002	0.007	0.016	0.010	0.028

Table 7. Effect of biofertilizer treatment on calcium and magnesium of *Acacia nilotica*

Biofertilizer		Calcium (%)			Mean	Magnesium (%)			Mean
		Stage				Stage			
		60	90	120		60	90	120	
Rhizobium	(T_1)	0.66	0:84	1.26	0.92	0.10	0.34	1.16	0.53
Phosphobacteria	(T_2)	0.57	0.67	0.94	0.73	0.09	0.40	0.63	0.37
VA mycorrhiza	(T_3)	0.44	0.71	1.34	0.83	0.11	0.25	0.78	0.38
Rhizobium + phosphobacteria	(T_4)	0.70	0.85	1.29	0.94	0.11	0.17	0.94	0.41
Rhizobium + VAM	(T_5)	0.66	0.93	1.62	1.07	0.12	0.24	1.04	0.46
Phosphobacteria + VAM	(T_6)	0.44	0.51	1.49	0.81	0.10	0.17	1.12	0.46
Rhizobium + phosphobacteria + VAM	(T_7)	0.52	0.53	1.11	0.72	0.07	0.26	0.89	0.41
Uninoculated control	(T_8)	0.29	0.42	0.90	0.53	0.04	0.14	0.44	0.21
Stage mean		0.53	0.68	1.24		0.09	0.25	0.87	
		T	S	T x S		T	S	T x S	
Sed		0.008	0.005	0.014		0.011	0.022	0.029	
CD (P=0.05)		0.016	0.010	0.028		0.022	0.013	0.038	

Results and Discussion

In *Acacia nilotica* the highest seed germination percentage (96%) is induced by *Rhizobium* and phosphobacteria treatments individually. The relative enhancement of seed germination might be attributed to the role of phosphorus solubilizing bacteria known as phosphobacteria in enhancing the availability of phosphorus in the soil (Cooper, 1979) and making it available to the germinating seed with consequent enhancement in the metabolic activity resulting in higher germination.

In *Acacia nilotica* seedlings, longer roots are produced by phosphobacteria and longer shoots are produced by *Rhizobium* + VAM. The possible mechanisum by which P_ solubilizing microorganisms influence P availability to plants include solubilize P from insoluble form by the production of organic acids and these organic acids may chelate P and make it more available to plants and by producing cytokinins which stimulate root growth that inturn may permit P absorption from large volume of soil (Barea *et al.*, 1976).

Increase in shoot height in seedlings of *Artocarpus integrifolia* inoculated with VAM is reported recently (Srivastava *et al.*, 2001). It is suggested that VAM can improve plant growth through increased uptake of P especially in low fertile soils (Gerdemann, 1975). The increase in length of shoot and root due to combined inoculation of biofertilizers is documented in several shola species (Anon, 1978; Rangarajan and Narayanan, 1990; Sekar *et al.*, 1995). The increase in plant growth attributes might be due to increased uptake of nutrients in mycorrhizal associated plants and its synergistic effect with other inoculum (Srinivas, 1987). Phosphobacteria + VAM inoculation have shown the heighest fresh and dry weights in the seedlings studied. The work of Gurumurthy *et al.*, supports this view. Increase in biomass production in seedling may be strongly correlated with accumulation of P due to mycorrhizal fungi and phosphobacteria inoculation (Sanders *et al.*, 1975). Total chlorophyll content was very high in the seedlings inoculated with VAM in *Acacia nilotica*.

In our study, the content of total soluble carbohydrates was maximum in *Rhizobium* + phosphobacteria and the maximum contents of reducing sugars, total free amino acids, total proteins and total free phenolics were noticed in phosphobacteria inoculation. The enhanced biochemical attributes due to single and dual inoculations were supported by the work of several investigators (McArthur and Kowlis, 1993; Sivaprasad and Rai, 1988) in several forest tree species. Higher N content due to triple inoculation (*Rhizobium* + phosphobacteria + VAM), P & K content due to single inoculation (VAM and phosphobacteria respectively), Ca uptake due to dual inoculation (*Rhizobium* + VAM) and Mg content due to single inoculation (*Rhizobium*) were noticed in our present study.

Various reasons are propounded to explain the increased uptake of N in VAmycorrhizal plants viz., the increased uptake of N due to N fixation which is induced secondarily by increased P uptake rather than to direct uptake of N compounds from the soil (Punj and Gupta, 1987) and the increased N uptake also might be due to the reduction of nitrate to nitrite by VAM fungi (Theobold and Smith, 1974). The increased N uptake in VA mycorrhizal plants might also be due to the increased P uptake which in turn might enhance the activity of NAD dependent enzyme, which might contribute to nitrate reductase activity (Trappe and Fogel, 1977).

Punj and Gupta (1987) confirm the role of mycorrhiza in supplying P to the host tree. Sanders *et al* (1975) observe that in mycorrhizal plants, incrased P uptake is due to existence and continued growth of extramatrical mycelium into soil extending beyond the phosphate depletion zone around the root and exploiting a greater and less depleted zone. Increased K uptake due to phosphobacteria inoculation in neem is reported by Kalavathy *et al.* (2000). The enhancement in levels of micronutrients due to biofertilizer inoculation in this study might be due to symbiotic effect of biofertilizers with host tree seedlings, which effects an improvement in micronutrient uptake and mineral status (Buwalda *et al.*, 1983).

The results of the present study reveal that almost all the employed biofertilizers (singly and in combination) enhance seed germination, seedling growth and most of the biochemical parameters. Among the biofertilizers, phosphobacteria inoculation is found to be more effective in general and VAM inoculation is found to be effective in chlorophyll pigments accumulation. From this study, it is concluded that biofertilizer inoculations will improve the plant growth, biomass and biochemical constituents of the seedlings and thereby help to produce elite seedlings and improve the survival rate of planted seedlings. Production of elite seedlings is a pre requisite for the successful implementation of extensive planting programmes such as afforestation, social forestry and agroforestry.

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