EFFECTS OF BIOFERTILISERS ON SEED GERMINATION, AND SEEDLING GROWTH AND CHEMICAL CONSTITUENTS IN NEEM (*AZADIRACHTA INDICA*)

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There is a growing need for utilisation of waste lands in agriculture and forestry in India. Many degraded lands and mined soils are brought to productivity, for example by using biofertilisers and organic manures. In recent years, not only in India but also in several other developing countries, biofertilisers have become an effective supplement to conventional chemical fertilisers. There is also a widening gap between the production and demand of chemical fertilisers in India and biofertilisers are able to reduce this gap and help produce high returns even from marginal and low productivity lands (Chakrabarthy 1990).

Neem (*Azadirachta indica*) belongs to the family Meliaceae. It is a tree of global importance not only due to its beautiful foliage but also for its importance in environmental conservation, now commonly introduced in agroforestry and afforestation programmes. However, there are problems in germinating neem seeds. Only fresh seeds germinate and the seeds lose viability after three months. A study was conducted to determine the effect of biofertilisers on seed germination, seedling growth and biochemical changes in neem.

Seeds collected from a 30-year-old plantation of neem were used in the study. Biofertilisers, namely, *Azospirillum*, phosphobacteria and AM fungi were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. *Azospirillum* and phosphobacteria were loaded on sterilised lignite as carrier while AM fungi were loaded on vermiculite. The concentration for *Azospirillum* was 2×10^8 and for phosphobacteria, 5×10^7 cells g^{-1} of lignite on dry weight basis. The inoculum load of AM fungi was 10^5 spores g^{-1}. The soil used in the pot culture study was a typical red sandy loam with pH 7.13, E.C. 0.26 mM/dsm and organic matter 1.20%. The pots used had 9 kg capacity.

The following were the treatments studied:

T_1 *Azospirillum*, 18 g/pot
T_2 Phosphobacteria, 18 g/pot
T_3 AM fungi, 45 g/pot
T_4 *Azospirillum* + phosphobacteria, 9 + 9 g/pot respectively
T_5 *Azospirillum* + AM fungi, 9 + 22.5 g/pot respectively
T_6 Phosphobacteria + AM fungi, 9 + 22.5 g/pot respectively
T_7 *Azospirillum* + phosphobacteria + AM fungi, 6 + 6 + 15 g/pot respectively
T_8 Uninoculated as control

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The biofertilisers were mixed in the soil at a rate of 2 g kg^{-1} of soil and AM fungi at 5 g kg^{-1} of soil. These are as per the recommendations of Tamil Nadu Agricultural University, Coimbatore.

A total of 50 seeds were sown in each pot. All pots received proper management practices and per cent seed germination was recorded a month after sowing while other growth parameters of the seedlings were recorded 120 days after sowing.

Chlorophyll content, carbohydrate level, reducing sugars, proteins, amino acids and plant phenolics were also determined 120 days after sowing. Leaf samples were estimated for chlorophyll a and b following the method of Yoshida *et al.* (1971). Total soluble carbohydrate were estimated by the anthrone reagent method (Hedge & Hofreiter 1962). The amount of total reducing sugar was estimated following the method of Somogyi (1952) while total free amino acids were estimated using the method of Moore and Stein (1948). The estimation of buffer-soluble proteins was carried out following the method of Lowry *et al.* (1951) and total phenolics were estimated by the method of Sadasivam and Manickam (1992). Data recorded were subjected to analysis of variance using completely randomised design (Panse & Sukhatme 1967).

The study revealed that seed inoculation with biofertilisers and AM fungi recorded 97% germination (Table 1). This observation is in agreement with the earlier investigations of Ponnuswamy (1993) and Vanangamudi *et al.* (1993) who reported that seed pelleting with VA mycorrhiza or phosphobacteria or *Azospirillum* significantly enhanced the germination of neem seeds. *Azospirillum*, phosphobacteria and AM fungi individually and collectively enhanced the percentage of germination by making available the nutrients, namely, phosphorus and nitrogen to help improve germination (Cooper 1979).

Inoculation with biofertilisers, namely, *Azospirillum*, phosphobacteria and AM fungi enhanced root and shoot lengths significantly compared with the rest of the treatments (Table 1). Dela Cruz *et al.* (1988) also recorded similar observations with biofertilisers. The increase in seedling biomass may be attributed to improved accumulation of nitrogen due to *Azospirillum* and of phosphorus due to AM fungi and phosphobacteria inoculation (Sanders *et al.* 1975).

Significant differences were observed in the concentrations of chlorophyll a and b due to inoculation with biofertilisers (Table 2). This may be attributed to more chlorophyll synthesis following inoculation with biofertilisers (Narayanan *et al.* 1990).

Table 1 Effects of biofertilisers on seed germination, shoot and root length and fresh and dry weight of neem seedlings 120 days after sowing

Treatment	Germination after 30 days (%)	Shoot length (cm)	Root length (cm)	Fresh weight/ seedling (g)	Dry weight/ seedling (g)
T_1	91	12.23	11.69	1.43	0.66
T_2	94	11.89	11.18	1.54	0.67
T_3	97	13.12	13.01	1.88	0.86
T_4	77	12.78	12.42	1.86	0.74
T_5	93	15.08	15.79	2.13	0.96
T_6	75	13.48	15.32	1.85	0.83
T_7	94	15.47	16.48	2.36	1.09
T_8	81	10.52	7.21	1.11	0.42
SED	0.8	0.05	0.08	0.05	0.02
CD (p = 0.05)	1.6**	0.10**	0.17**	1.10**	0.03**

SED = Standard error deviation, CD = critical difference
** Significant at $p < 0.01$

Table 2 Effects of biofertilisers on chlorophyll a and b, total soluble carbohydrates, reducing sugars, buffer-soluble protein, amino acid and phenolic of neem seedlings 120 days after sowing*

Treatment	Chlorophyll a	Chlorophyll b	Total soluble carbohydrate	Reducing sugar	Buffer-soluble protein	Amino acid	Phenolic
T_1	1.83	0.99	3.62	3.69	37.21	5.54	5.11
T_2	1.86	1.00	4.33	5.54	40.54	3.87	5.68
T_3	2.39	1.14	3.51	5.65	38.50	5.97	5.26
T_4	2.13	1.26	3.21	4.90	40.13	5.24	4.30
T_5	2.54	1.16	4.68	4.57	53.88	4.85	6.05
T_6	2.00	0.94	3.77	3.40	51.42	5.63	5.08
T_7	2.57	0.85	4.67	7.05	60.83	5.09	5.05
T_8	1.61	0.99	2.78	2.88	33.54	3.23	3.68
SED	0.027	0.02	0.09	0.09	0.53	0.07	0.06
CD (p = 0.05)	0.054**	0.04**	0.18**	0.18**	1.05**	0.15**	0.12**

Data represent mean of three determinations
* Expressed in mg per g of tissue on fresh weight basis
** Highly significant ($p < 0.01$)
SED = Standard error deviation, CD = critical difference

Besides total chlorophyll, this study revealed clearly that a combined inoculation of *Azospirillum*, phosphobacteria and AM fungi significantly enhanced carbohydrates, reducing sugars, buffer-soluble proteins, amino acids and phenolics. Similar observations have been made by Niranjan *et al.* (1990) and Kanakadurga and Ramarao (1995).

From the foregoing study it is clear that the use of biofertilisers, namely, *Azospirillum*, phosphobacteria and AM fungi greatly improved seed germination and establishment and growth of neem seedlings.

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