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RHIZOBIUM INOCULATION OF *ACACIA CATECHU* AND *ACACIA MOLLISSIMA*: EFFECT ON SEEDLING GROWTH AND BIOMASS PRODUCTION

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Planting of nitrogen-fixing trees to maintain soil productivity and to achieve the goal of sustainability is considered an essential aspect of afforestration programmes. In the recent past considerable attention has been given to symbiotic nitrogen fixation in forest trees. In developing countries, particularly India, the use of chemical fertilisers in forestry is not possible because of their large demand in agriculture and their high prices. The total chemical fertiliser production in India is 13.14 million tonnes and the consumption in the agriculture sector is 16.91 million tonnes (Anonymous 1990). To overcome this lacuna, the use of rhizobia has been recommended to increase the productivity of soils and plants. Kenny (1981) summarised the potential of adverse environmental and health impacts of excessive fertiliser applications. Thus, it is imperative to find a cheap and safe alternative source to maintain fertility of the forest soils.

Acacia catechu (khair) and *A. mollissima* (black wattle) are the most valuable commercial acacia species found in India and are known mainly for their products as well as shade and forage for animals. These two nitrogen-fixing trees are widely found throughout the peninsula ascending up to 90 m and sometimes found as high as 1200 m above mean sea-level (Troup 1921). The ability of these two species to establish symbiotic associations with *Rhizobium* has long been recognised, but little information is available about the effect of rhizobial inoculation on seedling growth and biomass. Such an effect was studied with different *Rhizobium* strains at the nursery stage of growth of those two species.

The experimental area (35° 51'N, 76° 11'E; 1200 m above sea-level) was located at the Dr. Y. S. Parmar University of Horticulture and Forestry (UHF), Solan (H.P.), India. Three rhizobial strains with one mixed strain were selected to inoculate the seeds of *A. catechu* and *A. mollissima*. Rhizobial strains were procured from the Department of Basic Sciences (Microbiology Division), UHF. The strains used were uninoculated (control), Uhf 76, Sln 20, Nhn 63 and mixed strain (Uhf 76 + Sln 20 + Nhn 63).

Broth cultures of these strains of *Rhizobium* were prepared by growing them in yeast extract medium. The separate inoculum of each strain was prepared by mixing broth culture in a charcoal carrier. The bacteria were multiplied in yeast extract mannitol broth (Dalton 1980). The broth culture was inoculated at 28 ± 2 °C on an electric shaker (120 rpm) until 10^8 viable rhizobia ml^{-1} count were obtained. The characteristics of nursery soil were: pH (7.23), available N (272.24 kg ha^{-1}) and available P (44.80 kg ha^{-1}). The mixture was fumigated with 8% formalin at 250–300 ml m^{-2} for 7 days.

Seeds were surface sterilised with mercuric chloride (0.02%) for 5 min and sown in polythene bags containing sand, soil and farmyard manure (FYM) in the ratio of 1:2:1 after subjecting them to standard pre-sowing treatments, viz.

A. catechu: soaking in cold water for 24 h,
A. mollissima : boiling water dip for 5 min and then soaking in cold water for 12 h.

The seeds were inoculated as given below (Somasegaran & Haben 1985):

Species	Seed wt. (g)	Charcoal inoculant (g)	Gum arabic solution (ml)
Acacia catechu	50	10.0	3.0
Acacia mollissima	100	9.0	3.5

The seeds were sown in the first week of March 1996 and all the cultural practices were followed. The experiment was laid out in randomised block design with three replications in a glasshouse under natural day length and light intensity (Spring-Summer) and watered regularly. Seedlings were uprooted after 120 days to evaluate the effect of the different strains on the growth and biomass in both the species.

Rhizobium inoculation of *A. catechu* significantly affected the growth and biomass parameters (Table 1). Among the various strains, the mixed strain (Uhf 76 + Sln 20 + Nhn 63) recorded the highest values for seedling height, (30.03 cm), root length (19.31 cm), number of branches (12.06) and nodule number per seedling (13.92), whereas for root : shoot ratio (0.59), the value was significantly higher than the control. Inoculation with the mixed strain resulted in maximum seedling biomass (3.59 g) and nodule biomass per seedling (62.64 mg) in comparison with the control (1.07 g and 0.00 respectively). Seedlings of *A. mollissima* were significantly affected by rhizobial inoculation with respect to growth and biomass production. The strain, Nhn 63, excelled over other strains as well as the control by registering the highest values of seedling height (33.76 cm), collar diameter (2.03 mm) and root length (30.07 cm). The total biomass per seedling (4.03 g) was also maximum with Nhn 63 but the nodule biomass per seedling was at par in all the strains studied.

Thus, the results showed that rhizobial inoculation had a positive symbiotic effect on these species, which was reflected in the enhanced seedling growth and biomass in comparison to uninoculated seedlings. The nitrogen fixation efficiency of rhizobia with host plant species often varies significantly and the most economical way of providing the nitrogen needed for optimum plant growth in legumes is to inoculate the plants with strains of rhizobia (Ham *et al.* 1971). The increase in growth may be attributed to the improved nitrogen status resulting from the symbiotic association between the host species and bacteria. Similar positive correlations between growth and biomass production due to symbiotic associations have been reported by Sharma *et al.* (1994) in *Acacia catechu.* These findings are in agreement with those of Diatloff (1985), Saliana *et al.* (1990) and Barua (1994).

Rhizobial inoculation in leguminous tree species is doubtlessly beneficial in the production of vigorous planting stock. Inoculation with the mixed strain in *A. catechu* and Nhn 63 in *A. mollissima* is recommended.

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Table 1. Effect of *Rhizobium* strains on growth and biomass of *Acacia catechu* and *Acacia mollissima* seedlings

Rhizobium strains	Seedling height (cm)	Collar diameter (mm)	Root length (cm)	No. of branches seedling^{-1}	Nodule number seedling^{-1}	Root: shoot	Biomass (dry weight)	
							Total biomass seedling^{-1} (g)	Nodule biomass seedling^{-1} (mg)
				Acacia catechu				
Control	15.92	1.23	9.89	6.63	0.00	0.20	1.07	0.00
Uhf 76	28.91	1.50	19.00	11.63	12.86	0.44	1.82	57.86
Sln 20	25.52	1.50	18.51	10.58	12.31	0.60	2.01	55.51
Nhn 63	21.33	1.27	13.74	8.56	12.09	0.70	1.76	54.38
Mixed	30.03	1.24	19.31	12.06	13.92	0.59	3.59	62.64
SE_D	1.50	0.08	1.16	0.66	0.12	0.03	0.06	0.54
$CD_{(0.05)}$	2.99	0.17	2.30	1.31	0.24	0.06	0.12	1.04
				Acacia mollissima				
Control	15.51	1.30	12.23	6.11	0.00	0.21	1.34	0.00
Uhf 76	25.55	1.75	22.74	9.57	9.56	0.34	3.08	52.51
Sln 20	27.21	1.74	23.96	8.32	9.35	0.43	2.80	52.38
Nhn 63	33.76	2.03	30.07	8.47	9.31	0.42	4.03	52.14
Mixed	27.62	1.79	25.56	8.16	9.48	0.72	3.06	53.06
SE_D	1.39	0.07	0.98	0.26	0.18	0.05	0.23	1.02
$CD_{(0.05)}$	2.75	0.14	1.95	0.52	0.36	0.09	0.46	2.03

$SE_{(Diff.)}$ = Standard error of difference.
CD = Critical value for comparison.

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