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Effect of bioinoculants on seedling vigour in tobacco (*Nicotiana tabacum*) nurseries

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Leaf being the end-product of tobacco (*Nicotiana tabaccum* L.), field planting of vigorous and healthy seedlings is the main pre-requisite for successful establishment in the field. Nutrition of seedlings plays an important role in improving the vigour of seedlings. In general, use of chemical fertilizers helps in raising healthy seedlings of various agricultural and horticultural crops and forest trees. Behl *et al.* (2007) and Sala *et al.* (2007) reported the use of the nitrogen-fixing bacteria, P-solubilizing bacteria and arbuscular mycorrhizal fungi in raising the seedlings and improving the production of various crops.

The present study was undertaken to investigate the influence of nitrogen-fixing bacterium *Azotobacter chroococcum* Beijerinck, growth-promoting bacterium *Pseudomonas fluorescens* Migula and phosphorus-solubilizing arbuscular mycorrhizal fungi on growth and nutrient uptake of seedlings in tobacco nursery.

The experiment was conducted for 2 seasons (2007 and 2008) in randomized block design with 8 treatments and 3 replications (T_1-control; T_2-VAM; T_3-*P. fluorescens;* T_4-*A. chroococcum;* T_5-VAM + *P. fluorescens*; T_6-VAM + *A. chroococcum;* T_7-*P. fluorescens* + *A. chroococcum;* T_8-VAM + *P. fluorescens* + *A. chroococcum)*. Tobacco seeds were sown on 1 m^2 seedbeds. Selected cultures of *A. chrococcum* was grown on Jensen's N_2- free medium for 3 days. The cells were centrifuged, washed thrice in sterile distilled water and suspended in 0.15 m phosphate buffer at *p*H 7.0. The cell suspension was having 10^9 cells/ml and 100 ml of such cell suspension was used to inoculate the m^2 seed-bed at the time of sowing. *Glomus intraradicus* was multiplied on maize plant roots under sterile conditions. Around 10 g soil including root bits containing 10–20 viable arbuscular micorrhizal fungi propagules g soil were used as inoculum and spread as a thin layer 1 cm below soil surface on each seed-bed. Three replications of each treatment were grown for a period of 60 days with everyday watering up to field capacity. King's B broth was prepared without addition of agar and *P. fluorescens* was inoculated aseptically to the broth Erlenmeyer flasks and allowed to multiply in a rotary shaker for 48 hr at room temperature.

The cultures were centrifuged at 600 rpm for 10 min and bacterial cells were resuspended in phosphate buffer and concentration adjusted to 10×10^{10} cfu/ml and used as bacterial inoculum. The inoculum was sprayed on tobacco seed-beds. Untreated seed-beds served as control. The seedlings were harvested for determining leaf area and total chlorophyll content (Hiscox *and* Israelstam 1979). Seedlings were dried at 70°C and made into fine powder after recording dry weight. This was used for analyzing total nitrogen by Microjeldhal's method, phosphorus by vanado molybdo phosphoric yellow colour method. Potassium by flame photometry and copper, iron, manganese and zinc contents in the plant samples were determined by employing atomic absorption spectrophotometer. The data were subjected to analysis of variance and means of inoculated and control treatments compared by the Scheffe's test for comparison. Similar trend was observed in both the seasons 2007 and 2008 with respect to the total number of transplantable seedlings and all the other parameters. Hence pooled data are presented in the Tables 1, 2.

Significant increase in the number of healthy transplantable seedlings was observed upon inoculation of tobacco nursery seed-beds with various beneficial microorganisms (Table 1). Higher seed germination was observed with AM fungi compared to that of nitrogen-fixing bacteria. Durgannavar *et al*. (2004) reported an improved seed germination in different crops upon inoculation with different nitrogen-fixing bacteria and AM fungi. The present study revealed that combined application of consortia of all the 3 microorganisms (*Glomus intraradices*, Schenck & Sm., *Pseudomonas fluorescens*, *Azotobacter chroococcum*) significantly improved germination percentage (Table 1) compared to the individual application or combination of 2 bioinoculants. Application of nitrogen-fixing bacteria and AM fungi and *P. fluorescens* significantly improved plant

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Table 1 Effect of biofertilizers on chlorophyll content and growth of FCV tobacco seedlings

Treatment	Seedling height (cm)	Germination count (%)	Shoot weight (g/seedling)		Leaf area (cm^2/plant)	Total chlorophyll (mg/g fresh weight)	Healthy trans-plantable seedlings/ m^2 bed
			Fresh	Dry			
Control	9.47	36.36	17.00	2.33	162.25	0.73	340
VAM	10.67	46.45	31.20	4.16	260.91	0.89	984
Pseudomonas fluorescens (*Pf*)	10.80	47.27	29.33	4.00	195.08	0.97	954
Azotobacter	9.20	45.95	28.83	3.66	328.99	0.89	826
VAM + *Pf*	10.83	60.24	29.83	4.83	331.99	1.09	1152
VAM + *Azotobacter*	10.33	66.60	34.50	4.83	375.56	1.16	1112
Pf + *Azotobacter*	11.03	65.90	37.83	6.33	292.21	1.20	1350
VAM + *Pf* + *Azotobacter*	12.23	83.09	47.33	6.83	610.32	1.35	1566
SEm±	0.49	0.61	1.12	0.27	55.33	0.06	103.43
CD (*P*=0.05)	1.48	1.86	3.42	0.84	167.84	0.18	313.76
CV (%)	8.04	8.55	6.11	10.38	30.22	10.21	43.54

Table 2 Nutrient concentration in FCV tobacco seedlings as influenced by biofertilisers

Treatment	N (%)	P (%)	K (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
Control	0.38	0.19	8.33	59.61	161.87	582.96	29.51
VAM	0.54	0.33	9.50	65.34	227.57	851.51	39.81
Pseudomonas flourescens (*Pf*)	0.54	0.28	9.73	66.27	232.73	951.67	42.32
Azotobacter	0.64	0.23	9.40	59.96	189.93	667.49	43.95
VAM + Pf	*0.58*	0.28	10.66	72.73	255.51	903.12	45.78
VAM + *Azotobacter*	0.55	0.29	10.20	70.64	248.82	918.65	47.84
Pf + *Azotobacter*	0.58	0.30	11.66	74.76	240.01	1031.88	48.49
VAM + *Pf* + *Azotobacter*	0.60	0.32	12.36	74.95	269.31	1107.88	50.02
SEm±	0.33	0.01	0.24	0.93	8.60	31.39	1.18
CD (*P*=0.05)	0.10	0.03	0.73	2.82	26.09	95.23	3.59
CV (%)	10.6	8.05	4.11	2.37	6.53	6.20	4.71

height, leaf area and shoot weight in 60 days old tobacco seedlings with maximum enhancement in all the parameters under dual and triple inoculation (Table 1). Similar observations were recorded with gooseberry (*Emblica officinalis* Gaertn.) by Aseri and Rao (2004) upon inoculation with AM fungi, *Azotobacter* and *P. fluorescens*. The enhancement in plant growth and biomass production might be due to increased rate of photosynthesis and higher metabolic activities, mobilization of some movable forms of important nutrients especially phosphorus, production of growth regulators and inhibition of soil-borne pathogens by *P. fluorescens*.

There was significant increase in total chlorophyll content and leaf area in tobacco nursery seedlings upon inoculation with all the 3 beneficial micro organisms. Application of VAM or *Azotobacter* or *P. fluorescens* separately did not significantly increase chlorophyll content. These observations are in conformity with those of Baqual *et al.* (2005) who reported higher chlorophyll content and photosynthesis in banana upon inoculation with nitrogen-fixing bacteria. Leaf area could not significantly increase due to the addition of VAM or *P. fluorescens* or *Azotobacter* individually (Table 1).

Nitrogen, phosphorus and potassium contents were significantly improved in tobacco seedlings upon inoculation with consortia of micro-organisms. Higher amount recorded was due to the synergistic effect leading to improved biomass and higher nutrient concentration (Table 2) in the inoculated plants. Nitrogen content was maximum in plants inoculated with nitrogen-fixing bacterium, while phosphorus content was more with AM fungus and multiple inoculation significantly improved NPK content of the plant (Table 2). The results are in conformity with those of Douds (2007) who had observed higher uptake of phosphorus in different fruit and forest crops upon inoculation with mycorrhizae. Inoculated seedlings are found to contain significantly higher amounts of various micronutrients, viz Cu, Fe, Zn and Mn, compared to that of uninoculated seedlings (Table 2). The results are in conformity with the observations of Cavagnaro (2008) that indicated an enhancement in the uptake of various micronutrients inoculated with AM fungi and nitrogen-fixing bacteria in apple and peach plants. The enhancement in growth might be due to the production of siderophores by *P. fluorescens* and its activity in biological control of soil-borne

pathogens (Haas and Defago 2005), ability of AM fungi for the uptake of immobile ions and enhanced absorbing surface area of the roots (Hamel and Strullu 2006).

From the results, it is suggested that the consortia of nitrogen-fixing bacteria *(A. chroococcum)*, AM fungi (*Glomus intraradicus*) and *P. fluorescens could* be successfully used in tobacco nurseries to produce healthy, vigorous and more number of transplantable tobacco seedlings.

SUMMARY

Tobacco *(Nicotiana tobacum* L.) seedbeds were raised at CTRI nursery site during 2007–08, to study the effect of nitrogen-fixing bacteria (*Azotobacter chroococcum*), phosphorus-solubilizing AM fungi (*Glomus intraradicus*) and growth-promoting bacteria, *Pseudomonas fluorescens* on growth and nutrient content of seedlings in tobacco nursery. The combined inoculation of *Azotobacter*, VAM and *P. fluorescens* resulted in an increase of plant biomass, dry matter, weight of seedlings, number of healthy transplantable seedlings, leaf area and chlorophyll content. The treatments with dual and triple inoculation had maximum content of NPK compared to that of single inoculation. A significant increase in the micronutrients content was observed with triple and dual inoculation as compared to uninoculated control and single inoculation.

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