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Influence of *Azotobacter chroococcum* Strains on Growth and Biomass of *Adathoda vasica* Nees.

Azotobacter chroococcum is an aerobic, free-living nitrogen fixing bacterium capable of synthesizing various plant growth promoting substances, which may augment its performance as an efficient inoculant for crop plants. Several studies have revealed the beneficial effects of these bacteria in the improvement of crop growth and yield. Abraham and Money (l994) reported that the strain TG_1 of *Azotobacter chroococcum* isolated from the soils of Laccadive Islands increased yields in crops like rice, tuber, tapioca and pepper and vegetative growth in *Acacia mangium,* a fast growing timber yielding legume tree. Sena and Das (1998) reported increased protein and curcumin content of Turmeric when inoculated both *Azotobacter* and *Azospirillum.* Paul *et al.* (2002) studied the effects of heterologus and homologus strains of *A. chroococcum* in improving yield of cotton. The heterologus strains showed increased cotton boll weight and total seed cotton yield compared to homologus strains. Sreeramulu and Srikantaiah (2003) studied the effect of N_2 fixers and P solubilizer on growth and yield of popularly grown banana varieties (Yalakki and Robusta) in both individual and in combination under field conditions. Among nitrogen fixers tested, *Azotobacter chroococcum* proved better in improving the yield compared to *Acetobacter diazotrophicus. Azotobacter chroococcum* inoculum prepared in silkworm excreta as carrier increased growth and yield of Okra (Jagadish, 2004). Vinutha (2005) reported increased growth, biomass, nitrogen, phosphorus, crude protein, soluble protein and phenol content in *Ocimum sanctum* and *Ocimum kilimandscharicum* inoculated with *Glomas fasiculatum, Azotobacter chroococcum* and *Aspergillus awamori* singly and in combinations. In this study we report the influence of *Azotobacter chroococcum* strains on growth and biomass of *Adathoda vasica* Nees. It is an important medicinal plant belongs to the family *Acanthaceae.* Leaves of the plant contain alkaloids effective against branchial asthama.

Azotobacter chroococcum strains were isolated and characterized from ten agroclimatic zones of Karnataka (Ananth Naik, 2006). These strains along with the reference strain maintained in the Department of Biotechnology, University of Agricultural Sciences, Bangalore-560065 were separately multiplied in Waksman No.77 N-free broth on a rotary shaker at room temperature for five days to reach maximum population. Thus grown cultures were mixed into sterilized lignite powder till moisture of the carrier reached to field capacity. The population of *Azotobacter chroococcum* stains in the carrier material was determined by serial dilution plate method (Table l). Then these carrier-based inoculants were used for inoculation in the pot experiment. A pot experiment was conducted in glasshouse for 90 days from April-June, 2005. The pots measuring 5 kg capacity were filled with red sandy loam soil having pH 6.5 up to 3/4th of the volume. The pots were watered one day prior to planting. A planting hole was made at the center to enable to place *Azotobacter* inoculum. Ten gram carrier based inoculum representing each zone *Azotobacter chroococcum* isolate was separately added to the pot as per the treatment allocation given in the table 2. Then, the rooted cuttings of *Adathoda vasica* plants having uniform height were planted in the pots. One plant per pot was maintained and there were three replications per each treatment. These pots were watered as and when required until harvest.

Table 1. Population of ten isolates of *A. chroococcum* used in pot experiment

A. chroococcum isolates	*Population in the carrier based inoculant (cfu/g)
Zone 1 isolate	3.0×10^7
Zone 2 isolate	2.2×10^8
Zone 3 isolate	4.1×10^8
Zone 4 isolate	5.0×10^7
Zone 5 isolate	6.3×10^8
Zone 6 isolate	5.4×10^7
Zone 7 isolate	3.5×10^7
Zone 8 isolate	4.5×10^8
Zone 9 isolate	8.4×10^7
Zone 10 isolate	4.1×10^7
Reference strain	7.4×10^8

*cfu : Colony forming units

The observations for growth parameters like plant height, number of leaves and number of branches were recorded at 30,60, 90days intervals. The plants were harvested at 90 days and the plant biomass was recorded after drying the harvested plants at 60°C in an hot air oven for 7 days to reach constant weight. The total nitrogen in plants was determined by Microkjeldal digestion and distillation method as described by Jackson (1973). The data obtained in the pot experiments were subjected to one-way analysis of variance using MSTAT-C software.

The plant height was significantly increased in the inoculated treatments at 30, 60 and 90 days after planting, compared to control (Table 2). At 30th day after planting, the zone-8 isolate recorded maximwn plant height, which is followed by the reference strain and the zone 5 isolate. The others were found similar to un-inoculated plants. Further, at 60th and 90th days after planting maximwn height (20.66 cm and 33.00 cm, respectively) was observed in the plants inoculated with zone 8 isolate. This was followed by the plants inoculated with the reference strain (20.00 cm and 26.00cm respectively). This indicated that the zone- isolate is more efficient compared to others including the reference strain. The increased growth might be attributed to the nitrogen fixation and production of growth hormones by *A. chroococcum.* Awasthi *et al.* (1996) observed increased growth of peach seedlings when inoculated with *Azotobacter* and *Glomus fasciculatum.* The enhanced growth was attributed to continuous production of growth substances

Table 2. Effect of inoculation of *A. chroococcum* isolates on growth of *Adathoda vasica* at 30, 60 and 90 days after planting

Treatments	Plant height (cm/plant)			Number of leaves/plant			Number of branches / plant		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
Control	8.00	12.00	14.33	3.00	4.66	7.00	0.33	1.66	3.00
Reference strain	12.66	20.00	26.00	6.00	11.33	15.00	2.00	3.66	4.66
Zone 1 isolate	8.33	17.66	24.66	4.33	6.66	12.00	1.33	2.33	3.66
Zone 2 isolate	10.00	17.33	23.33	7.66	12.00	13.00	1.33	3.00	4.00
Zone 3 isolate	10.00	18.00	24.66	5.00	11.00	10.66	0.66	2.33	3.66
Zone 4 isolate	8.33	14.00	20.00	4.00	8.00	11.66	0.66	2.33	3.33
Zone 5 isolate	11.00	18.33	24.33	4.00	8.33	12.00	1.66	3.00	4.00
Zone 6 isolate	9.00	14.33	19.33	4.66	9.33	12.33	1.33	2.33	3.66
Zone 7 isolate	10.60	15.66	20.33	4.66	9.33	13.00	1.33	2.33	3.33
Zone 8 isolate	11.66	20.66	33.00	6.33	12.33	17.66	2.33	4.00	5.33
Zone 9 isolate	8.66	14.33	19.66	5.33	11.33	12.33	1.33	2.33	3.33
Zone 10 isolate	9.33	16.00	21.66	5.00	10.00	13.66	2.00	3.33	4.33
S.Em±	0.95	1.40	1.72	1.73	1.55	1.92	0.35	0.40	0.30
CD at 5%	2.00	4.11	5.06	2.60	4.56	5.63	1.03	1.17	0.83

DAP - Days after planting

by *Azotobacter* spp. and its interaction with G. *fasciculatum* for better root colonization, which increased the ability of absorption of nutrients by the plants.

In all the inoculated treatments number of leaves per plant was increased signifIcantly compared to un-inoculated plants at all the growth intervals (Table 2). The average nwnber of leaves/plant was signifIcantly higher (7.66) in the plants inoculated with Zone 2 isolate at 30 DAP, which was followed by the isolate of zone 8 (6.33) and the reference strain (6.00) respectively. On 60th DAP, the plants inoculated with zone 8 isolate (12.33) and zone 2 isolate (12.00) recorded signifIcantly greater nwnber ofleaves, which were followed by the reference strain and isolates of zone 9, 3,10,6,7 respectively. The other isolates from zone 1,4 and 5, though showed increased number of leaves, did not differ significantly. Further, the isolate from the zone 8 recorded significantly higher number of leaves (17.66) at the time of harvest (90th DAP). The least number of leaves was observed in the control plants (7.0). The other isolates though, showed increased number of leaves did not differ significantly from the control. Das *et al.* (1990) evaluated the mulberry yield after inoculation with *Azotobacter* and *Azospirillum* biofertilizers. *Azotobacter* inoculated plants had greater number of leaves, leaf area, plant height and leaf nitrogen content compared to *Azospirillum* inoculated plants.

The higher number of branches were noticed in the plants inoculated with *A. chroococcum* isolates compared to uninoculated control at all the growth intervals (30,60 and 90 days after planting). Maximum number of branches were recorded (2.33, 4.0 and 5.33) in the zone 8 *A. chroococcum* isolate treated plants at 30th, 60th and 90th DAP and it is significantly superior to all other isolates including reference strain. The least number of branches were observed in the uninoculated plants. Greater number of branches was reported in *Ocimum sanctum* inoculated with *A. chroococcum* alone as well as its combinataion with *Glomus fasciculatum* and *Aspergillus awamori* (Vinutha, 2005).

The data pertaining to the total dry weight of shoot and root dry weight are presented in Table 3. The isolate from zone 8 enhanced the dry weight of the shoot (10.2 g) significantly, which is comparable to the reference culture inoculated plants (9.98 g). The other isolates except zone I though showed increased dry biomass did not differ significantly compared to control (Table 3). Similarly, the highest root dry weight was also observed in the treatments inoculated with zone 8 isolate and reference culture. The other isolates except zone 2 isolate were found on par with the uninoculated plants. Increased dry weight is due to enhanced growth, number of leaves and branches, which was influenced probably by greater availability of nitrogen in the soil to the plants inoculated with *A. chroococcum.* Sreeramulu and Srikantaiah (2003) reported improved yields of Banana varieties (yalakki and robusta) inoculated with *Azotobacter chroococcum* in southern parts of Karnataka.

Adathoda vasica plants inoculated with different isolates of *A. chroococcum* revealed significantly increased nitrogen content in shoot compared to the control plants (Table 4). The highest nitrogen in shoot (115.15 mg/plant) was observed in the plants treated with zone 8 isolate. The next best were the reference strain, zone 5, 6 and 10 isolates, which contained 112.25 mg, 101.22 mg, 102.14 mg and 105.35 mg, respectively. Similarly, the root nitrogen content was also significantly higher in *A. chroococcum* inoculated plants including reference strain compared to control plants. However, the highest nitrogen content was observed in the plants treated with the zone 8 isolate

Table 3. Effect of inoculation of *A. chroococcum* isolates on dry weight and nitrogen content in shoot and roots of *A. vasica*

Treatments	Dry weight (g/plant)		Nitrogen content (mg/plant)	
	Shoot	Root	Shoot N	Root N
Control	4.02	2.25	62.18	7.28
Reference strain	9.98	5.30	112.25	12.48
Zone 1 isolate	7.21	3.28	75.15	8.02
Zone 2 isolate	6.92	3.25	98.18	9.89
Zone 3 isolate	8.60	3.69	85.07	8.87
Zone 4 isolate	8.20	3.43	95.15	9.63
Zone 5 isolate	8.00	3.32	101.22	10.02
Zone 6 isolate	8.80	4.52	102.14	12.40
Zone 7 isolate	8.10	3.38	97.37	9.87
Zone 8 isolate	10.20	5.54	115.15	13.34
Zone 9 isolate	8.70	3.75	92.54	9.25
Zone 10 isolate	8.17	3.40	105.35	11.35
S.Em±	0.95	0.73	0.711	0.192
CD at 5%	3.30	2.00	2.085	0.564

(13.4 mg), which was followed by the reference strain (12.48). Least N content was observed in uninoculated plants. These results are in agreement with the earlier fmdings of Gopal *et al.* (2000) who reported increased N content in *Azotobacter* inoculated plants. Results of the preset study revealed enhanced growth, biomass and nitrogen content of *Adathoda vasica* due to inoculation with *Azotobacter chroococcum* strains isolated from different agro-climatic zones of Karnataka and the zone 8 isolate as the most efficient compared to others including the reference strain.

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