# **EFFECT OF BIOFERTILIZERS ON** *Jatropha curcas* L. UNDER TROPICAL CONDITIONS

G. RAJA AND B.D. RANJITHA KUMARI

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# SUMMARY

*Jatropha curcas* L. is a biofuel plant which substitutes the fossil fuels. A study was conducted to investigate the effects of Jatropha seeds inoculated with Vesicular arbuscular mycorrhizal (VAM) fungi, *Azospirillum, Azotobacter* and Phosphate solubilizing bacteria (PSB) at various combinations. The biofertilizer treated seeds were tested under field conditions and seedlings were uprooted at 30, 60 and 90 days. Combined microbial inoculations resulted in the significant increase of root and shoot length, shoot and root tolerance index, fresh and dry weight of shoot, root and leaves and leaf area of all treated plants compared to control. After 120 days, chlorophyll contents, total soluble sugars, free amino acids and total protein were analyzed and the results indicated that the plants inoculated with *Azospirillum* + *Azotobacter* + PSB +*VAM fungi* showed the significant increase. Morphological and biochemical contents of Jatropha plants were significantly increased by the effect of combined biofertilizers compared to either individual biofertilizer or control. Biofertilizers accelerated the assimilation of nutrients to the plants.

# Key words : Jatropha curcas L., VAM fungi, Azospirillum, Azotobacter and Phosphate solubilizing bacteria (PSB).

atropha is a shrub or small tree, and it grows up to 6 m U height with spreading branches and stubby twigs (Dehgan, 1984). It belongs to the family Euphorbiaceae and it grows as a tropical thorn and can be grown in areas of low rainfall and problematic soil. Interspecific hybridization has been attempted between different species of Jatropha with a limited success (Dehgan, 1984 Sujatha and Prabakaran, 1997). Possible uses of Jatropha plant parts, such as leaves are used as anti-inflammatory agents and the latex are believed to have anticancerous properties, which contains the alkaloids such as Jatrophine, Jatrophone, Jatropham and Curcain (Duke and Ayensu, 1985). Bark, fruits, leaf, root and wood have also been reported to contain HCN (Watt and Breyer - Brandwijk 1962). Tannins and dyes are obtained from Jatropha bark. Jatropha seeds have been used as economically important products such as biodiesel, illuminators, edible oil, soap production, other cosmetics, medicinal uses, lubricant, biopesticides, animal feed and organic fertilizers. The seeds have been used in oil, press-cake and biogas production and in controlling breeding in guinea pigs (Makonnen et al., 1997; Staubmann et al., 1997). Whole plant is used for erosion control, living hedge, shelter plant for other crops and it is used in rodant repellent and folk medicinal uses, in the treatment of cancer, antiseptic, cough, diarrhoea, dysentery, fever, gonorrhea, inflammation, jaundice, paralysis, pneumonia, stomach ache, tooth ache, syphilis, tumors, ulcers and yellow fever. IcantCorrespondence to :hoot,**B.D. RANJITHA KUMARI,**phyllDepartment of Plant Science,bwedBharathidasan University,triRUCHIRAPPALLI (T.N.)wereINDIAidualAccepted : May, 2008

See end of the article for

authors' affiliations

Inoculation of *Glomus intraradices*, *G. geosporum*, Azospirillum brasilense and Phosphate solubilizing bacteria combination could be used for the production of healthy and vigorously growing seedlings (Muthukumar et al., 2001). Dual inoculation of AM fungi and PSB might be stimulated the plant growth and better than inoculation with individual organism (Kim et al., 1997). Similar effect also reported for AM fungi, Azospirillum inoculations in some plant species (Pacovsky et al., 1985 and Pacovsky, 1989). No report is available on the interaction between Azospirillum, Azotobacter, Phosphate solubilizing bacteria (PSB) and Vesicular Arbuscular Mycorrhizal (VAM) fungi on the growth and development of Jatropha plants. Inoculation with Azospirillum, Azotobacter, Phosphate solubilizing bacteria (PSB) and Vesicular Arbuscular Mycorrhizal (VAM) fungi could enhance the growth of the Jatropha seedlings in nurseries. Hence the present study was undertaken to evaluate the synergistic effects of indigenous VAM fungi, PSB, Azospirillum and Azotobacter on the growth and biochemical changes in Jatropha.

# MATERIALS AND METHODS

Plant material and Bioinoculants :

Jatropha seeds were collected from the Forest College and Research Institute, TNAU, Mettuppalayam, Tamil Nadu, India. Biofertilizers like *Azospirillum*, Phosphate solubilizing bacteria mixed with carrier based material were collected from biofertilizer production unit, Trichy Division, Tiruchirappalli and *Azotobacter*, vesicular arbuscular mycorrizhae (VAM) fungi mixed with carrier based material were purchased from the Stan's biofertilizer company, Coimbatore, respectively.

#### Pre-sowing soaking treatment :

The seeds were soaked in tap water for overnight (10 to 12 h) and seeds were washed with 0.1% HgCl, (10 to 20 min.). Then seeds were washed with 70% ethanol for removing the HgCl<sub>2</sub> from the seeds. Finally seeds were thoroughly washed with distilled water (3 to 5 times). Seeds were mixed with different type of biofertilizers in various combinations (10 to12 h) as listed below-

- Τ, -Control (uninoculation)
- $T_{2}^{1} T_{3}^{1} T_{3$ Azospirillum + VAM
- Phosphate solubilizing bacteria (PSB) + VAM
- Τ<sub>4</sub> -Azotobacter + VAM
- $T_5 -$ Azospirillum + Phosphate solubilizing bacteria (PSB)
- T<sub>6</sub> -Azospirillum + Azotobacter + Phosphate solubilizing bacteria (PSB)
- $T_7 -$ Azospirillum + Azotobacter + Phosphate solubilizing bacteria (PSB) + VAM

Then the seeds were dried under shade place and sowed in the field.

## Experimental Field :

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The experiment was conducted at the Department of Plant Science experimental garden, Bharathidasan University, Tiruchirappalli on dry season between February to May, 2006. This study was carried out in a Randomized Block Design (RBD) with seven treatments with five replicates.

# Analysis of Agro botanical characters and biochemical contents :

After 30, 60 and 90 days, plants were uprooted and washed with running tap water and then washed with distilled water to remove the dust particles from the plants. The plants were blotted with Whatman filter paper No: 47. Agro-botanical characters like shoot and root length, leaf area, shoot and root tolerance index, fresh and dry weight of the plants. The leaf materials were dried at 80°C in a hot air oven for 48 hrs and dry weights were measured. During the experiment, the leaf area was measured for the fourth and fifth leaves from the apex by using leaf area meter (Systronic, India). Shoot and root tolerance index were calculated in between the treated and control plants using the following formulae (Taylor and Foy, 1985) :-

$$RTI = \frac{\text{Underground (root) biomass of the treated plant}}{\text{Underground (root) biomass of the control plant}}$$
  
*Isian J. Envl. Sci., Vol. 3 (1) (June, 2008)*]

$$STI = \frac{Aerial (shoot) biomass of the treated plant}{Aerial (shoot) biomass of the control plant}$$

The biochemical parameters of the leaf samples were analyzed from 120 days old plants. The plant leaves chlorophyll content was estimated using the method of Arnon (1949). The leaves were dried and powdered and which were used to analyze the total soluble sugars (Dubois et al., 1951), free amino acids (Troll and Canon, 1956) and total proteins were estimated by Lowry et al. (1951).

#### Statistical analysis :

The morphological and biochemical parameters of the treated and control plants were analysed by standard error and the Duncan's multiple range test methods at  $P \leq 0.05$  significant level.

# **RESULTS AND DISCUSSION**

### Plant morphology :

The shoot and root length of Jatropha plants increased in all bioinoculants treated plants than control (Table 1). Among the various combinations, Azospirillum + Azotobacter + Phosphate solubilizing bacteria (PSB)  $(T_c)$ and VAM fungi with Azospirillum + Azotobacter + Phosphate solubilizing bacteria (PSB)  $(T_{2})$  which highly increased the shoot and root length of the plants. Increase in plant growth, nodulation and nutrient uptake by combined inoculation of Rhizobium and Phosphate solubilizing bacteria (PSB) on chickpea and some other plants has been reported by Alagawadi and Gaur (1988), Gupta and Namdeo (1997) and Khurana and Sharma (2000). Azospirillum and Azotobacter to change root morphology and plant growth rates has been widely described and commonly related to the production of biologically active substances by these bacteria (Bashan and Levanony, 1990; Becking, 1992).

With the use of biofertilizers, the leaf area of Jatropha plants was increased in all treated plants than control plants. The treatment of Azospirillum + Azotobacter + PSB (T<sub>c</sub>) and Azospirillum + Azotobacter + PSB + VAM $(T_{7})$ , highly significantly increased the leaf area of the treated plants (Table 1). Shoot and root tolerance index of biofertilizer treated plants were increased than uninoculated plants. Among the various combinations  $T_{s}$ ,  $T_6$  and  $T_7$  treatments were significantly increased the shoot and root tolerance index from the other treated and control plants. AM fungi inoculated plantlets had significantly increased the leaf area, leaf dry mass, fruit number, leaf area ratio and decreased the shoot/root ratio than Non AM fungi on ancho pepper plantlets (Estrada-

Treatments	Days	Shoot length (cm)	Root length (cm)	Leaf area (cm <sup>2</sup> )	Shoot tolerance index	Root tolerance index
	30	17.68±0.58 <sup>cd</sup>	8.1±1.2 <sup>cd</sup>	$24.77 \pm 1.59^{f}$	1 <sup>d</sup>	1 <sup>e</sup>
$T_1(con)$	60	$15.88 \pm 1.09^{d}$	$13.27 \pm 0.58^{d}$	33.75±3.65 <sup>ef</sup>	$1^d$	1 <sup>de</sup>
	90	16.15±0.94 <sup>e</sup>	$14.82{\pm}0.8^{e}$	53.4±5.0 <sup>e</sup>	$1^{e}$	$1^{e}$
	30	$15.03 \pm 0.39^{d}$	$6.98 \pm 0.72^{d}$	29.08±0.7 <sup>ef</sup>	0.61±0.03 <sup>e</sup>	0.94±0.23 <sup>ef</sup>
$T_2$	60	21.9±2.14 <sup>cd</sup>	12.48±0.75 <sup>de</sup>	40.57±0.96 <sup>e</sup>	$0.40\pm0.04^{e}$	$1.22 \pm 0.31^{d}$
	90	18.73±0.8 <sup>de</sup>	18.32±0.46 <sup>de</sup>	84.2±1.71 <sup>de</sup>	$1.35 \pm 0.46^{de}$	$2.1\pm0.33^{d}$
	30	18.48±1.65 <sup>c</sup>	$10.97 \pm 0.7^{bc}$	$46.9 \pm 2.45^{d}$	1.36±0.17 <sup>cd</sup>	$1.76\pm0.46^{a}$
$T_3$	60	22.53±1.12 <sup>bc</sup>	21.43±1.3 <sup>ab</sup>	$61.9 \pm 1.12^{d}$	$1.17\pm0.17^{a}$	2.6±0.85°
	90	$19.95 {\pm} 0.69^{d}$	$18.48 {\pm} 0.61^{d}$	96.6±3.28 <sup>d</sup>	$1.4 \pm 0.32^{d}$	1.38±0.34 <sup>de</sup>
	30	$20.52 \pm 1.0^{b}$	8.57±0.4 <sup>c</sup>	35.67±1.5 <sup>e</sup>	$1.39\pm0.18^{\circ}$	$1.28 \pm 0.39^{d}$
$T_4$	60	22.95±1.1 <sup>b</sup>	20.03±1.57 <sup>bc</sup>	51.98±3.89 <sup>de</sup>	$1.21 \pm 0.16^{cd}$	2.76±0.89 <sup>bc</sup>
	90	23.38±0.57 <sup>c</sup>	21.85±0.68 <sup>cd</sup>	103.5±2.45 <sup>cd</sup>	$3.53 \pm 0.56^{\circ}$	3.24±0.41 <sup>cd</sup>
	30	20.23±1.6 <sup>bc</sup>	$10.98 {\pm} 0.75^{b}$	55.8±4.4°	1.64±0.21 <sup>b</sup>	$1.58\pm0.49^{bc}$
$T_5$	60	22.08±1.72 <sup>c</sup>	$20.08 \pm 0.76^{b}$	71.45±1.35°	$1.29\pm0.17^{\circ}$	$2.76 \pm 0.62^{bc}$
	90	23.05±0.8 <sup>cd</sup>	22.47±0.98°	107.07±2.24 <sup>c</sup>	2.83±0.81 <sup>cd</sup>	3.6±0.57 <sup>bc</sup>
	30	22.3±0.92 <sup>ab</sup>	11.28±0.69 <sup>ab</sup>	$68.67 \pm 5.64^{b}$	$1.83 \pm 0.35^{ab}$	$1.45\pm0.44^{\circ}$
$T_6$	60	24.98±0.99 <sup>ab</sup>	17.38±2.64 <sup>c</sup>	89.87±2.15 <sup>b</sup>	$1.54{\pm}0.11^{ab}$	4.31±1.3 <sup>a</sup>
	90	31.75±0.68 <sup>ab</sup>	$40.62 \pm 1.81^{b}$	171.75±4.14 <sup>b</sup>	6.62±1.15 <sup>b</sup>	5.36±0.32 <sup>b</sup>
	30	$22.75 {\pm} 0.72^{a}$	$11.84{\pm}0.76^{a}$	$85.25 \pm 3.56^{a}$	$1.95{\pm}0.26^{a}$	$1.61 \pm 0.48^{b}$
$T_7$	60	$25.45 \pm 0.57^{a}$	$22.0\pm0.7^{a}$	$116.1 \pm 7.08^{a}$	$1.48 \pm 0.08^{bc}$	3.34±0.66 <sup>b</sup>
	90	33.87±0.76 <sup>a</sup>	$64.68 \pm 1.28^{a}$	$208.5 \pm 2.17^{a}$	$9.74{\pm}2.39^{a}$	$12.33 \pm 1.49^{a}$

 Table 1 : Effect of biofertilizers on shoot and root length, leaf area and shoot root and tolerance index of Jatropha plants (30, 60 and 90 days old plants)

Values are means  $\pm$  SE of five replicates of three experiments. Means within a column followed by the same letter are not significant at P $\leq$  0.05 according to DMRT. T<sub>1</sub> - Control (uninoculation); T<sub>2</sub> - *Azospirillum* + VAM; T<sub>3</sub> - Phosphate solubilizing bacteria (PSB) + VAM; T<sub>4</sub> - *Azotobacter* + VAM; T<sub>5</sub> - *Azospirillum* + Phosphate solubilizing bacteria (PSB); T<sub>6</sub> - *Azospirillum* + *Azotobacter* + Phosphate solubilizing bacteria (PSB); T<sub>7</sub> - *Azospirillum* + *Azotobacter* + Phosphate solubilizing bacteria (PSB) + VAM

Luna and Devies, 2003). The highest leaf area was obtained in  $N_{120} P_{60}$  at knee high stage of maize in 2002 and 2003, respectively. But in second year it was at par with  $N_{120}$  SSP<sub>30</sub> with VAM fungi the related results reported by Banerjee *et al.* (2006). The inoculation of *Glomus intraradices, Glomus geosporum*, phosphate solubilizing bacteria and *Azospirillum*, vigorously increased seedling growth of neem trees in tropical condition (Muthukumar *et al.*, 2001). Plants could change ions uptake characteristics of roots due to a modification of root morphology or alteration of uptake mechanisms, relative growth rate or internal composition of plants can affect by soil and Rhizosphere bacteria (Tinker, 1984).

Fresh weight of shoot, root and leaves of Jatropha plants treated with bioinculants were highly significantly increased than control plants. Treated plants of  $T_5$ ,  $T_6$  and  $T_7$  highly increased the fresh weight of shoot, root and leaves than control plants (Table 2). The shoot, root and leaves dry biomass of treated plants increased from  $T_2$  to  $T_7$  biofertilizers inoculated plants and among the different concentrations  $T_6$  and  $T_7$  treated plants were highly significant than control plants (Table 2). Mycorrhizae with *Rhizobium* and *Azotobacter* have highest significant effect on seed germination, number of nodules, nodule

dry weight, plant height and nutrient content of cowpea (Rakeshkumar *et al.*, 2001). Shoot, root and total plant biomass, plant height and leaf number were significantly different between AM fungi and non AM fungi on ancho pepper plantlets by Estrada-Luna and Davies (2003). The dry weight of the maize plants were increased by the treated of VAM fungi with N<sub>120</sub> and SSP<sub>30</sub> and PSB with N<sub>120</sub> RP<sub>30</sub> (Banerjee *et al.*, 2006).

#### Biochemical assay :

Chlorophyll content was estimated at 120 days old treated plants. In treatments  $T_2$  and  $T_3$ , the chlorophyll a content was slightly varied from the control plants ( $T_1$ ) and  $T_4$  and  $T_5$  treatments were moderately differentiated from the control plants and  $T_2$  and  $T_3$  treated plants.  $T_6$  and  $T_7$  bioinoculants treated plants significantly increased the chlorophyll a with compared to control plants ( $T_1$ ). The significant increase of chlorophyll b content in  $T_5$  and  $T_6$  treated plants was observed than control and other treatments. Moreover, the total chlorophyll content gradually increased from  $T_2$  to  $T_7$  inoculated plants (Table 3). AM fungi and non AM fungi on ancho pepper plants had comparable leaf chlorophyll during acclimatization, however during post-acclimatization, AM fungi had higher

Treatments	Days	Shoot (g)		Root (g)		Leaves (g)	
		Fresh wt	dry wt	Fresh wt	dry wt	Fresh wt	dry wt
$T_1(con)$	30	4.95±0.9 <sup>cd</sup>	0.45±0.1 <sup>cd</sup>	0.54±0.12 <sup>cd</sup>	0.07±0.03 <sup>cd</sup>	4.43±0.34 <sup>d</sup>	0.64±0.06°
	60	$8.98 \pm 1.19^{d}$	$1.2\pm0.17^{cd}$	$0.98{\pm}0.04^{d}$	$0.25 \pm 0.05^{d}$	5.37±0.5 <sup>de</sup>	0.69±0.06
	90	7.89±1.06 <sup>e</sup>	$1.44 \pm 0.31^{f}$	1.82±0.29 <sup>e</sup>	$0.42 \pm 0.06^{de}$	$2.8 \pm 0.45^{f}$	0.65±0.04
T <sub>2</sub>	30	$4.58 \pm 0.5^{d}$	$0.39 \pm 0.07^{d}$	0.56±0.03 <sup>cd</sup>	$0.07 \pm 0.09^{cd}$	5.15±0.76 <sup>cd</sup>	0.55±0.07
	60	9.93±0.51 <sup>cd</sup>	$0.87 \pm 1.15^{d}$	$0.96 \pm 0.07^{de}$	$0.24 \pm 0.03^{de}$	5.24±2.94 <sup>e</sup>	$0.85 \pm 0.06$
	90	$8.92 \pm 0.7^{de}$	1.93±0.17 <sup>ef</sup>	1.78±0.13 <sup>ef</sup>	$0.63 \pm 0.06^{cd}$	11.21±1.21 <sup>e</sup>	$1.8\pm0.27^{\circ}$
T <sub>3</sub>	30	5.97±0.39 <sup>bc</sup>	$0.55 \pm 0.1b^{c}$	$0.85 \pm 0.09^{a}$	$0.13 \pm 0.02^{ab}$	5.64±0.5°	0.76±0.05
	60	14.71±1.3 <sup>b</sup>	2.58±0.31 <sup>b</sup>	$2.01 \pm 0.2^{bc}$	$0.54 \pm 0.04^{bc}$	$11.77 \pm 0.87^{bc}$	$3.06\pm0.5^{a}$
	90	$11.1 \pm 0.88^{d}$	1.94±0.19 <sup>e</sup>	$1.74{\pm}0.14^{\rm f}$	$0.55 \pm 0.09^{d}$	11.8±1.06 <sup>de</sup>	2.17±0.2°
$T_4$	30	5.49±0.57 <sup>c</sup>	$0.54{\pm}0.05^{\circ}$	0.73±0.06 <sup>c</sup>	0.09±0.006 <sup>c</sup>	6.68±0.34 <sup>ab</sup>	0.86±0.03
	60	13.77±0.96 <sup>bc</sup>	2.43±0.4 <sup>bc</sup>	$2.38 \pm 0.19^{b}$	0.51±0.06 <sup>c</sup>	$12.06 \pm 0.75^{b}$	2.06±0.26
	90	19.1±0.68 <sup>cd</sup>	4.3±0.32 <sup>c</sup>	5.06±0.38 <sup>c</sup>	1.37±0.16 <sup>c</sup>	$10.93 \pm 0.66^{d}$	2.77±0.34
T <sub>5</sub>	30	6.44±0.45 <sup>b</sup>	$0.61 {\pm} 0.06^{ab}$	$0.82{\pm}0.08^{ab}$	0.13±0.01 <sup>b</sup>	6.46±0.47 <sup>bc</sup>	0.84±0.12
	60	11.8±0.85 <sup>c</sup>	$2.19\pm0.32^{\circ}$	1.96±0.19 <sup>c</sup>	$0.54{\pm}0.05^{b}$	$7.87 \pm 0.75^{d}$	1.22±0.2
	90	19.5±0.66 <sup>c</sup>	3.5±0.21 <sup>cd</sup>	5.04±0.26 <sup>cd</sup>	$1.42 \pm 0.14^{bc}$	15.26±0.54 <sup>c</sup>	4.28±0.5 <sup>t</sup>
$T_6$	30	$6.63 \pm 0.52^{ab}$	$0.59{\pm}0.08^{b}$	$0.78 \pm 0.08^{b}$	$0.12 \pm 0.01^{bc}$	$6.48 \pm 0.44^{b}$	1.13±0.19
	60	15.3±0.66 <sup>ab</sup>	$2.66 \pm 0.2^{ab}$	3.33±0.17 <sup>ab</sup>	$0.69 \pm 0.09^{a}$	12.83±0.65 <sup>ab</sup>	2.74±0.29
	90	$37.74 \pm 0.5^{b}$	$7.87 \pm 0.45^{b}$	$8.7 \pm 0.82^{b}$	$2.51 \pm 0.34^{b}$	$27.76 \pm 2.08^{b}$	5.72±0.36
$T_7$	30	7.55±0.37 <sup>a</sup>	$0.74 \pm 0.05^{a}$	$0.77 \pm 0.06^{bc}$	$0.14 \pm 0.01^{a}$	$7.59{\pm}0.5^{a}$	1.06±0.06
	60	$16.28 \pm 0.8^{a}$	$2.77 \pm 0.33^{a}$	$3.41 \pm 0.57^{a}$	$0.56 \pm 0.04^{ab}$	13.73±0.67 <sup>a</sup>	3.35±0.36
	90	54.7±1.51 <sup>a</sup>	$12.1\pm0.28^{a}$	$11.27\pm0.7^{a}$	4.25±0.23 <sup>a</sup>	38.01±3.02 <sup>a</sup>	13.17±0.8

Table 2 : The fresh and dry weight of shoot, root and leaves of biofertilizers treated Jatropha plants (30, 60 and 90 days old plants)

Values are means  $\pm$  SE of five replicates of three experiments. Means within a column followed by the same letter are not significant at P $\leq$ 0.05 according to DMRT. T<sub>1</sub> - Control (uninoculation); T<sub>2</sub> - *Azospirillum* + VAM; T<sub>3</sub> - Phosphate solubilizing bacteria (PSB) + VAM; T<sub>4</sub> - *Azotobacter* + VAM; T<sub>5</sub> - *Azospirillum* + Phosphate solubilizing bacteria (PSB); T<sub>6</sub> - *Azospirillum* + *Azotobacter* + Phosphate solubilizing bacteria (PSB); T<sub>7</sub> - *Azospirillum* + *Azotobacter* + Phosphate solubilizing bacteria (PSB) + VAM.

chlorophyll than non AM fungi (Estrada-Luna and Davies, 2003). Banerjee *et al.* (2006) reported that the magnitude of increase in chlorophyll content over that of the preceding year was highest in treatment  $N_{120} RP_{30}$  with VAM fungi followed by  $N_{120} RP_{30}$  with PSB on maize plants.

Some biochemical studies were carried out at 120 days old plants. The total soluble sugar content of

Azotobacter + VAM ( $T_4$ ) and Azospirillum + Phospho bacteria (PSB) ( $T_5$ ) treated plants showed highly significant increase over the control plants ( $T_1$ ) and other treatments, where as in the treatments  $T_6$  and  $T_7$ , higher total soluble sugars content was recorded than control plants (Table 3). There was great response in biochemical (Free amino acids) attributes of Jatropha to the increasing the treatments of Azospirillum + Azotobacter + PSB

 Table 3 : Effect of biofertilizers on the chlorophyll contents, total soluble sugars, free amino acids and total protein of 120 days old Jatropha plant

Treatments	Chlorophyll a (mg g <sup>-1</sup> fw)	Chlorophyll b (mg g <sup>-1</sup> fw)	Total Chlorophyll (mg g <sup>-1</sup> fw)	Total soluble Sugars (mg g <sup>-1</sup> dw)	Free amino acids (mg g <sup>-1</sup> dw)	Total Protein (mg g <sup>-1</sup> dw)
$T_1(con)$	$1.674 \pm 0.28^{d}$	$0.497 {\pm} 0.07^{b}$	$1.02\pm0.14^{ef}$	197.2±8.33 <sup>f</sup>	1.54±0.13 <sup>de</sup>	36.78±0.78 <sup>g</sup>
$T_2$	$0.992 \pm 0.14^{ef}$	$0.392 \pm 0.08^{\circ}$	1.30±0.12 <sup>de</sup>	235.34±5.89 <sup>e</sup>	$1.5\pm0.14^{e}$	49.15±2.9 <sup>cd</sup>
T <sub>3</sub>	0.995±0.14 <sup>e</sup>	$0.409 \pm 0.09^{bc}$	$1.13\pm0.12^{e}$	277.55±13.0 <sup>cd</sup>	$1.79 \pm 0.07^{d}$	$54.85 \pm 3.7^{ab}$
$T_4$	$1.528 \pm 0.27^{de}$	$0.219{\pm}0.07^{d}$	$1.57{\pm}0.24^{d}$	352.86±9.38 <sup>ab</sup>	2.53±0.11 <sup>c</sup>	$42.46 \pm 1.5^{ef}$
T <sub>5</sub>	3.164±0.29 <sup>c</sup>	$0.358 \pm 0.08^{cd}$	$2.40\pm0.08^{c}$	370.56±12.7 <sup>a</sup>	$2.62\pm0.15^{b}$	42.98±1.08 <sup>e</sup>
$T_6$	4.36±0.11 <sup>ab</sup>	$0.675 \pm 0.04^{ab}$	$3.71 \pm 0.08^{ab}$	$278.27 \pm 5.76^{\circ}$	2.73±0.13 <sup>ab</sup>	50.23±0.96°
T <sub>7</sub>	$4.67 \pm 0.09^{a}$	$0.734 \pm 0.04^{a}$	$4.0\pm0.08^{a}$	260.69±12.89 <sup>d</sup>	$2.73\pm0.2^{a}$	57.71±2.43 <sup>a</sup>

Values are means  $\pm$  SE of five replicates of three experiments. Means within a column followed by the same letter are not significant at  $P \le 0.05$  according to DMRT.  $T_1$  - Control (uninoculation);  $T_2$  - *Azospirillum* + VAM;  $T_3$  - Phosphate solubilizing bacteria (PSB) + VAM;  $T_4$  - *Azotobacter* + VAM;  $T_5$  - *Azospirillum* + Phosphate solubilizing bacteria (PSB);  $T_6$  - *Azospirillum* + *Azotobacter* + Phosphate solubilizing bacteria (PSB);  $T_7$  - *Azospirillum* + *Azotobacter* + Phosphate solubilizing bacteria (PSB) + VAM.

(T<sub>6</sub>) and *Azospirillum* + *Azotobacter* + PSB + VAM (T<sub>7</sub>) treated plants. Free amino acids of T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> treated plants were significantly increased than the other treatements. Total protein gradually increased from T<sub>2</sub> to T<sub>7</sub> treatments. Among this, the higher amount of total protein was recorded in T<sub>7</sub> treated plants (Table 3). In T<sub>3</sub> and T<sub>6</sub> treated plants, the total protein content was increased from the other inoculation (T<sub>2</sub>, T<sub>4</sub> & T<sub>5</sub>) and control plants (Table 3). Inoculation of compost with *Azospirillum* spp. individually or together enhanced the nitrogen and phosphorus accumulation in plants (Sompong, *et al.*, 2005). The qualitative and quantitative effects of inoculations on the mineral composition of *Vicia faba* varied largely among *Azotobacter* or *Azospirillum* strains (Rodelas *et al.*, 1999).

Mycorrhizal symbiosis also resulted in a significant increase in chlorophyll content, sugar contents, free amino acid contents and protein content in Ziziphus mauritiana plants under water stress conditions as compared with non-mycorrhizal plants (Mathur and Vyas, 2000). Protein content was substantially higher in mycorrhizal plants. The protein content of shoot and root in mycorrhizae treated plant (Medicago sativa) were highly significant than nonmycorrhizal plants (Vazquez et al., 2002 and Tejera et al., 2005). In natural environments, the sugarcane plants were not under nitrogen stress, the production of stimulatory factors by PGPR like Azospirillum could be considered beneficial for sugarcane plants. Mycorrhizal fungus absorbed the scarce nutrients from a large area of ground, which it supplied to the plants, afforded the plant protection against water and thermal stresses and resistance against soil borne pathogens (Maheshwari, 2006).

Inoculations of *Azospirillum*, *Azotobacter*, PSB with VAM fungi increased the seedling growth and plant quality. It is suggested that this combination was the best over the other combinations. Among these seven treatments,  $T_6$  and  $T_7$  combinations highly enhanced the growth and development of Jatropha plant.

# Authors' affiliations

**G. RAJA,** Department of Plant Science, Bharathidasan University, TIRUCHIRAPPALLI (T.N.) INDIA

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