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SHORT COMMUNICATION

INFLUENCE OF *VAM FUNGI* AND MICROBIAL INOCULANTS ON GROWTH, NUTRIENTS AND BIOCHEMICAL CONSTITUENTS IN *JATROPHA CURCAS* L.

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Field experiment was conducted to study the effect of *VAM fungi* and its interaction with other microbial inoculants, *Azospirillum* spp, *Azotobacter* spp. and phosphate solubilizing bacteria on plant biomass, nutrients and biochemical constituents in *Jatropha curcas* L. Application of combined microbial inoculants, *Azospirillum* + Phosphobacteria + *Azotobacter* + *VAM fungi* significantly enhanced the fresh biomass, total soluble protein and phenols as well as relative water content over other inoculants and uninoculated control. The study indicated effectiveness of biofertilizers in increasing plant growth, nutrients and biochemical constituents of *Jatropha* plants.

Key words*: Biochemical constituents, biofertilizers, biomass, *Jatropha

Jatropha curcas L. is widely cultivated in central and South America, Africa, India and South East Asia. It grows in a number of climatic zones in tropical and subtropical regions of low rainfall and problematic soil being drought tolerant. *Jatropha* plants were mainly cultivated for the purpose of oil (curcas oil). The seeds contain 40-50 % for semidrying oil which is an efficient substitute fuel for diesel (Bhasabutra and Sutiponpeibum 1982, Banerji *et al*. 1985). *Jatropha* plants were mainly cultivated for wasteland reclamation. The latex of *Jatropha* contains the alkoloids such as Jatrophine, Jatropham, Jatrophone and Curcain, which are believed to have anticancerous properties. The free living bacteria (*Azotobacter),* associate (*Azospirillum*) and symbiotic (*Rhizobium*) bacteria are gaining much popularity and such practices being encouraged to save the chemical fertilizers, national economy and the environment (Subba Rao 1993). *Azospirillum* spp. are bacteria known for their ability to promote the growth and yield of various crop plants under various soil and climatic conditions. *Arbuscular mycorrhizal fungi* with *Azospirillum brasilense* and phosphate- solubilization bacteria (PSB) individually or in various combination in unsterile soil resulted in increased mycorrhizae colonization, greater plant height, leaf area and number, root collar diameter, biomass, nitrogen, phosphorous and potassium content and seedling quality (Muthukumar *et al*. 2001).

Mycorrhizal fungi allow plants to live in wasteland wherein they could not grow otherwise. Inoculation with *Rhizobium* and *Paenibacillus polymyxa* strain loutit (L) stimulated nodulation as well as nitrogen fixation. PGPR also stimulated nodulation (number of nodules per gram of root dry weight), that translated into higher levels of accumulated nitrogen (Figueiredo *et al*. 2008). Hence, the present study was undertaken in field conditions to investigate the effect of combined bio-inoculation of nitrogen fixers and phosphate solubilizers with *VAM fungi* on the growth, nutrients and biochemical constituents in *Jatropha curcas* L.

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The experiment was conducted at Plant Science Department Garden, Bharathidasan University, Tiruchirappalli, under the field condition (unsterilized soil) in randomized block design with 3 replications. The size of the individual plot was 1×1 m. The space between the blocks and plots were 1m × 1m, respectively. The soil samples were collected from the experimental field randomly, 0-30 cm depth. Soil samples analysed prior to experiments following standard procedures (Davis 1962 and Jackson 1971) showed available nitrogen 46.135 kg/ ha, total phosphorus 1.214 kg/ha, available potassium 50.587 kg/ha and pH 8.24.

The *Jatropha curcas* L. seeds were procured from the Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Coimbatore. The carrier based biofertilizers like *Azospirillum, Azotobacter*, phosphobacteria and *VAM fungi* were collected from Tamil Nadu Agricultural office, Biofertilizers Production Unit, Tiruchirappalli and Stan's Company Ltd. Tiruchirappalli, respectively. The seeds were inoculated overnight with these bioinoculants and dried in a shade place. The treatments details are C = Control (uninoculated seed), T1 = *Azospirillum* + *VAM fungi*, T2 = Phosphobacteria + *VAM fungi*, T3 = *Azotobacter* + *VAM fungi*, T4 = *Azospirillum* + Phosphobacteria, T5 = *Azospirillum* + Phosphobacteria + *Azotobacter* (3) and T6 = *Azospirillum* + Phosphobacteria + *Azotobacter* + *VAM fungi* (3+1).

For the growth and fresh biomass analysis, plants with intact roots were carefully dug at random. These plants were thoroughly washed with distilled water to remove soil from the roots. The seed germination (%) and morphological characteristics, *viz*. length of root and shoot, number of leaves per plants and fresh weight of plants were recorded on 30, 60 and 90 days after sowing. Relative water content (RWC) of leaves was determined at 90 days after sowing. Total soluble proteins was determined according to the method of Lowry *et al*. (1951), Free proline and total phenol were estimated in 6 month old plants by using UV spectrophotometer as adopted by Bates *et al*. (1973) and Sadasivam and Manickam (1996). Total N, P and K were determined using procedures followed by Jackson (1971). The data was statistically analysed following the Duncan's Multiple Range Test (DMRT).

The highest percentage of seed germination rate to 80%, 78% and 80% was obtained in T4, T5 and T6, respectively (Fig. 1). The combination of biofertilizers (*Azospirillum* + PSB) with the soil has shown a significant improvement in the seed germination and biochemical parameters with an improvement in soil quality. *Azosprillum* + *Azotobacter* + PSB + *VAM fungi* (3+1) combination registered the longest root and shoot (11.84 cm, 22.0 & 64.68 and 22.75 cm, 25.45 & 33.87) at 30, 60 and 90 DAS, respectively over the other treatment and control (Table 1). *Azosprillum* + *Azotobacter* + PSB + *VAM fungi* (T6) also produced the significantly higher number of leaves per plant (Table 1). Results reported by Kibria (2000), Delacruz *et al*. (1988). Mohammad and Hossain (2003) also suggested longest root and shoot with combined bio- inoculation than the control.

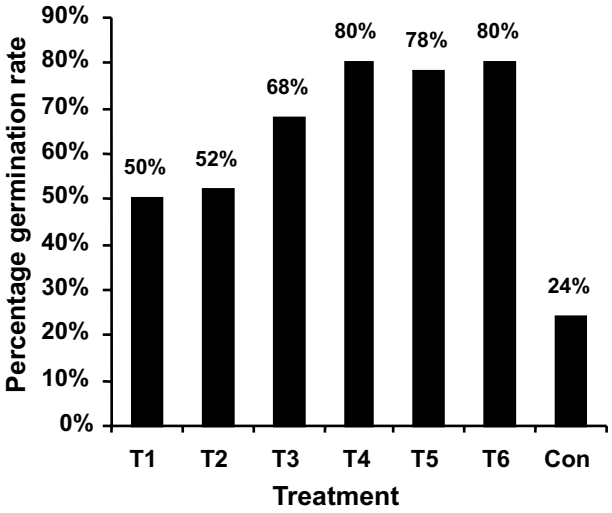

Fig. 1. Effect of biofertilizers with VAM fungi on seed germination in *Jatropha curcas* L.

The inoculation of *Azosprillum* + *Azotobacter* + PSB + *VAM fungi* (T6) significantly increased fresh weight of whole plant at all the stages examined. The fresh weight of root, shoot and leaves were significantly higher at 30, 60 and 90 DAS when *Azospirillum* + *Azotobacter* + PSB + *VAM fungi* (T6) applied in combination whereas T2 treatment (PSB + *VAM fungi*) increased the root fresh weight more than other treatments at 30 days. The similar results are also reported by Vijaykumari and Punitha (1998). The increase in seedling biomass may be strongly correlated

Table 1. Effect of biofertilizers with *VAM* fungi treatments on root and shoot length and number of leaves in *Jatropha* plants 30, 60 and 90 days after sowing (DAS). Each value represents mean ± SE.

Treatments	Root length (cm)			Shoot length (cm)			Number of leaves (cm)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Control	8.1^{cd}± 1.2	13.27^{d}± 0.58	14.82^{e}± 0.8	17.68^{cd}± 0.6	15.88^{d}± 1.1	16.15^{e}± 0.94	5.5^{ef}± 0.5	6.33^{e}± 0.49	8.33^{f}± 0.56
T1	6.98^{d}± 0.7	12.48^{de}± 0.75	18.3^{de}± 0.46	15.03^{d}± 0.4	21.93^{cd}± 2.1	18.73^{de}± 0.8	5.5^{e}± 0.76	7.67^{d}± 0.67	10.17^{e}± 0.8
T2	10.97^{bc}± 0.7	21.43^{ab}± 1.33	18.48^{d}± 0.6	18.48^{c}± 1.7	22.53^{bc}± 1.12	19.95^{d}± 0.69	6.3^{d}± 0.49	9.17^{c}± 0.48	11.17^{d}± 0.6
T3	8.57^{c}± 0.4	20.03^{bc}± 1.57	21.85^{cd}± 0.68	20.52^{b}± 1.0	22.95^{b}± 1.1	23.38^{c}± 0.57	7.0^{ab}± 0.37	10.5^{ab}± 0.76	11.83^{c}± 0.5
T4	10.98^{b}± 0.8	20.08^{b}± 0.76	22.47^{c}± 0.98	20.23^{bc}± 1.6	22.08^{c}± 1.7	23.05^{cd}± 0.8	6.7^{c}± 0.67	9.83^{bc}± 0.95	13.17^{b}± 0.7
T5	11.28^{ab}± 0.7	17.38^{c}± 2.64	40.62^{b}± 1.8	22.3^{ab}± 0.9	24.98^{ab}± 1.0	31.75^{ab}± 0.68	6.83^{c}± 0.4b	10.17^{b}± 1.0	12.5^{bc}± 0.76
T6	11.84^{a}± 0.8	22^{a}± 0.7	64.68^{a}± 1.28	22.75^{a}± 0.7	25.45^{a}± 0.57	33.87^{a}± 0.76	7.17^{a}± 0.3	10.67^{a}± 0.9	14.33^{a}± 1.1

Treatment details are given in text, within a column, means ± SE followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's Multiple Range Test (DMRT).

with improved accumulation of nitrogen due to *Azospirillum* + phosporous due to PSB inoculation (Sanders *et al*. 1993, Narayana 1991). The growth attributes exhibited maximum values in treatments PSB in combination with *Rhizobium* application (Gupta *et al*. 1997 and Rakeshkumar *et al*. 2001).

The higher relative water content (RWC) values of 88.92%, 88.27% and 87.81% were observed in treatments such as *Azotobacter* + *VAM* (T3), *Azospirillum* + *Azotobacter* + PSB (T5) and *Azospirillum* + *Azotobacter* + PSB + *VAM fungi* (T6) respectively. However, these values were statistically on par with other combinations and uninoculated control (Table 3). According to Andres *et al*. (2003) leaf RWC increased from 83% to 93% and remained constant through out the duration of the experiment. *VAM fungi* significantly enhanced RWC at day 6 during peak leaf dehydration. Nitrogen, phosphorus and potassium concentrations in *Azospirillum* + *Azotobacter* + PSB + *VAM fungi* (T6) were significantly higher over the other inoculants and uninoculated control (Table 3). Phosphate solubilizing bacteria are also known to increase phosphorus uptake resulting in better growth and

Table 2. Effect of biofertilizers with *VAM* fungi on fresh biomass of *Jatropha curcas* L. 30, 60 and 90 days after sowing (DAS).

Treatments	Root fresh weight (g)			Shoot fresh weight (g)			Leaves fresh weight (g)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Control	0.54^{cd}±0.12	0.98^{d}±0.04	1.82^{e}±0.29	4.95^{cd}±0.9	8.98^{d}±1.19	7.89^{e}±1.1	4.43^{d}±0.34	5.36^{de}±0.51	2.8^{f}±0.45
T1	0.56^{cd}±0.03	0.96^{de}±0.07	1.78^{ef}±0.13	4.58^{d}±0.5	9.93^{cd}±0.51	8.92^{de}±0.7	5.15^{cd}±0.76	5.24^{e}±0.94	11.21^{e}±1.2
T2	0.85^{a}±0.09	2.01^{bc}±0.19	1.74^{f}±0.14	5.97^{bc}±0.39	14.7^{b}±1.32	11.05^{d}±0.9	5.64^{c}±0.5	11.77^{bc}±0.9	11.8^{de}±1.06
T3	0.73^{c}±0.06	2.38^{b}±0.19	5.06^{c}±0.38	5.49^{c}±0.57	13.77^{bc}±1.0	19.1^{cd}±0.68	6.68^{ab}±0.34	12.06^{b}±0.8	10.93^{d}±0.66
T4	0.82^{ab}±0.08	1.96^{c}±0.19	5.04^{cd}±0.26	6.44^{b}±0.45	11.8^{c}±0.85	19.5^{c}±0.66	6.46^{bc}±0.47	7.87^{d}±0.75	15.26^{c}±0.54
T5	0.78^{b}±0.08	3.33^{ab}±0.17	8.72^{b}±0.8	6.63^{ab}±0.5	15.3^{ab}±0.66	37.74^{b}±0.5	6.48^{b}±0.44	12.83^{ab}±0.7	27.76^{b}±2.08
T6	0.77^{bc}±0.06	3.41^{a}±0.57	11.27^{a}±0.7	7.55^{a}±0.4	16.3^{a}±0.77	54.7^{a}±1.5	7.59^{a}±0.5	13.7^{a}±0.67	38.01^{a}±3.02

Treatment details are given in text, within a column, means ± SE followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's Multiple Range Test (DMRT).

Table 3. Relative water content and N, P and K concentration in *Jatropha curcas* L. as influenced by biofertilizers with *VAM* fungi treatments. Each value represents mean ± SE.

Treatments	Relative water content (%)	N (mg g^{-1} dw)	P (mg g^{-1} dw)	K (mg g^{-1} dw)
Control	84.99^{f}±0.36	1.70^{e}±0.022	0.575^{f}±0.017	0.335^{e}±0.014
T1	85.12^{ef}±0.37	3.047^{d}±0.082	0.802^{de}±0.016	0.758^{c}±0.027
T2	86.37^{de}±0.22	3.645^{c}±0.15	0.842^{d}±0.01	0.53^{d}±0.022
T3	88.92^{a}±0.26	3.704^{bc}±0.071	1.042^{c}±0.02	0.96^{bc}±0.026
T4	86.6^{d}±0.54	3.20^{cd}±0.054	0.883^{cd}±0.017	0.988^{b}±0.017
T5	88.27^{b}±0.24	3.736^{b}±0.15	1.465^{b}±0.041	1.042^{ab}±0.026
T6	87.81^{bc}±0.43	6.46^{a}±0.5	1.80^{a}±0.067	1.056^{a}±0.039

Treatment detail are given in text, within a column, means ± SE followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's Multiple Range Test (DMRT).

Table 4. Effect of bioinoculants with *VAM* fungi on soluble protein, phenol and proline content in *Jatropha* plants. Each value represents mean ± SE.

Treatments	Total soluble protein (mg g^{-1} dw)	Total phenol (mg g^{-1} dw)	Free proline (mg g^{-1} dw)
Control	35.8^{g}±0.33	2.538^{e}±0.07	0.208^{c}±0.012
T1	48.3^{cd}±1.22	2.83^{cd}±0.02	0.208^{cd}±0.02
T2	50.76^{ab}±1.67	3.008^{bc}±0.026	0.282^{a}±0.003
T3	43.08^{e}±0.65	3.095^{b}±0.033	0.226^{bc}±0.012
T4	42.14^{ef}±0.32	3.298^{a}±0.033	0.153^{e}±0.008
T5	49.88^{c}±0.346	2.801^{d}±0.02	0.242^{b}±0.011
T6	56.44^{a}±1.09	2.845^{c}±0.021	0.178^{de}±0.0122

Treatment details are given in text, within a column, means ± SE followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's Multiple Range Test (DMRT).

higher yield of crop plants (Bajpai and Sundra Rao 1971, Gaur *et al*. 1980, Alagawadi and Gaur 1988). *Azospirillum* can produce nitric acid (NO) and IAA, which has a direct role in the lateral root development of plant (Creus *et al*.2005). Our results are in conformly with what was reported by Bashan and Levanony (1990) and Okon and Vanderleyden (1997) with *Azospirillum* inoculated plants. Inoculation of cereal with free living nitrogen-fixing *Azotobacter* and the associative nitrogen fixing *Azospirillum* and the phosphate solubilizing organisms are gaining popularity. Nitrogen and phosphorous are the two major plant nutrients and combined inoculation of nitrogen fixers and phosphate solubilizers may benefit the plants better than either group of organisms. Rathore and Singh (1995) reported that phosphorus application and *VAM* inoculation increased dry matter accumulation and uptake of N, P and K by maize shoots at 30 and 45 days of growth. It was reported that mycorrhizal inoculums may substitute phosphatic fertilizer equivalent to about 30 kg /ha.

The highest total soluble protein content of 56.44 mg g^{-1} dry weight was recorded in *Azospirillum* + *Azotobacter* + PSB + *VAM* (T6) which was statistically at par with that of other inoculants and uninoculated control (Table 4). The total phenol content of 3.30 and 3.10 mg g^{-1} dry weight was recorded in *Azotobacter* + *VAM* (T3) and *Azospirillum* + PSB (T4), respectively, these combinations were significantly higher than the other combination and uninoculated control (Table 4). The free proline 0.282, and 0.308 mg g^{-1} dry weight was observed in treatments of PSB + *VAM* (T2) and uninoculated control. However, these values were statistically similar to with other inoculated plants (Table 4). Mycorrhizal colonization has been shown to increase accumulation of amino acids, protein, chlorophyll and sugar contents as compared with non mycorrhizal plants (*Zizuphus mauritiana*) under water stress conditions (Mathur and Vyas 2000).

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