International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 1729-1732

Received: 07-01-2018
Accepted: 09-02-2018

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# Application of bio-fertilizers for quality seedling production of (*Jatropha carcus* L.)

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**Abstract**
*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, and Phosphate solubilizing bacteria (PSB) at various combinations. The biofertilizer treated seeds were tested under field conditions and seedlings were uprooted at 45, 90, 135 and180 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 180 days, chlorophyll contents were analyzed and the results indicated that the plants inoculated with Azospirillum + 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. Bio fertilizers accelerated the assimilation of nutrients to the plants.

**Keywords:** *Jatropha carcus* L., VAM, azospirillum, and phosphobacterium

## Introduction

Jatropha is a shrub or small tree, and it grows up to 6 m height with spreading branches and stubby twigs (Dehgan, 1984) [5]. It belongs to the family Euphorbiaceous 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) [5, 20]. Possible uses of Jatropha plant parts, such as leaves are used as anti-inflammatory agents and the latex are believed to have anti cancerous properties, which contains the alkaloids such as Jatrophine, Jatrophone, Jatropham and Curcain (Duke and Ayensu, 1985) [7]. Bark, fruits, leaf, root and wood have also been reported to contain HCN (Watt and Breyer – Brandwijk 1962) [24]. 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, bio pesticides, 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) [12, 21]. Biodiesel contains no petroleum but it can be blended at any level with petroleum diesel to create a biodiesel blend. It can be used in compression-ignition (diesel) engines with little or no modification. Biodiesel is simple to use biodegradable, nontoxic and essentially free of sulphur and aromatics (Savitha and Naik, 2011) [22]. 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, gonorrhoea, inflammation, jaundice, paralysis, pneumonia, stomach ache, tooth ache, syphilis, tumors, ulcers and yellow fever.

Inoculation of Azospirillum brasilense, Phosphobacterium (Bacillusmegaterium var. phosphaticum) and Vesicular Arbuscular Mycorrhizal fungi (Glomusfasiculatum) combination could be used for the production of healthy and vigorously growing seedlings (Muthukumar *et al*., 2001) [15]. Dual inoculation of AM fungi and PSB might be stimulated the plant growth and better than inoculation with individual organism (Kim *et al*., 1997) [10]. Similar effect also reported for AM fungi, Azospirillum inoculations in some plant species (Pacovsky *et al*., 1985 and Pacovsky, 1989) [16, 17]. No report is available on the interaction between Azospirillum, Phosphate solubilizing bacteria (PSB) and Vesicular Arbuscular Mycorrhizal (VAM) fungi on the growth and development of Jatropha plants. Inoculation with Azospirillum, Phosphate solubilising 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, and Azospirillum on the growth changes in Jatropha.

## Materials and Methods

### Plant material and Bio inoculants

The experiment was conducted at the College of Forestry and Environment, Allahabad Agricultural Institute –Deemed University, Allahabad, India It is situated at $25^0$ 87'N Latitude, $81^0$ 5'E Longitude,78m above m.s.l. with mean annual rainfall of 1100mm. The genetically superior seeds of *Jatropha carcus* were collected from elite trees and used for the study. Biofertilizers like Azospirillum, Phosphate solubilising bacteria and Vesicular Arbuscular Mycorrhizae fungi mixed with carrier based material were collected from biofertilizer production unit, Division, of Microbiology, AAI-DU, and Allahabad, 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% $HgCl_2$ (10 to 20 min.). Then seeds were washed with 70% ethanol for removing the $HgCl_2$ 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

Details of treatments

### Treatment details

$T_1$ - VAM
$T_2$ - Azospirillum
$T_3$ - Phosphobacteria
$T_4$ - VAM + Azospirillum
$T_5$ - VAM + Phsphobacteria
$T_6$ - Azospirillum + Phophobacteia
$T_7$ - VAM + Azospirillum + Phophobacteria
$T_8$ - Control

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

### Experimental Field

The experiment was conducted at the Department of Forestry and Environment, Allahabad Agricultural Institute –Deemed University, This study was carried out in a Randomized Block Design (RBD) with eight treatments with three replicates

### Analysis of Agro botanical characters and biochemical contents

After 45, 90, 135 and180 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 What man filter paper No: 47. Agro-botanical characters like shoot and root length, leaf area, fresh and dry weight of the plants. The leaf materials were dried at 800C 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). After 180 days of treatment the plants were harvested and the growth assessments were made. All harvested plants after measurement were oven dried at $70^0$C for 48 h for the determination of dry weight. The Seedling Quality Index (SQI) was calculated according to Dickson *et al.* (1960) [6] by using the following formula

$$\text{Quality index} = \frac{\text{Total dry weight (g)}}{\dfrac{\text{Height (cm)}}{\text{Diameter (mm)}} + \dfrac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

The biochemical parameters of the leaf samples were analyzed from 180 days old plants. The plant leaves chlorophyll content was estimated using the method of (Yoshida *et al.*, 1971) [25] and The Vesicular arbuscular mycorrhizae (VAM) colonization in roots was measured by following the method suggested by (Phillips and Haymann 1970) [18].

$$\text{VAM colonization Formula} = \frac{\text{Number of infected root bits}}{\text{Total number of root bits examined}} \times 100$$

### 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, VAM + Azospirillum + Phosphate solubilizing bacteria ($T_7$) and VAM + Azospirillum ($T_4$) 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) [1], Gupta and Namdeo (1997) [9] and Khurana and Sharma (2000) [11]. Azospirillum and VAM 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 (Bashanand Levanony, 1990; Becking, 1992) [3, 4].

With the use of biofertilizers, the leaf area of Jatropha plants was increased in all treated plants than control plants. The treatment of VAM + Azospirillum + Phosphate solubilizing bacteria ($T_7$) highly significantly increased the leaf area of the treated plants (Table 1). VAM 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 VAM fungi on ancho pepper plantlets (Estrada- Luna and Devies, 2003) [8]. 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 $N_{120}$ $SSP_{30}$ with VAM fungi the related results reported by Banerjee *et al*. (2006) [2]. 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) [15]. 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) [23].

Fresh weight of shoot, root and leaves of Jatropha plants treated with bioinculants were highly significantly increased than control plants. Fresh weight of shoot, root and no.of leaves of Jatropha plants treated with bioinculants were highly significant increased than control plants. Treated plants of $T_7$,

$T_5$ and $T_4$ highly increased the fresh weight of shoot, root and leaves than control plants (Table 1). The shoot and root dry biomass of treated plants increased from $T_2$ to $T_5$ biofertilizers inoculated plants and among the different concentrations $T_4$ and $T_7$ treated plants were highly significant than control plants (Table 1). Mycorrhizae with *Rhizobium* and PSB have highest significant effect on seed germination, number of nodules, nodule dry weight, plant height and nutrient content of cowpea (Rakesh kumar *et al.,* 2001) [19]. Shoot, root and total plant biomass, plant height and leaf number were significantly different between VAM fungi and non VAM fungi on ancho pepper plantlets by Estrada-Luna and Davies (2003) [8]. The dry weight of the maize plants were increased by the treated of VAM fungi with $N_{120}$ and $SSP_{30}$ and PSB with $N_{120}$ $RP_{30}$ (Banerjee *et al*., 2006) [2]. Application of bio fertilizers, organic manure with 50% RDF recorded highest plant height, plant spread, fresh weight and dry weight of chrysanthemum (Mridubhashini Patanwar *et al.,* 2014) [14].

**Biochemical assay**

Chlorophyll content was estimated at 180 day's old treated plants. In treatments $T_2$ and $T_3$, the chlorophyll-a content was slightly varied from the control plants $T_8$ 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 $T_7$ bio inoculants treated plants significantly increased the chlorophyll-a with compared to control plants ($T_8$). The significant increase of chlorophyll-b content in $T_6$ and $T_7$ 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 2).VAM fungi and non AM fungi on ancho pepper plants had comparable leaf chlorophyll during acclimatization, however during post-acclimatization, VAM fungi had higher chlorophyll than non VAM fungi (Estrada-Luna and Davies, 2003) [8]. Banerjee *et al*. (2006) [2] 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. Mycorrhizal symbiosis also resulted in a significant increase in chlorophyll content, in *Ziziphus mauritiana* plants under water stress conditions as compared with non-mycorrhizal plants (Mathur and Vyas, 2000) []13. Inoculations of *Azospirillum*, Phosphate solubilizing bacteria 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 eight treatments, $T_6$ and $T_7$ combinations highly enhanced the growth and development of Jatropha plant.

**Table 1:** Effect of biofertilizers on shoot and root length, collar dia, leaf area, Dry shoot/root weight and R/S ratio of Jatropha plants (45, 90, 135 and 180 days,)

| Treatments | Shoot leng th | Root length | Collar dia | No. of leaves | Shoot fresh Weight (g) | Leaf area ($cm^2$) | Root fresh Weight (g) | Shoot dry weight (g) | Root dry Weight (g) | Total biomass (g) | R/S Ratio | SQI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 71.60 | 54.8 | 25.90 | 28.10 | 118.30 | 78.0 | 38.75 | 57.75 | 21.15 | 78.90 | 0.366 | 14.33 |
| $T_2$ | 69.72 | 52.9 | 25.20 | 27.15 | 98.50 | 77.9 | 37.15 | 48.30 | 19.70 | 68.00 | 0.408 | 13.03 |
| $T_3$ | 66.21 | 49.7 | 24.45 | 26.25 | 89.70 | 72.6 | 35.50 | 44.80 | 18.95 | 63.75 | 0.423 | 12.58 |
| $T_4$ | 81.35 | 65.8 | 27.05 | 30.75 | 155.00 | 84.2 | 46.75 | 75.05 | 24.75 | 99.80 | 0.330 | 16.53 |
| $T_5$ | 78.90 | 61.8 | 26.65 | 30.10 | 141.05 | 96.6 | 43.55 | 69.25 | 22.75 | 92.00 | 0.330 | 15.35 |
| $T_6$ | 74.60 | 57.7 | 26.25 | 29.65 | 130.90 | 106.07 | 40.70 | 64.40 | 21.45 | 85.85 | 0.333 | 14.69 |
| $T_7$ | 85.70 | 69.5 | 27.05 | 31.10 | 166.95 | 205.5 | 52.25 | 81.75 | 26.20 | 107.95 | 0.320 | 17.16 |
| $T_8$ | 62.20 | 13.10 | 24.10 | 24.15 | 81.70 | 53.4 | 33.65 | 40.25 | 18.50 | 58.75 | 0.461 | 12.37 |

$T_1$ – VAM, $T_2$ - *Azospirillum,* $T_3$ - Phosphate solubilizing bacteria, $T_4$ –VAM + Azospirillum,
$T_5$ - VAM+ Phosphate solubilizing bacteria, $T_6$ - *Azospirillum* + Phosphate solubilising bacteria,
$T_7$- VAM + Phosphate solubilising bacteria +*Azospirillum*, $T_8$- Control

**Table 2:** Effect of biofertilizers on the chlorophyll contents, (mg/g) and Vam colonization (%) of *Jatropha curcus* L. at 180 days old plants

| | Treatments | Chlorophyll-a (mg g-1 fw) | Chlorophyll-b (mg g-1 fw) | Total Chlorophyll (mg g-1 fw) | VAM colonization |
|---|---|---|---|---|---|
| $T_1$ | VAM | 1.44 | 0.43 | 1.85 | 19.90 |
| $T_2$ | Azospirillum | 1.70 | 0.44 | 2.05 | 13.75 |
| $T_3$ | PSB | 1.50 | 0.41 | 1.79 | 15.20 |
| $T_4$ | VAM + Azospirillum | 1.85 | 0.47 | 2.25 | 20.15 |
| $T_5$ | VAM + PSB | 1.75 | 0.45 | 2.11 | 20.60 |
| $T_6$ | Azospirillum + PSB | 1.89 | 0.48 | 2.43 | 15.90 |
| $T_7$ | VAM + Azospirillum + PSB | 1.94 | 0.51 | 2.45 | 21.20 |
| $T_8$ | Control | 1.42 | 0.37 | 1.58 | 10.05 |

## References


1. Algawadi AR, Gaur AC. Associative effect of *Rhizobium* and PSB on the yield and nutrient uptake of chickpea. Plant and Soil. 1988; 105:241-246.
2. Banerjee M, Rai RK, Srivastava GC, Maiti D, ShivaDhar. Influence of nitrogen and phosphate solubilising bacteria and phosphorus sources on growth, chlorophyll and yield of maize. Indian J Physiol. 2006; 11(4):373-378.
3. Bashan Y, Levanony H. Current status of *Azospirillum* inoculation technology: Azospirillum as a challenge for agriculture. Can. J Microbiol. 1990; 67:1317-1324.
4. Becking JH. The family *Azotobacteraceae*. In Balous, A., Truper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., (eds) The prokaryotes, 2nd edn. *A handbook on the biology of bacteria*: ecophysiology, Isolation,I dentification, applications. Vol.4. Springer, Berlin Heidelberg, New york, 1992, 3144-3170.
5. Dehgan B. Phylogenetic significance of inter specific hybridization in Jatropha (Euphorbiaceae). Sys. Bot. 1984; 9:467-478.
6. Dickson A, Leaf AL, Hosner JF. Quality appraisal of white spruce and white pine seedling stock innurseries. Forestry Chronicles. 1960; 36:10-13.
7. Duke JA, Ayensu ES. Medicinal plants of the China. Reference publications. Inc. Algonac, MI, 1985.
8. Estrada- Luna AA, Devies Jr FT. AM fungi influence water relations, gas exchange, absisic acid and growth of micro propagated chile ancho pepper (*Capsicum*

*annuum*)plant lets during acclimatization and post-acclimatization. J Plant Physiol. 2003; 160:1013-1018.
9. Gupta SC, Namdeo SL. Effect of *Rhizobium*, phosphate solubilizing bacteria and FYM on nodulation, grain yield and quality of chickpea. Indian J Pulses. Res. 1997; 10:171-174.
10. Kim DS, Weller DM, Cook RK. Population dynamics of Bacillus sp. L324 -92R12 and Pseudomonas fluorescence 2-79RN10 in the rhizosphere of wheat. Phyto pathology. 1997; 87:559-564.
11. Khurana AS, Sharma P. Effect of dual inoculation of phosphate solubilizing bacteria, *Bradyrhizobium* sp. (*Cicer*) and Phosphorus on nitrogen fixation and yield of chickpea. Indian J Pulses. Res 2000; 13:66-67.
12. Makonnen E, Roston AAH, Assefa ZL. Anti fertility effect of *Jatropha curcas* L. seeds in Guinea pigs. Eth. J Health. Dev. 1997; 11:145-148.
13. Mathur N, Vyas A. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and Physiological changes in *Ziziphus mauritiana* Lam. under water stress. J Arid. Environ. 2000; 45:191-195.
14. Mridubhashini Patanwar, Gaurav Sharma, Chetna Banjare, Deepika Chandravanshi, Eshu Sahu. Growth and development of chrysanthemum (*dendranthema grandi floratzvelev*) as influenced by integrated nutrient management. The Ecoscan. 2014; 4:459-462
15. Muthukumar T, Udaiyan K, Rajeshkannan V. Response of Neem to indigenous arbuscular mycorrizal fungi, Phosphate solubilizing and Asymbiotic nitrogen fixing bacteria under tropical nursery conditions. Bio. Fertile. Soil. 2001; 34:417-426.
16. Picovsky RS, Fuller G. Influence of soil on the interactions between endomycorrhizae and Azospirillum in sorghum. Soil Biology and Biochemistry. 1985; 17:525-535.
17. Picovsky RS. Diazotroph establishment and maintenance in the sorghum-Glomus-Azospirillum association. Can. J Microbiol. 1989; 35:977-981.
18. Phillips JA, Haymann DS. Improved procedures for clearing roots and staining parasitic and Vesicular-Arbuscular fungi for rapid assessment of infection, Mycol. Soc. 1970; 55:158-101.
19. Rakesh KPP, Gupta D, Jalali BL. Impact of *VA*-Mycorrhiza, Azotobacter and Rhizobium on growth and nutrition of cowpea. J Mycol. Pl. Pathol. 2001; 31(1):38-41.
20. Sujatha M, Prabhakaran AJ. Characterization and utilization of Indian Jatrophas. Indian J Genet. Resources. 1997; 10:123-128
21. Staubmann R, Foidl G, Foidl N, Gibitz GM, Lafferty RM, Arbizu VMV, *et al*. Biogas production from *Jatropha curcas* press-cake. Energy Eng. Biotech. 1997; 65:63-65.
22. Savitha G, Naik GR. Production of biodiesel from *jatropha curcas* L. Oil. The Bioscan. 2011; 6:87-88.
23. Tinker PB. The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. Plant & Soil 1984; 76:77-91.
24. Watt JM, Breyer-Brandwijk MG. The medicinal and poisonous plants of southern and eastern Africa. 2nd ed. E&S. Livingstone, ltd., Edinburgh and London, 1962.
25. Yoshida S, Forno DA, Coa JH. Laboratory manual of physiological studies of rice. IRRI publication, Philippines, 1971, 36-37