

Influence of AMF Inoculants on Biochemical and Morphological Traits of *Jatropha curcas* L.K.G. Bansode^{1*}, R.S. Bhagade², Afreen Begum Y. Attar³ and P.P. Sarwade⁴^{1,2,3}Department of Botany, S.G.R.G. Shinde Mahavidhyalaya Paranda, Dharashiv - 413 503, Maharashtra, India.⁴Department of Botany, S.G.R.G. Shinde Mahavidhyalaya Paranda, Dist. Dharashiv - 413 503, Maharashtra, India.

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ABSTRACT

Jatropha curcas L. Seedlings were taken in eight different pots, in which soil was treated with *Glomus fasciculatum* (Gf). In eight different pots soil was treated by *G. fasciculatum* along with *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma viride* + *Trichoderma harzianum*, *Rhizobium meliloti*, Bacterial Combination of *Bacillus megaterium* + *Fraturia aurentia*, *Bacillus megaterium* + *Fraturia aurentia* + *Rhizobium meliloti* and *Bacillus megaterium* + *Fraturia aurentia* + *Rhizobium meliloti* + *Trichoderma Spp.* Observations on number of leaves, size of leaf, shoot length, shoot diameter, root length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weight were taken after six months of sowing of the plants. Observations on macro and micro elements i.e. Total nitrogen, ammonical nitrogen, nitrate nitrogen, phosphorus, potash, calcium, magnesium, sulphur, zinc, copper, ferrous, manganese, boron, molybdenum, and sodium were estimated and calculated. Data shown that number of leaves, shoot length, shoot diameter, fresh root weight, dry shoot weight, phosphorous, calcium, magnesium, zinc, copper, manganese and sodium were found highest in *Gf* + *Tv* treatment; size of leaf (width), root length, fresh root weight, and nitrate nitrogen in *Gf* + *Th* Treatment; ferrous in *Gf* + *Tv* + *Th* treatment; dry root weight, total nitrogen, molybdenum in *Gf* + *Rhz* and *Gf* + bacterial combination; potash in *Gf* + bacterial combination; size of leaf (length), boron, sodium in *Gf* + *Tv* + *Th* + *Rhz* + bacterial combination; ammonical nitrogen, sulphur in *Gf* treatment.

Keywords: *Jatropha curcas* L., *Glomus fasciculatum*, AMF, *Trichoderma Spp.*, *Rhizobium meliloti*, *Bacillus megaterium* (PSB) and *Fraturia aurentia* [KMB]

INTRODUCTION

Jatropha curcas L. is an important biofuel tree found in arid and semi-arid areas. Necessity for reduced use of fossil fuels has been intensified research to invent technologies for sustainable and renewable energy (Sharma and Singh, 2009). Biodiesel is technically feasible, economically cheaper and sustainable instead of petro-diesel (Demirbas, 2007). In this regard, *Jatropha curcas* L. is an important plant due to excellent seed quality for oil (Behera et al., 2010). *J. curcas* is native of tropical America and now broadly dispersed through the tropical as well as subtropical areas of Africa and Asia (Schmook and Serralta-Peraza, 1997; Openshaw, 2000). *J. curcas* is a perennial shrub having 5 m to 7 m height (Achten et al., 2008; Drumond et al., 2009). It has average lifespan of about 50 years (Achten et al., 2008). Plant is drought tolerant and can grow in soils with less nutrient contents. It requires almost no manual labor for cultivation and it does not compete along with other crops. *J. curcas* tolerates pests and diseases (Openshaw, 2000; Jongschaap et al., 2007; Achten et al., 2008; Behera et al., 2010). The beneficial association of *J. curcas* and AMF has been

established (Openshaw, 2000; Achten et al., 2008; Charoenpakdee et al., 2010). AMF can enhance wheat growth by improving phosphorus uptake, especially in nutrient-poor soils (Abdul and Wahab, 2023). Effect of different AM -fungi on growth, nutrition and forskolin (active ingredient) content of *C. forskohlii* after AM inoculation was recently reported by Borkotoki et al. (2019). Inoculation with VA mycorrhizal fungi and *Rhizobium meliloti* can synergistically enhance nodulation, nitrogen fixation, and plant growth (Athanasia Kavadia et al., 2021). Most agricultural soils contain native AMF spores, whose role in crop productivity has been extensively studied (Pushpa Soti, 2023). Therefore, appropriate host-endophyte combinations are necessary to achieve optimal results.

Considering these facts, the present research was undertaken to observe the capability of *Glomus fasciculatum* with *J. curcas*. This can be achieved by better understanding the interaction between *Gf*, AMF fungi and *Trichoderma viride*, *Trichoderma harzianum*, *Rhizobium meliloti*, *Bacillus megaterium* and *Fraturia aurentia*. *Bacillus megaterium* is

phosphate solubilizing bacteria (PSB) and *Fraturia aurentia* potassium mobilizing bacteria (KMB).

MATERIALS AND METHODS

Rhizosphere sample of *Jatropha curcas* L. was collected from the Yedshi Ramling Wildlife Sanctuary. Root segments were cleared with 10% KOH, stained with Trypan blue, and assessed for mycorrhizal colonization using the methods of (Phillips and Hayman, 1970) and (Giovannetti and Mosse, 1980). AMF spore density was determined using the wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). AMF fungal species were identified using the keys of Schenck and Perez (1990). Soil was sterilized in autoclave. This sterilized soil is used for pot experiments. Seeds of *Jatropha curcas* L. were planted in pots containing sterilized soil. These pots were maintained in a greenhouse and watered as per the need. In eight different pots soil was treated with eight different treatments viz. 1) *G. fasciculatum*, 2) *G. fasciculatum* + *Trichoderma viride*, 3) *G. fasciculatum* + *Trichoderma harzianum*, 4) *G. fasciculatum* + *Trichoderma viride* + *Trichoderma harzianum* 5) *G. fasciculatum* + *Rhizobium meliloti*, 6) *G. fasciculatum* + Bacterial Combination of *Bacillus megaterium* + *Fraturia aurentia*, 7) *G. fasciculatum* + *Bacillus megaterium* + *Fraturia aurentia* + *Rhizobium meliloti* and 8) *G. fasciculatum* + Bacterial Combination of *Bacillus megaterium* + *Fraturia aurentia* + *Rhizobium meliloti* + *Trichoderma viride* + *Trichoderma*

harzianum. All the bacterial species used in this experiment were sourced from College of Agriculture, Shivajinagar, Pune.

A mycorrhizal inoculum (native i.e. isolated from samples) containing spores was thoroughly mixed and applied to each pot. Eight treatments were applied including non-mycorrhizal treatment. For treatment, 5g of *Trichoderma* Spp. and 25g of bacteria (*PSB* & *KMB*) were applied to kg⁻¹ of seeds. The entire experiment was replicated in 3 sets. Plants were harvested six months after sowing. Number of leaves, size of leaves, shoot height, shoot diameter, root length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weights were recorded at harvest. After harvest, all the samples were sent to laboratory for bio-chemical analysis. Total Nitrogen, Ammonical Nitrogen, Nitrate Nitrogen and Phosphorus were estimated as per method suggested by (Jackson, 1973), Potash by (Humphry Davy, 1807) Calcium and magnesium by (Humphry Davy, 1808), sulphur, Zinc, Copper, Ferrous and Manganese by (Issac and Kerber, 1971), Boron by (Capelle, 1961), Molybdenum by (Robinson, 1948), Sodium by (Jacob Volhard, 1858).

RESULTS AND DISCUSSION

Experiment was conducted on *Jatropha curcas* L. using eight different treatments. The readings on 10 different parameters were noted for the period of six month as shown in **Table 1**.

Table 1: Influence of (AMF) Inoculants on Growth, Physiology, and Biochemical Traits of *Jatropha curcas* L.

Sr. No.	PARAMETER	TREATMENTS							
		<i>Gf</i>	<i>Gf</i> + <i>Tv</i>	<i>Gf</i> + <i>Th</i>	<i>Gf</i> + <i>Tv</i> + <i>Th</i>	<i>Gf</i> + <i>Rhz</i>	<i>Gf</i> + <i>Comb</i>	<i>Gf</i> + <i>Rhz</i> + <i>Comb</i>	<i>Gf</i> + <i>Tv</i> + <i>Th</i> + <i>Rhz</i> + <i>Comb</i>
1	No. of Leaf	24	27	26	24	18	22	20	18
2	Width of Leaf (cm)	13.1	13.1	15.6	12.4	14.2	14.4	13.6	13.3
3	Length of Leaf (cm)	14.6	14.1	16	13.2	13.9	14.2	16.2	17.2
4	Shoot Length (in cm)	82.6	97.8	93.1	75.4	92.3	77.7	73.1	73.4
5	Shoot Diameter (in cm)	8.7	9.3	9.1	8.4	9.1	7.6	8.2	8.2
6	Root Length (cm)	24.8	24.6	39.9	30.6	31.9	30.3	27.3	30.3

Sr. No.	PARAMETER	TREATMENTS							
		<i>Gf</i>	<i>Gf</i> + <i>Tv</i>	<i>Gf</i> + <i>Th</i>	<i>Gf</i> + <i>Tv</i> + <i>Th</i>	<i>Gf</i> + <i>Rhz</i>	<i>Gf</i> + <i>Comb</i>	<i>Gf</i> + <i>Rhz</i> + <i>Comb</i>	<i>Gf</i> + <i>Tv</i> + <i>Th</i> + <i>Rhz</i> + <i>Comb</i>
7	Fresh Root Weight (g)	50.07	65.67	60.63	47.55	62.1	23.8	38.27	28.49
8	Dry Root Weight (g)	9.18	14.37	7.91	8.8	18.49	4.57	2.23	4.74
9	Fresh Shoot Weight (g)	280.09	311.04	321.39	231.24	283.17	208.38	182.65	180.82
10	Dry Shoot Weight (g)	36.95	41.75	41.4	29.76	36.63	12.39	27.89	22.97

Gf, *Glomus fasciculatum*; *Tv*, *Trichoderma Viride*; *Th*, *Trichoderma harzianum*; *Rhz*, *Rhizobium meliloti*; *Bacterial Combination*: *Bacillus megaterium*, *Rhizobium meliloti* and *Fraturia aurentia*

Data recorded that number of leaves were found highest in *Gf*+*Tv* treatment and lowest in *Gf*+*Rh* and *Gf*+*Tv*+*Th*+*Rh*+ bacterial combination. Shoot length and shoot diameter was highest in *Gf*+*Tv* treatment and lowest in *Gf* + bacterial combination and *Gf*+*Rhz*+ bacterial combination. Root length was highest in *Gf* + *Th* treatment and lowest in *Gf* + *Tv* treatment. Fresh root weight recorded highest

in *Gf* + *Tv* treatment lowest in *Gf*+ bacterial combination treatment. Dry root weight was highest in *Gf* + *Rhz* and lowest in *Gf* + *Rhz* + bacterial combination treatment. Fresh shoot weight found highest in *Gf* + *Th* and lowest in *Gf*+*Tv*+*Th*+*Rh*+ bacterial combination treatments. Dry shoot weight was highest in *Gf* + *Tv* and lowest in *Gf*+ bacterial combination treatment.

Table 2: Effect of (AMF) Inoculants on chemical parameters of *Jatropha curcas* L. Merr.

Sr. No.	Parameter	Unit	TREATMENTS							
			<i>Gf</i>	<i>Gf</i> + <i>Tv</i>	<i>Gf</i> + <i>Th</i>	<i>Gf</i> + <i>Tv</i> + <i>Th</i>	<i>Gf</i> + <i>Rhz</i>	<i>Gf</i> + <i>Comb</i>	<i>Gf</i> + <i>Rhz</i> + <i>Comb</i>	<i>Gf</i> + <i>Tv</i> + <i>Th</i> + <i>Rhz</i> + <i>Comb</i>
1	Total Nitrogen	%	0.56	0.56	0.90	0.84	1.01	1.01	0.73	0.78
2	Ammonical Nitrogen	ppm	683	674	665	679	651	651	6.81	668
3	Nitrate Nitrogen	ppm	451	444	464	460	451	410	450	438
4	Phosphorus	%	0.10	0.18	0.11	0.08	0.10	0.09	0.09	0.10
5	Potash	%	2.36	2.03	1.80	2.00	1.73	3.03	2.10	1.47
6	Calcium	%	0.80	1.08	0.92	0.72	0.84	0.80	0.84	0.80
7	Magnesium	%	0.51	0.61	0.46	0.41	0.54	0.39	0.19	0.19
8	Sulphur	%	0.06	0.04	0.04	0.03	0.02	0.03	0.01	0.03
9	Zinc	ppm	22	39	29	18	21	22	22	30
10	Copper	ppm	22	33	23	27	30	21	29	32
11	Ferrous	ppm	139	150	107	169	103	104	107	139
12	Manganese	ppm	59	127	74	49	43	39	28	63
13	Boron	ppm	68.10	60.30	55.30	48.30	51.40	60.40	61.30	71.40
14	Molybdenum	ppm	0.68	0.61	0.71	0.78	0.83	0.71	0.65	0.63
15	Chloride	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	Sodium	%	0.08	0.09	0.06	0.05	0.04	0.04	0.07	0.09

Gf, *Glomus fasciculatum*; *Tv*, *Trichoderma Viride*; *Th*, *Trichoderma harzianum*; *Rhz*, *Rhizobium*; *Combination*: *Rhizobium meliloti* + *Bacillus megaterium* + *Fraturia aurentia*

Chemical analysis of *Jatropha curcas* L. showed highest in total nitrogen and molybdenum *Gf* + *Rhz* treatment and lowest total nitrogen in *Gf* and molybdenum in *Gf* + *Tv* treatment. While highest ammonical nitrogen was found in *Gf* and lowest in *Gf* + *Rhz*. Nitrate nitrogen was highest recorded in *Gf*+*Th* treatment and lowest in *Gf*+ bacterial combination treatment. Plant treated with *Gf* + *Tv* showed highest in phosphorus, calcium, magnesium, zinc, copper, manganese, and sodium while lowest phosphorus, calcium, and zinc was found in *Gf*+*Tv*+*Th*, magnesium in *Gf*+*Rhz*+ bacterial combination, copper in *Gf*+ bacterial combination, manganese in *Gf*+*Rhz*+ bacterial combination and sodium in *Gf*+*Rhz* treatment. Highest potash was recorded in *Gf* + bacterial combination treatment and lowest potash was observed in *Gf* + *Tv* + *Th* + *Rhz* + bacterial combination treatment. sulphur was found highest in *Gf* treatment and lowest in *Gf* + *Rhz* + bacterial combination treatment. While ferrous were found highest in *Gf* + *Tv*+*Th* treatment and lowest ferrous was showed in *Gf* + *Rhz* treatment. molybdenum was highest in *Gf* + and lowest was recorded in *Gf* + *Tv* treatment. Boron was highest in *Gf*+*Tv*+*Th*+*Rhz* + bacterial combination and lowest was recorded in *Gf* + *Tv*+*Th* treatment. When compared with other remaining treatments.

By observing the parameters studied it is seen that, combined application of different microorganisms (fungi and bacteria) resulted in synergistic effects on plant growth. It had positive impact on growth and development of leaves and shoot. Roots were seen aggressively grown when treated with *Gf* and combination of *Rhizobium meliloti* + *Bacillus megaterium* + *Fraturia aurentia*. Therefore, by observing the data recorded in the first six months, *Gf* + bacterial combination treatment had positive impact on growth and development of *Jatropha curcas* L. Merr. in its initial stage. However, more extensive study and research is required to observe the impact of the said treatment throughout the life cycle of *Jatropha curcas* L.

The results of this study demonstrate the positive impact of mycorrhizal fungi on the growth and development of *Jatropha curcas* L. These investigations have stressed the importance of AM fungal relationships in allowing successful recolonization and plant growth. There are several reports on the role of AM fungi in the revegetation of

mining (Hazarika et al., 2010, 2014; Agus et al., 2019). The outcome of the current study showed that environmental aspects, host species, and soil form strongly influenced spore making and AM fungal colonization. Related results have been noted from earlier studies (Muthukumar et al., 1998; Sivakumar, 2013; Vieira Junior et al., 2020). The present examination observed that AM colonization was condensed during the dry season (pre-monsoon). Results described in the present study approve with Halder et al. (2015), The structure and strength of AM colonization differ within plant populations and be influenced by on factors such as form and spatial accessibility of inoculum, season, phase of plant growth, susceptibility to inoculation, and plant nutritious status. Seasonal dissimilarities in spore populations in altered plant species have been reported previous (Halder et al., 2015). Eight strains boosted growth by ~50% over nitrogen-fertilized controls, highlighting their strong symbiotic efficiency (Wolde-Meskel et al., 2016). To establish a functioning N₂-fixing symbiosis, an ample number of root-nodulation bacteria in the soil is required, or they must be provided during sowing (Bhat and Bhatt, 2020). Further research is needed to elucidate the specific mechanisms underlying the observed growth responses and to explore the potential for optimizing the combined application of these fungi.

The study established that PSBs are active bio-fertilizers and found a sustainable alternative to chemical phosphorus fertilizers in recent agriculture (Kumar et al., 2023). Phosphate solubilizing bacteria have the capability to convert insoluble phosphorus compounds into bioavailable forms, helping better nutrient uptake and crop development (Sharma et al., 2023). PSB play a vital role in mobilizing insoluble phosphorus in the soil, assembly it nearby to plants and improving complete soil fertility (Rao et al., 2023). Phosphate solubilizing bacteria improve the bioavailability of phosphorus in soils by flouting down complex inorganic phosphate compounds over biological processes (Patel et al., 2023). PSBs are key bio-fertilizers that increase plant phosphorus uptake by changing insoluble forms into plant-available forms over microbial activity (Begum et al., 2023).

From pot experiment it has been observed that, *Jatropha curcas* L. showed good growth and development when *G. fasciculatum* was used with consortium of bacteria like *Rhizobium meliloti*, (PSB

and KMB). Dual inoculation with *Rhizobium* and *Glomus fasciculatum* significantly enhanced biomass, nitrogen, and phosphorus content, as well as nodule nitrogenase activity and chlorophyll content in *Sesbania grandiflora* compared to controls (Lalitha, 2015). The association of AMF helps nutrient uptake, soil stability, control root diseases and lowers water stress problems (Begum *et al.*, 2019). There are reports of enhanced correlation in between spore number and root colonization (Visen *et al.*, 2016). Keserwani *et al.* (2018) reported that seed borne mycoflora i. e. *Rhizopus* sp. and *Fusarium* sp. reduced the seedling vigour index of pea varieties whereas, fungicidal and biocontrol agent *Trichoderma* sp. increased the seedling vigour index by keeping seed borne mycoflora under check. Therefore, further study is required on association of *G. fasciculatum* with *Rhizobium meliloti*, other bacteria and *Trichoderma* Spp.

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