

Full Length Research Paper

## Influence of vesicular arbuscular mycorrhiza (VAM) and phosphate solubilizing bacteria (PSB) on growth and biochemical constituents of *Marsdenia volubilis*

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A field experiment was carried out to find out the effect of biofertilizers, vesicular arbuscular mycorrhiza (VAM), and phosphate solubilising bacteria (PSB) individually and in combination on growth and physiological attributing properties of *Marsdenia volubilis* plant under nursery conditions. The plant seedlings were harvested at various intervals: 30, 60 and 120 days after transplantation. The inoculation of microbial cultures VAM and PSB resulted in enhancement of growth parameters like plant height, root length, fresh weight and dry weight of shoot and root, leaves/plant, leaf area/plant, chlorophyll content, reducing and non-reducing sugars, starch, lipid and protein contents in root and shoot samples. These parameters were maximum with dual inoculation than individually. The results emphasize the importance of microbial biofertilizers inoculations for rapid growth of seedlings of plant (*M. volubilis*) in nurseries and illustrate the advantage of inoculating soils of low microbial population with indigenous microbes.

**Key words:** Biofertilizers, vesicular arbuscular mycorrhiza (VAM), phosphate solubilising bacteria (PSB), *Marsdenia volubilis*, growth and biochemical parameters.

### INTRODUCTION

Soil fertility is diminishing gradually due to soil erosions, loss of nutrients, accumulation of salts and toxic elements, water logging and unbalanced nutrient compensation. Organic wastes and biofertilizers are alternate sources to meet the nutrient requirement of crops. In recent years, biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture. Thus, biofertilizers are organic products containing specific microorganisms in concentrated forms, derived from the soil root zone (rhizosphere) (Mishra and Dadhich, 2010). Consequently, microbial fertilizers are considered as an important part of environment friendly

sustainable agricultural practices, with low cost inputs; mainly including nitrogen fixing, phosphate solubilizing, potash mobilizing and plant promoting microorganisms. Vesicular arbuscular mycorrhizal (VAM) fungi improve plant growth through phosphorous nutrition. In addition to phosphorous, they also help in the uptake of other nutrient elements. Nutrient absorption by fungal symbionts is due to external hyphae of the fungus proliferating beyond the nutrient depletion zone and reaching the source of nutrients. Mycorrhizal fungi appear to be extremely advantageous to crops grown in soils with low fertility. The improved plant growth is also attributed to the production

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**Abbreviations:** VAM, Vesicular arbuscular mycorrhizal; PSB, phosphate solubilizing bacteria.

of growth promoting substances, tolerance to drought, salinity and transplantation shock, resistance to soil-borne plant pathogens and synergetic interactions with other beneficial rhizosphere microorganisms. Phosphorous is one of the major plant nutrients limiting plant growth. Most agricultural soils contain large reserves of P, a considerable part of which has accumulated as a consequence of regular application of chemical fertilizers. However, a large proportion of soluble inorganic phosphate added to soil is rapidly fixed as insoluble forms soon after application and becomes unavailable to plants. Phosphorus and other major nutrients are involved in cell division and development, photosynthesis, breakdown of sugar, energy transfer, nutrient transfer within the plant and cell signal transduction (Sharma and Namdeo, 1999). There are several microorganisms which can solubilize the unavailable phosphorous. Bacteria like *Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomonas straita* are important phosphate solubilizing microorganisms. Many fungi, *Aspergillus* and *Pencillium* species are potential solubilizers of bound phosphates. They solubilize the bound phosphorous through secretion of organic acids and make it available to the plant, resulting in the improved plant growth and yield. Therefore, phosphate dissolving microorganisms play some part in correcting phosphorous deficiency in plantation soils. They may also release soluble inorganic phosphate into soil through decomposition of phosphate rich organic compounds. These microbial inoculants can substitute almost 20-25% of the phosphorous requirement of plants. In view of this, the supply of these elements to plant is essential for achieving optimum growth and crop yield.

In the present study, *Marsdenia volubilis* plant was selected due to its high medicinal value. *M. volubilis* is an important medicinal plant belonging to the family *Aselepiadaceae*. It is a tall woody climber, grows 11 m height and 95 cm in girth with dense lenticillate and pustular branches. This plant is widely used in ayurvedic medicine in India. The leaves are used for snake bites and to cure boils and abscesses as it has potent antimicrobial activity against a wide range of fungal and bacterial species which causes the diseases in human beings. The plant bark is widely used in the case of anorexia and nervous dyspepsia and roots and tender stalks are considered emetic and expectorant. The flowers and unripe fruits are eaten as vegetable. In view of medicinal importance of *M. volubilis*, there is a need to develop efficient, low cost cultivation methods for this plant which are suitable to various climatic conditions to obtain higher yield, hence there is a need to improve plantation of this tree, with implementation of organic farming and application of biofertilizers.

## MATERIALS AND METHODS

### Location of the study

The plants of *M. volubilis* were maintained under glass house

conditions in the medicinal plant garden of Botany Department, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The climate was warm and humid at the time of starting the experiment. There was monsoon rain for few days which gave the favorable climate for the seed germination. The weekly average maximum and minimum temperatures ranged between 27.1 to 36.2°C and 14.6 to 23.7°C, respectively, during the experimental period.

### Collection of biofertilizers

*Glomus mosseae* and *Bacillus megaterium* were obtained from Regional Biofertilizers Development Centre, Bangalore Division, India.

### Experimental design

The pot culture experiment was carried out under greenhouse conditions to know the response of *M. volubilis* plant to *G. mosseae* and *B. megaterium* inoculation. The *M. volubilis* plants were grown in plastic pots containing a sterilized mixture of soil and sand (1/1 w/w). The pots were placed according to a completely randomized design. Seeds of *M. volubilis* were surface sterilized with 0.05% sodium hypo chloride for 45 min before sowing them into a 5 cm depth of growth media. Five to six seeds were sown in each pot and after a week of germination time, they were thinned to one plant per pot. The plants were grown in a greenhouse under natural photoperiods (23.5/18°C day/night, 6000/4000 lux light intensity) for three months. Inoculum of *G. mosseae* (20 g/kg soil), and 20 ml of *B. megaterium* was laid around the seed.

The following treatments were established to know the response of *M. volubilis* to the inoculation with VAM fungi and phosphate solubilising bacteria (PSB): T<sub>1</sub>, Control (without inoculation of microorganisms); T<sub>2</sub>, inoculated with VAM (*G. mosseae*); T<sub>3</sub>, inoculated with PSB (*B. megaterium*); T<sub>4</sub>, inoculated with both *G. mosseae* and *B. megaterium*.

### Growth parameters

The growth parameters of *M. volubilis*, shoot length, root length, number of leaves, leaf area, fresh and dry biomass of shoot and root were measured on every 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day of the plant growth in all the treatments with or without biofertilizers.

### Physiological parameters

The physiological characteristics such as chlorophyll content, reducing and non-reducing sugars, starch, lipid and protein contents in root and shoot samples on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day were studied with and without inoculum treated plants. The biochemical properties, chlorophyll content (Arnon, 1949), starch (Mc Cready et al., 1950) carbohydrates (Highkin and Frankel, 1962) total lipids (Bligh and Dyer, 1959), and total proteins (Lowry et al., 1951) were estimated.

### Statistical analysis

Two-way analysis of variance (ANOVA) was carried out at a 0.05 level of significance on the data and SPSS version 13.0 was used. The values corresponded to each table in the results.

## RESULTS AND DISCUSSION

Influence of biofertilizers both VAM and PSB showed

**Table 1.** Effect of VAM fungi and PSB on shoot length (cm) of *M. volubilis*.

Treatment	Incubation days (after treatment)		
	30	60	90
T <sub>1</sub>	3.15 (0.40)	5.40 (0.36)	10.80 (0.60)
T <sub>2</sub>	5.67 (0.25)	10.40 (0.40)	21.10 (0.56)
T <sub>3</sub>	4.63 (0.45)	9.87 (0.50)	18.77(0.45)
T <sub>4</sub>	6.23 (0.65)	11.60 (0.60)	24.37 (0.61)
LSD	0.87	0.90	1.05
SE	0.37	0.72	1.52

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 2.** Effect of VAM fungi and PSB on root length (cm) of *M. volubilis*.

Treatment	Incubation days (after treatment)		
	30	60	90
T <sub>1</sub>	5.13 (0.40)	9.57 (0.31)	17.37 (0.45)
T <sub>2</sub>	14.10 (0.46)	20.53 (0.55)	27.83 (0.55)
T <sub>3</sub>	12.60 (0.50)	17.53 (0.65)	22.00 (2.00)
T <sub>4</sub>	14.77 (0.65)	22.87 (0.35)	30.50 (0.40)
LSD	0.96	0.92	2.04
SE	1.17	1.28	1.41

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 3.** Effect of VAM fungi and PSB on leaf number of *M. volubilis*.

Treatment	Incubation days (after treatment)		
	30	60	90
T <sub>1</sub>	3.87 (0.31)	5.33 (0.31)	8.47 (0.31)
T <sub>2</sub>	5.43 (0.45)	8.43 (0.31)	12.0 (0.26)
T <sub>3</sub>	4.83 (0.25)	7.40 (0.30)	11.60 (0.20)
T <sub>4</sub>	6.0 (0.20)	9.63 (0.40)	13.60 (0.40)
LSD	0.60	0.63	0.57
SE	0.13	0.38	0.52

Values with in the brackets indicate standard deviation. Each value represents mean of six replications.

significant effect on growth and physiological characteristics of *M. volubilis*. The data presented in Tables 1, 2, 3, 4, 5 and 6 indicate that biofertilizers had significant effect on shoot length, root length, fresh weight of shoot and root, dry weight of shoot and root, leaves / plant, leaf area of plant. All characteristics under study were significantly higher in combined inoculation of VAM and PSB (T<sub>4</sub>), than other inoculations and control. The biofertilizers treated plants exhibited increased shoot length compared to un-inoculated plants. The maximum shoot length was recorded in T<sub>4</sub> plants (24.37 cm) at 90 days of plant whereas the co-inoculation of biofertilizers (T<sub>4</sub>) exhibited maximum root length (30.50 cm) and the root length was

found minimum (5.13 cm) in T<sub>1</sub> treatment after 90 days. The maximum number of leaves were observed in T<sub>4</sub> treatment (13.60) followed by T<sub>2</sub> (12.00) and T<sub>3</sub> (11.60). In contrast, least leaves were counted in the control (T<sub>1</sub>). The leaf area differed significantly in treated plants compared to the control. On the 30<sup>th</sup> day, the maximum leaf area was found in T<sub>4</sub> plants (34.1 cm<sup>2</sup>) and the minimum in T<sub>1</sub> (22.42 cm<sup>2</sup>). On the 60<sup>th</sup> and 90<sup>th</sup> day, inoculated individually PSB or VAM or in combination performed better compared to control (T<sub>1</sub>). The maximum leaf area was recorded in T<sub>4</sub> plants whereas the least leaf area was observed with control. The plant biomass was improved along with increasing the incubation periods.

**Table 4.** Effect of VAM fungi and PSB on leaf area (cm<sup>2</sup>) of *M. volubili*.

Treatment	Incubation days (after treatment)		
	30	60	90
T <sub>1</sub>	22.42 (0.00)	29.05 (0.56)	36.52 (1.14)
T <sub>2</sub>	30.66 (0.00)	42.57 (0.99)	57.40 (1.50)
T <sub>3</sub>	26.54 (0.00)	35.37 (0.66)	48.56 (0.97)
T <sub>4</sub>	34.10 (0.00)	45.99 (1.52)	59.43 (1.90)
LSD	0.00	1.90	2.68
S E	0.00	1.99	2.75

Values with in the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 5.** Effect of VAM fungi and PSB on fresh biomass of *M. volubilis*.

Treatment	Incubation days (after treatment)								
	Shoot fresh biomass (g)			Root fresh biomass (g)			Total fresh biomass (g)		
	30	60	90	30	60	90	30	60	90
T <sub>1</sub>	0.64 (0.08)	1.01 (0.03)	1.88 (0.04)	0.34 (0.07)	0.64 (0.10)	1.14 (0.08)	0.96 (0.14)	1.32 (0.12)	3.15 (0.06)
T <sub>2</sub>	1.06 (0.10)	2.63 (0.06)	3.96 (0.13)	0.51 (0.02)	1.09 (0.15)	1.60 (0.09)	1.60 (0.17)	3.63 (0.14)	5.64 (0.19)
T <sub>3</sub>	1.00 (0.11)	2.58 (0.23)	3.92 (0.10)	0.49 (0.01)	0.93 (0.07)	1.44 (0.05)	1.48 (0.12)	3.21 (0.23)	5.24 (0.20)
T <sub>4</sub>	1.37 (0.17)	3.19 (0.29)	4.33 (0.14)	0.60 (0.02)	1.23 (0.11)	1.73 (0.07)	1.93 (0.13)	4.15 (0.27)	6.07 (0.21)
LSD	0.23	0.36	0.21	0.08	0.21	0.14	0.27	0.37	0.33
SE	0.08	0.25	0.29	0.03	0.07	0.05	0.11	0.33	0.34

Values with in the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 6.** Effect of VAM fungi and PSB on dry biomass of *M. volubilis*.

Treatment	Shoot dry biomass (g)			Root dry biomass (g)			Total dry biomass (g)		
	Incubation days (after treatment)								
	30	60	90	30	60	90	30	60	90
T <sub>1</sub>	0.13 (0.04)	0.25 (0.05)	0.74 (0.09)	0.07 (0.01)	0.11 (0.03)	0.20 (0.01)	0.18 (0.02)	0.39(0.11)	0.89 (0.06)
T <sub>2</sub>	0.45 (0.09)	0.83 (0.05)	1.46 (0.11)	0.14 (0.01)	0.25 (0.06)	0.57 (0.08)	0.55 (0.07)	1.03 (0.16)	1.97 (0.13)
T <sub>3</sub>	0.37 (0.09)	0.65 (0.05)	1.25 (0.11)	0.11 (0.01)	0.24 (0.06)	0.55 (0.13)	0.51 (0.13)	0.99 (0.22)	1.77 (0.11)
T <sub>4</sub>	0.50 (0.11)	0.92 (0.06)	1.65 (0.24)	0.15 (0.01)	0.35 (0.10)	0.65 (0.09)	0.63 (0.10)	1.23 (0.22)	2.15 (0.17)
LSD	0.16	0.10	0.28	0.02	0.12	0.17	0.17	0.35	0.23
SE	0.05	0.07	0.11	0.01	0.03	0.06	0.06	0.11	0.15

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 7.** Effect of VAM and PSB on chlorophyll content of *M. volubilis*.

Treatment	Chlorophyll 'a' (mg/g)			Chlorophyll 'b' (mg/g)			Total Chlorophyll (mg/g)		
	Incubation days (after treatment)								
	30	60	90	30	60	90	30	60	90
T <sub>1</sub> (Control)	0.58 (0.04)	0.69 (0.04)	0.85 (0.02)	0.95 (0.02)	1.05 (0.03)	1.15 (0.03)	1.53 (0.21)	1.95 (0.01)	2.15 (0.03)
T <sub>2</sub> (VAM)	0.77 (0.03)	0.96 (0.02)	1.23 (0.03)	1.26 (0.04)	1.39 (0.03)	1.63 (0.03)	2.11 (0.03)	2.38 (0.02)	2.45 (0.03)
T <sub>3</sub> (PSB)	0.68 (0.02)	0.86 (0.03)	1.10 (0.04)	1.19 (0.04)	1.34 (0.03)	1.44 (0.04)	2.09 (0.03)	2.28 (0.02)	2.41 (0.02)
T <sub>4</sub> (VAM +PSB)	0.86 (0.03)	1.0 (0.04)	1.34 (0.08)	1.31 (0.03)	1.45 (0.02)	1.77 (0.03)	2.39 (0.03)	2.48 (0.02)	2.91 (0.03)
LSD	0.05	0.06	0.09	0.06	0.05	0.06	0.20	0.03	0.05
S E	0.02	0.02	0.03	0.03	0.03	0.06	0.06	0.03	0.07

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

The improvement of growth parameters in the present study may be due to functions of biofertilizers, availability of nitrogen, phosphorous, and certain growth hormones like auxins, gibberlins, vitamins and organic acid secreted by bioinoculants which increase the surface area per unit root length and were responsible for root hair. Similarly, reports were made by Gupta et al. (1999), Ahmad et al., (2004), Nandre et al. (2005), Chadrasekar et al. (2005), Nabila et al. (2009), Zaki et al. (2010) and Abou El-Yazeid and Abou-Aly (2011).

The influence of biofertilizers on biochemical properties was studied. Content of Chlorophyll a, b and total chlorophyll were estimated and shown in Table 7. Maximum chlorophyll a, b were observed in T<sub>4</sub> (1.34, 1.77) and least in T<sub>1</sub> (0.85, 1.15). There was a significant difference in chlorophyll-a, chlorophyll-b and total chlorophyll content among the treatments and different days. This may be due to the increase in stomatal conductance and carbon assimilation (Levy and Krikun, 1980). Krishna and Bagyaraj (1981) observed that bundle sheath chloroplasts were larger and numerous in mycorrhizal plant. Increased chlorophyll 'a', chlorophyll 'b' and total chlorophyll content were also reported by Mathur and Vyas, (2000) Bhoopander Giri et

al. (2003), Kate et al. (2005) and Senthilkumar and Sivagurunathan (2012).

Maximum amount of carbohydrates were observed in plants treated with biofertilizers individually and combined form. With increasing plant incubation days, the reducing sugar content also improved ranging from 1153.42 to 1317.07 µg/g in T<sub>4</sub> plants whereas the least in T<sub>1</sub> in all treatments except control as shown in Tables 8 and 9. The polysaccharide starch content was also maximum in T<sub>4</sub> in all incubation days than the control. Improvement in carbohydrate content in all biofertilizers treated plants may be due to Increased carbon fixation, activation of enzymes and increased photosynthetic rate increased reducing and non reducing sugar contents in different mycorrhizal plants was observed by Krishna and Bagyaraj (1981), Mathur and Vyas (2000) and Nelson and Achar (2001).

The influence of biofertilizers on lipid content in shoot and root parts of *M. volubilis* is shown in Table 10. The maximum lipid content was recorded in shoot samples of T<sub>4</sub> (9.61, 17.39 and 26.77 mg/g) and minimum in the control plants. Similarly, the lipid contents in roots of *M. volubilis* studied in selected plants results are shown in Table 10. The increments in lipid content of bio-

fertilizer inoculated plants were due to the formation of lipid bodies in arbuscular trunks and intercellular hyphae. In this mutualistic symbiosis, the fungus acquires carbon as hexose within the root and stores predominantly as triacylglycerol. Stimulation of mycorrhizal activity in presence of PSB may attributes for more lipid content in dual inoculated plants.

The total protein content was estimated in plants parts treated with biofertilizers individually or in combination of both. The protein content in shoot and roots of T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> plants on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> days were significantly higher when compared to protein content of Control (T<sub>1</sub>) plants. Maximum shoot protein content was recorded in T<sub>4</sub> plants and minimum in control (Table 11). Significant increase in the protein content of both shoot and root tissue of inoculated plants over control plants attributes to the accumulation of more Nitrogen and phosphorous in treated plants. Maximum protein content in dual inoculated plants is due to the increase of plant membrane proteins and/or to the presence of proteins from the fungal partner. Similar reports were made by Mathur and Vyas (2000), Nelson and Achar (2001) and Shehata and Khawas (2003), Senthilkumar and Sivagurunathan (2012).

**Table 8.** Effect of VAM fungi and PSB on carbohydrate content in shoot of *M. volubilis*.

Treatment	Reducing sugar ( $\mu\text{g/g}$ )			Non-reducing sugar ( $\mu\text{g/g}$ )			Starch ( $\text{mg/g}$ )		
	Interval days ( after treatment)								
	30	60	90	30	60	90	30	60	90
T <sub>1</sub> (Control)	709.24 (10.27)	850.24 (15.20)	926.58 (28.65)	433.22 (11.90)	474.39 (21.12)	554.08 (6.79)	14.35 (0.39)	19.33 (0.38)	24.94 (0.70)
T <sub>2</sub> (VAM)	888.10 (10.95)	1099.21 (10.00)	1112.91 (1.52)	524.92 (8.59)	571.23 (9.45)	595.89 (4.45)	20.29 (0.12)	28.14 (0.19)	31.42 (0.66)
T <sub>3</sub> (PSB)	762.43 (9.05)	1040.03 (10.92)	1105.44 (10.53)	521.87 (6.86)	542.70 (10.07)	582.62 (7.23)	19.84 (0.14)	26.30 (0.39)	30.53 (0.14)
T <sub>4</sub> (VAM +PSB)	1153.42 (7.40)	1184.80 (6.19)	1317.07 (15.09)	542.00 (6.48)	574.70 (8.09)	602.37 (7.78)	25.46 (0.49)	31.71 (0.16)	37.63 (0.17)
LSD	17.94	20.84	32.14	16.46	24.99	12.61	0.61	0.56	0.92
SE	51.79	37.16	41.89	12.97	12.57	5.83	1.19	1.36	1.36

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 9.** Effect of VAM fungi and PSB on carbohydrate content in roots of *M. volubilis*.

Treatment	Reducing sugar ( $\mu\text{g/g}$ )			Non-reducing sugar ( $\mu\text{g/g}$ )			Starch ( $\text{mg/g}$ )		
	Interval days ( after treatment)								
	30	60	90	30	60	90	30	60	90
T <sub>1</sub> (Control)	298.94 (2.51)	496.09 (5.99)	668.03 (5.41)	315.69 (2.91)	351.03 (4.33)	446.84 (5.59)	4.88 (0.31)	5.72 (0.12)	6.07 (0.15)
T <sub>2</sub> (VAM)	464.00 (4.37)	764.08 (4.56)	825.84 (3.81)	550.97 (3.84)	566.66 (3.34)	618.00 (3.00)	6.17 (0.07)	11.26 (0.04)	13.40 (0.13)
T <sub>3</sub> (PSB)	397.87 (2.69)	714.69 (5.89)	831.00 (3.61)	534.61 (3.21)	558.14 (4.72)	606.27 (0.49)	5.98 (0.06)	8.56 (0.05)	9.35 (0.12)
T <sub>4</sub> (VAM +PSB)	555.03 (2.38)	775.49 (6.17)	851.55 (2.74)	584.45 (6.70)	619.39 (5.16)	653.81 (4.44)	6.65 (0.10)	12.68 (0.05)	14.84 (0.09)
LSD	5.84	10.73	7.56	8.35	8.37	7.31	0.32	0.13	0.23
SE	28.21	34.07	22.16	31.94	30.92	24.00	0.20	0.80	1.04

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 10.** Effect of VAM fungi and PSB on total lipid content of *M. volubilis*.

Treatment	Shoot lipid content ( $\text{mg/g}$ )			Root lipid content ( $\text{mg/g}$ )		
	Interval days ( after treatment)					
	30	60	90	30	60	90
T <sub>1</sub> (Control)	5.72 (0.04)	9.44 (0.08)	15.63 (0.09)	4.07 (0.08)	6.53 (0.10)	10.52 (0.09)
T <sub>2</sub> (VAM)	8.44 (0.07)	15.46 (0.05)	25.22 (0.41)	7.13 (0.11)	10.18 (0.06)	13.98 (0.15)
T <sub>3</sub> (PSB)	8.33 (0.07)	15.49 (0.06)	23.44 (0.08)	5.15 (0.05)	9.65 (0.07)	13.17 (0.07)
T <sub>4</sub> (VAM +PSB)	9.61 (0.06)	17.39 (0.06)	26.77 (0.07)	8.18	11.54 (0.12)	15.66 (0.10)
LSD	0.11	0.12	0.41	0.15	0.17	0.20
SE	0.21	0.42	0.69	0.40	0.44	0.45

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

**Table 11.** Effect of VAM fungi and PSB on total protein content of *M. volubilis*.

Treatment	Shoot protein content (mg/g)			Root protein content (mg/g)			Total protein content (mg/g)		
	Interval days ( after treatment)								
	30	60	90	30	60	90	30	60	90
T <sub>1</sub> (Control)	3.52 (0.05)	4.57 (0.06)	5.20 (0.05)	0.82 (0.07)	1.12 (0.05)	1.34 (0.03)	4.33 (0.11)	5.88 (0.02)	6.84 (0.02)
T <sub>2</sub> (VAM)	6.28 (0.06)	6.55 (0.07)	6.81 (0.06)	1.56 (0.08)	1.77 (0.04)	1.99 (0.06)	7.84 (0.14)	8.32 (0.11)	8.80 (0.12)
T <sub>3</sub> (PSB)	5.28 (0.09)	5.89 (0.05)	6.21 (0.07)	1.44 (0.05)	1.65 (0.03)	1.78 (0.04)	6.72 (0.05)	7.54 (0.08)	7.99 (0.11)
T <sub>4</sub> (VAM +PSB)	6.53 (0.06)	7.20 (0.05)	8.10 (0.09)	1.69 (0.05)	1.98 (0.05)	2.32 (0.07)	8.22 (0.10)	8.85 (0.05)	10.42 (0.10)
LSD	0.12	0.11	0.13	0.11	0.08	0.09	0.20	0.14	0.17
S E	0.24	0.16	0.23	0.10	0.07	0.08	0.34	0.23	0.31

Values within the brackets indicate standard deviation. Each value represents mean of six replications.

## Conclusion

In this study, treatment of biofertilizers in combination with *G. mosseae* and *B. megaterium* significantly enhanced the growth parameters which included, shoot length, root length, leaves, leaf area, biomass of root and shoot and biochemical constituents, total chlorophyll, carbohydrate lipid and protein content in *M. volubilis* when compared to the control.

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