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Co-Inoculation Studies of Vesicular Arbuscular Mycorrhizal Fungi (VAM) and Phosphate Solubilizing Bacteria (PSB) On Nutrient Uptake of *Marsdenia volubilis* (T. Cooke)

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Abstract: The co-inoculation effect of vesicular arbuscular mycorrhizal (VAM) fungi, and phosphate solubilizing bacteria (PSB) on nutrient uptake of *Marsdenia volubilis* was carried out in this study. The plant seedlings harvested at various incubation days (30, 60, and 90) transplantation with two different treatments (individual VAM and PSB) resulted in uptake of shoot and root nutrients content, the dual inoculation (VAM and PSB) showed an excellent improvements in uptake of nutrients like N, P, K, Ca, Mg, Fe, Mn and Zn concentrations in *Marsdenia volubilis* than single applications.

Keywords: VAM, PSB, *G. mosseae*, *B. megaterium*, nutrient uptake

Introduction

Rhizosphere is a heterogenous, continuous and natural habitat in which different types of interactions occur between soil microbes and plants. The beneficial plant microbe interaction in the rhizosphere is the primary determinants of plant health and soil fertility. Microbial fertilizers are considered as an important part of environmental friendly sustainable agricultural practices, with low cost inputs; nitrogen fixing, phosphate solubilizing, potash mobilizing and plant growth promoting microorganisms [1]. Application of biofertilizers to the soil/seed accelerates the extent of nutrient availability supplements the demand of chemical fertilizer to some extent and enhances the growth and biomass of plant. Nutrient absorption by VAM fungi is due to external hyphae of the fungus proliferating beyond the nutrient depletion zone and reaching the source of nutrients [2]. Impact of microorganisms may not be very prominent under normal conditions, because the microorganisms occurring in the soil are generally insufficient. Efficiency of biofertilizers can be increased using mixtures of bio preparations as N-fixers, Phosphate solubilizers, Potash solubilzers as well as mycorrhizal fungi [3, 4]. Recently VAM and PSB are most widely spread biofertilizers significantly contributing N, P, K and other nutrients to plants and also providing resistance to drought situation [5].

Marsdenia volubilis is an important medicinal plant belongs to the family *Aselepiadaceae.* It is tall woody climber, grows 11m height and 95cm in girth with dense lenticillate and pustular branches. This is widely used in ayurveda system of medicine in India. The leaves are used for snake bites and to cure boils and abscesses as it has antimicrobial activity against a wide range of fungal and bacterial cells. The bark is widely used in the case of anorexia and nervous dyspepsia. The roots and tender stalks are considered emetic and expectorant. The flowers and unripe fruits are eaten as vegetable. However very limited or no information is available regarding the effect of biofertilizers on the yield and major nutrient contents in *Marsdeniavolubilis*. In view of the above facts the present investigation was undertaken to recommend improved technology of biofertilizers application on the yield and nutrient content in *Marsdenia volubilis.*

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 favourable climate for the seed germination. The weekly average maximum and minimum temperatures ranged between 27.1° C to 36.2° C and 14.6° C to 23.7°C respectively during the experimental period.

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Collection of biofertilizers

The VAM fungi, *Glomus mossae* and PSB, *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 *Marsdenia volubilis* plant to co-inoculation of *G.mosseae* and *Bacillus megaterium*. The *Marsdenia volubilis* plants were grown in plastic pots containing a sterilized mixture of soil and sand (1/1 w/w) ratio. 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 (23.5/18ºC day / night, 6000 / 4000 lux light intensity) for three months. Inoculum of *Glomus mosseae* (20 gms/kg soil), and 20 ml of *B. megaterium* was laid around the seed.

The following treatments were conducted to know the response of *Marsdeniavolubilis*to the inoculation with VAM fungi and PSB.

T_1: Control (without VAM and PSB)
T_2: Inoculated with Vesicular Arbuscular Mycorrhizal fungi (VAM) (*G.mosseae*)
T_3: Inoculated with Phosphate Solubilizing Bacteria (PSB) (*B. megaterium*)
T_4: Inoculated with both *G.mosseae and B.megaterium*

Nutrient analysis

Periodical data on nutrient parameters were recorded at 30, 60, 90 days interval. The shoot samples were randomly collected per treatment plot (from all the replicates) using a clean knife, and the roots were carefully dug up with a hand-held hoe. The plant tissues were separated into root and shoot thoroughly washed with distilled water, oven-dried at 70 °C for 48 hours and ground using a Thomas stainless-steel milling machine. The total nitrogen was estimated according to Markham method [6]. Phosphorous content of plants was analyzed colorimetrically following the Vanadomolybolate method [7]. The potassium content in plants was determined by flame photometry. The concentrations of Ca and Mg in each plant digestion sample were measured using an AAS, whereas the concentration of K was measured using a flame photometer. The micro elements (Fe, Mn and Zn) concentrations in the roots and shoots were determined using an AAS. The nutrient concentrations obtained were multiplied by the mean dry weights of the roots and shoots to obtain the nutrient uptake per treatment.

Results and Discussion

The effect of the different treatments (single, VAM and PSB) on shoot and root nutrients content of plant is presented in Table 1, 2, 3, 4. All nutrient concentrations were significantly influenced by the application of the microbial inoculations. The dual inoculation (T_4) resulted in significantly ($p < 0.05$) higher N, P, K, Ca, Mg, Fe, Mn and Zn concentrations than the single application. The control (T_1) treatment gave the lowest concentrations of N, P, K, Ca, Mg, Fe, Mn and Zn compared to the other treatments. The increase in the nutrient contents by the application of biofertilizers might be due to their influence in enhancing the reactive surface of the soil [8].

Seedlings inoculated with PSB+VAM displayed improved maximum nitrogen uptake (1.73% in shoots and 1.32% in roots) over control and other treatments. Seedlings inoculated with VAM, PSB, VAM+PSB improvement in phosphorous uptake was maximum in T4 (0.72%) followed by T2 (0.62%) and T3 (0.66%) and T1 (0.45%). Potassium uptake was (0.33%) higher in T4 inoculated seedlings but at the same time it was also found lower (control) in some cases (Table 1). Calcium uptake was improved in all treatments under study and recorded maximum improvement (1.39%) in seedlings inoculated with VAM+PSB. Magnesium concentration was maximum in dual inoculated seedlings. The improvement in uptake of iron was maximum (913.03 ppm) in VAM+PSB inoculated seedlings followed by VAM, PSB alone. Manganese and zinc uptake was also found higher in seedlings inoculated with different biofertilizers and improvement was noticed maximum up to 142.50 ppm and 26.23 ppm in VAM+PSB inoculated seedlings respectively. The same trend was observed in root samples also.

Table 1: Effect of VAM fungi and PSB on major nutrient contents (%) in shoots of *M.volubilis*

Treatments	Nitrogen			Phosphorous			Potassium			Calcium			Magnesium		
	Incubation days after treatment														
	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
T1	1.15 (0.04)	1.25 (0.03)	1.37 (0.06)	0.27 (0.03)	0.38 (0.03)	0.45 (0.04)	0.08 (0.02)	0.14 (0.01)	0.22 (0.03)	1.06 (0.03)	1.17 (0.03)	1.22 (0.03)	0.12 (0.02)	0.25 (0.02)	0.32 (0.03)
T2	1.48 (0.02)	1.58 (0.04)	1.70 (0.06)	0.51 (0.04)	0.60 (0.02)	0.66 (0.05)	0.17 (0.03)	0.21 (0.03)	0.27 (0.03)	1.23 (0.03)	1.35 (0.03)	1.40 (0.01)	0.20 (0.04)	0.34 (0.02)	0.48 (0.03)
T3	1.40 (0.02)	1.51 (0.03)	1.65 (0.05)	0.44 (0.02)	0.54 (0.02)	0.62 (0.03)	0.15 (0.04)	0.19 (0.04)	0.24 (0.04)	1.19 (0.03)	1.29 (0.03)	1.34 (0.04)	0.27 (0.03)	0.31 (0.02)	0.40 (0.02)
T4	1.55 (0.04)	1.65 (0.03)	1.73 (0.05)	0.56 (0.02)	0.63 (0.03)	0.72 (0.04)	0.19 (0.04)	0.28 (0.04)	0.33 (0.03)	1.31 (0.03)	1.39 (0.03)	1.49 (0.05)	0.34 (0.02)	0.39 (0.02)	0.51 (0.03)
LSD	0.05	0.06	0.10	0.05	0.05	0.08	0.06	0.06	0.06	0.05	0.05	0.06	0.05	0.04	0.05
SE	0.02	0.02	0.02	0.03	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01

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

Table 2: Effect of VAM fungi and PSB on major nutrient contents (%) in roots of *M.volubilis*

Treatments	Nitrogen			Phosphorous			Potassium			Calcium			Magnesium		
	Incubation days after treatment														
	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
T1	0.72 (0.07)	0.82 (0.03)	0.92 (0.04)	0.18 (0.04)	0.26 (0.04)	0.42 (0.04)	0.17 (0.02)	0.22 (0.03)	0.30 (0.01)	0.29 (0.03)	0.38 (0.01)	0.42 (0.05)	0.18 (0.04)	0.25 (0.04)	0.38 (0.04)
T2	0.95 (0.04)	1.09 (0.03)	1.18 (0.04)	0.38 (0.06)	0.53 (0.06)	0.62 (0.03)	0.23 (0.03)	0.26 (0.02)	0.36 (0.04)	0.38 (0.02)	0.46 (0.02)	0.6 (0.02)	0.25 (0.03)	0.35 (0.03)	0.45 (0.05)
T3	0.88 (0.04)	1.00 (0.06)	1.17 (0.07)	0.29 (0.03)	0.49 (0.05)	0.57 (0.03)	0.21 (0.02)	0.24 (0.02)	0.33 (0.04)	0.35 (0.03)	0.52 (0.02)	0.64 (0.02)	0.23 (0.03)	0.34 (0.02)	0.44 (0.02)
T4	1.13 (0.05)	1.22 (0.05)	1.32 (0.02)	0.35 (0.04)	0.52 (0.06)	0.62 (0.03)	0.27 (0.02)	0.31 (0.03)	0.42 (0.03)	0.43 (0.03)	0.58 (0.03)	0.68 (0.03)	0.33 (0.02)	0.45 (0.04)	0.55 (0.04)
LSD	0.09	0.08	0.09	0.08	0.10	0.06	0.04	0.04	0.06	0.05	0.04	0.06	0.06	0.06	0.07
SE	0.05	0.05	0.05	0.03	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Value with in the brackets indicates standard deviation.
Each value represents mean of six replications.

TABLE 3: Effect of VAM fungi and PSB on iron, manganese and zinc contents (ppm) in shoot of *M.volubilis*

Treatments	Iron			Manganese			Zinc		
	Incubation days after treatment								
	30	60	90	30	60	90	30	60	90
T_1	869.77 (0.93)	878.07 (0.31)	884.27 (5.06)	127.80 (0.60)	131.83 (0.75)	137.10 (0.70)	7.87 (0.70)	11.20 (0.70)	16.20 (0.66)
T_2	891.43 (1.05)	903.40 (0.82)	910.53 (0.70)	134.03 (0.45)	136.47 (0.60)	141.13 (0.70)	12.87 (0.65)	17.13 (0.75)	20.00 (0.56)
T_3	886.37 (1.17)	903.97 (52.63)	915.33 (0.45)	131.97 (0.50)	134.63 (0.75)	139.20 (0.30)	14.30 (0.46)	18.43 (0.65)	21.90 (0.26)
T_4	897.30 (0.66)	906.53 (0.70)	913.03 (0.55)	136.07 (0.50)	137.83 (0.60)	142.50 (0.46)	17.10 (0.70)	21.20 (0.80)	26.23 (0.55)
LSD	1.83	49.64	4.87	0.98	1.28	1.07	1.20	1.37	1.00
S E	3.10	8.23	3.46	0.93	0.70	0.63	1.02	0.89	0.91

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

Table 4: Effect of VAM fungi and PSB on iron, manganese and zinc contents (ppm) in roots of *M.volubilis*

Treatments	Iron			Manganese			Zinc		
	Incubation days after treatment								
	30	60	90	30	60	90	30	60	90
T_1	1481.43 (2.57)	1495.53 (4.69)	1505.43 (0.60)	138.47 (0.61)	146.40 (0.20)	158.17 (0.70)	25.13 (0.61)	32.27 (0.61)	38.33 (0.31)
T_2	1528.40 (0.56)	1546.47 (3.70)	1563.33 (1.53)	144.73 (0.50)	158.33 (0.25)	171.30 (0.82)	37.30 (0.66)	45.53 (0.45)	53.10 (0.36)
T_3	1505.57 (1.11)	1518.20 (0.60)	1532.67 (3.06)	142.37 (0.23)	154.60 (0.40)	165.20 (0.60)	34.70 (0.36)	42.83 (0.40)	50.77 (0.45)
T_4	1531.43 (0.80)	1564.70 (4.36)	1589.00 (1.00)	151.00 (0.56)	165.80 (0.40)	172.13 (0.47)	39.57 (0.35)	48.70 (0.36)	57.67 (0.42)
LSD	2.79	7.00	3.40	0.94	0.61	1.25	0.97	0.88	0.73
SE	6.08	8.02	9.50	1.38	2.11	1.70	1.06	1.16	1.24

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

In the present study enhanced nitrogen levels in VAM and PSB treated plants attributes to the enhanced nitrate reductase activity in host, release of hydrolytic enzymes which mobilize nitrogen and decompose organic material present in the soil in presence of phosphate solubilizing bacteria. In addition the extra radical hyphae which extend beyond the depletion zones of the host root increases the transfer rate of nutrients such as N [9]. In this study the enhanced levels of phosphorous in the plant tissue in VAM treated plants attributes to the production of phosphate solubilizing enzymes which catalyzes the release of inorganic phosphorous from organic complexes in the soil. In addition the increased surface area of the external mycelium resulted in more phosphorous acquisition. The further increase of P in dual inoculation was due to the further solubilization of the P by PSB through the production of organic acids, ion chelation, increasing root cell permeability, and interacting with VAM supporting its establishment and function. The studies carried out by Lal [10] also emphasized the enhanced P uptake in mixed inoculation compared to VAM alone. Apart from well known effect of VAM fungal associations on phosphate uptake, the external hyphae of VAM can also provide a good delivery system for Mg, Fe and Mn [11]. In the present study low concentration of K, Zn, Ca, Mg, Fe and Mn in control plants is because of absence of mycorrhizal association with the root system of these plants. Greater mobilization of these nutrients in mycorrhizal plants attributes to mass flow and root interception to the extent permitted by greater absorption of water and improved root growth [12]. It has been shown that mycorrhizal plants can extract more water from soil and have higher root hydrolytic conductivity than non mycorrhizal plants [13, 14, 15]. In addition, the mycorrhizal plants are able to extract more nutrients from the soil through their extra matricular mycelium which facilitates the easier absorption of these nutrients. Enhanced Ca and Mg acquisition probably resulted from chelation of Ca^{2+} and Mg^{2+} by organic acids. Plants may secrete compounds that complex Fe-sideorophores. These Fe-complexes may be absorbed intact by the plant increasing the supply of Fe to the plant. The Mycorrhizal plants are known to differ from their non-mycorrhizal counterparts in the rate of exudation and some contribution of these components to improved uptake of nutrients can be expected. These components of the exudates such as amino, phenolic and carboxylic acids can promote easier diffusion of these nutrients by forming soluble complexes. The plant roots because of mycorrhizal establishment change the chemical composition of root exudates and these are often source of nutrients to associate in mycorrhizosphere [17, 18]. The high nutrient concentration in the plants treated with VAM and PSB is because of stimulation of VAM fungi by the PSB as some soil bacteria have been shown to directly influence AM fungal germination and growth rate. Specific bacteria together with AM fungi may create a more direct synergism that supports more nutrient acquisition [19]. The present findings are in consistence with other author's findings [20, 21, 22, 23,].

Conclusion

The present study clearly shown that the co-inoculation of AM fungi, and PSB played a significant role in improving the growth and nutrient uptake of *M.volubilis* seedlings there by producing good quality planting stock. The plants which were treated with VAM and PSB exhibited more levels of macronutrients like nitrogen, phosphorous, potassium, calcium and magnesium and micronutrients such as iron, manganese and zinc. These seedlings may perform better growth, survival and more biomass production in nutrient impoverished soil.

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