Research Article

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SYNERGISTIC INTERACTION BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI, *RHIZOBIUM* AND PHOSPHATE SOLUBILISING BACTERIA ON *VIGNA UNGUICULATA* (L) VERDC.

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Abstract: Green house pot experiments were conducted to evaluate the effect of Arbuscular Mycorrhizal (AM) Fungi (*Funneliformis mosseae*) along with the dual inoculation of AM fungi (*Funneliformis mosseae*) with *Rhizobium*, Phosphate solubilising bacteria (PSB) and a triple inoculation of AM Fungi *(Funneliformis mosseae)*, *Rhizobium*, and PSB in *Vigna unguiculata* (L) Verdc. Growth parameters such as plant height, dry weight of root and shoot, spore number, per cent root colonization, number of nodules was recorded and P and N uptake were estimated at the intervals of 15, 30 and 45 days. Results revealed that inoculation of AM Fungi (*Funneliformis mosseae*) + *Rhizobium*+ PSB showed an increase in all the growth parameters when compared with dual inoculation. The combined inoculation of bacteria and AM fungi evidence provide that these two organisms are synergistically involved in the beneficial effects of *Vigna unguiculata* (L) Verdc.

Keywords: *Vigna unguiculata*, *Funneliformis mosseae*, phosphate solubilising bacteria, growth parameters.

INTRODUCTION

Arbuscular mycorrhiza (AM) is one of the most efficient bioinoculant in improving growth and N content in legumes. Legumes play a fundamental role in natural ecosystems (Jeffries and Barea, 2001). Legumes have a higher P requirement for nodule formation, nitrogen fixation and optimum growth. Mycorrhizal condition of legume crops found to increase its vegetation in addition to improve nodulation. However, legumes grow rapidly but the success of these species will depend on their ability to symbiotically fix nitrogen content of the plant along with the dual inoculation of AM fungi. Nitrogen is a non-metallic element needed for formation of amino acids, purines and pyrimidines, and thus indirectly involved in protein and nucleic acid synthesis. It is also a part of porphyrins and many coenzymes of the plant system. Soil microorganisms and their activities play important roles in transformation of plant nutrients from unavailable to available forms and also have many metabolic qualities related to soil fertility improvement. Mycorrhiza benefits the host through mobilization of phosphorus from non-labile sources, whereas rhizobia fixes N_2 (Scheublin and Vander Heijden, 2006). Biofertilizer have recently gained with momentum for effecting the sustainable increase in crop yield under various agroclimatic conditions

Arbuscular mycorrhizal fungi are significant plant-growth-promoting organisms, as they not only improve the nutritional status of their hosts but also protect these hosts from pathogens and allow the hosts to survive under adverse conditions (Aruna and Lakshman, 2007; Shwetha *et al.*, 2013). Though the use of biofertilizers optimizes the yield, the aim of this study was to determine the role played by bacteria associated with AM fungi in the interaction of AM fungi with its plant hosts.

MATERIALS AND METHODS

The experiment was arranged in completely randomized block design with three replication of each treatment. AM fungal spores of *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler comb. nov. were maintained in a greenhouse using Jowar (*Sorghum vulgare* L.) as host for mass multiplication in 30-cm diameter pots containing sterilized sand–soil mix (1:1 v/v) and were used as inoculum. The biofertilizers Phosphate solubilising bacteria (PSB) and *Rhizobium* were collected from the microbiology laboratory, UAS, Dharwad, India. *Rhizobium* inoculation was done by treating seeds with a peat based culture before sowing. 3 ml of culture suspension culture of PSB was inoculated and the treatments were as follows a. Control b. AMF + *Rhizobium* c. AMF+PSB d. AMF+*Rhizobium*+PSB. A non-inoculated control was maintained. The plants were exposed to sunlight and were kept free of weeds and irrigated properly. The plants were harvested after 15, 30 and 45 days. The percentage of AM fungal colonization was evaluated microscopically followed by clearing of roots in 10% KOH, neutralized in 2% HCL and stained with 0.05% tryphan blue in lactophenol according to the method described by (Phillips and Hayman, 1970) and root colonization was calculated by the formula mentioned below

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$$\% \text{ of Root colonization} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

The growth parameters like plant height, dry weight of shoot and root, number of nodules, spore number, per cent root colonization, P and N uptake was determined. AM fungal spores were counted in 50g of soil by wet sieving and decanting method (Gerdmann and Nicholson, 1963). The phosphorous content in the shoots in terms of percentage was determined according to Vandomolybolate phosphoric yellow colour method (Jackson, 1973). Total nitrogen content was determined by the Microkjeldahl method (Bremmer, 1960).

RESULTS AND DISCUSSION

The inoculation of AM fungi (*Funneliformis mosseae*) with PSB and *rhizobium* on growth parameters increased significantly over the uinoculated- control plants. After 15 days, plants inoculated with AMF+PSB+*Rhizobium* resulted in higher plant height (39.3 cms), dry weight of shoot (0.34 g) and root (0.26 g), spore number (61.0), number of nodules (38.3), per cent root colonization (64.9) compared to plants inoculated with dual inoculations of AMF+*Rhizobium*, AMF+PSB.

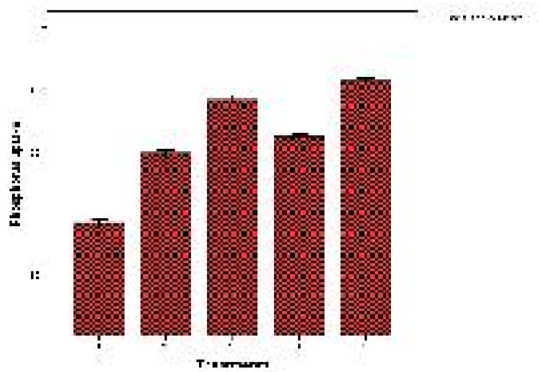
The phosphorous and nitrogen content was also recorded higher in plants inoculated with AMF+PSB+*Rhizobium* than the other treatments. In dual inoculation spore number, percentage of root colonization, number of nodules and P uptake was found to superior in the plants inoculated with AMF+*Rhizobium*, than AMF+PSB. After 45 days, the plant growth responded in the similar trend that is the triple inoculation of AMF+PSB+*Rhizobium* resulted in the highest plant height (66.7 cms), dry weight of shoot (0.55 g) and root (0.45 g), per cent root colonization (81.6 %), Spore number (85.3), no. of nodules (50.6) (Table 1), P (0.41%) and N (0.28%) uptake (Figures 2 and 3). All rhizobacterial+AM treatments showed a significant increase in shoot dry weight compared to dual inoculations after 45. This significant increase can be attributed to the positive interaction between rhizobacterial inoculants and AM fungus. Plant growth, shoot P concentration, and root colonization were evaluated colonized or not by several AM fungal species. Combined inoculation of PSB and *Rhizobium sp.* produces a positive response significantly increasing nodulation (Parmar and Dufresne, 2011).

The study showed that the degree to which each of these species was affected by mycorrhizal colonization varied with the host and the colonizing AM species (Burleigh *et al.*, 2002).

Figure 1. Effect of AM fungi and other microorganisms in *Vigna unguiculata* (L) Verdc.

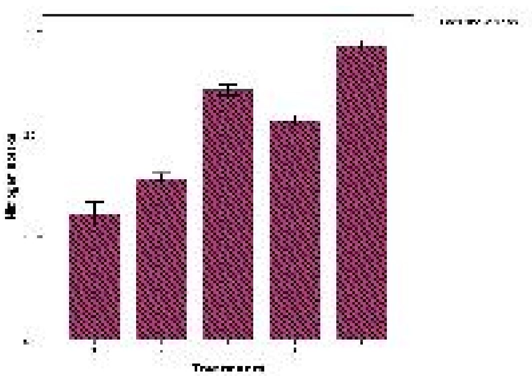
1. Control
2. AM fungi
3. AM fungi+PSB
4. AM fungi+*Rhizobium*
5. AM fungi+ *Rhizobium* + PSB

Figure 2: Showing P Uptake in *Vigna unguiculata* (L) Verdc.



1. Control 2. AM fungi 3. AM fungi+Rhizobium 4. AM fungi+PSB 5 .AM fungi+ *Rhizobium*+ PSB

Figure 3: Showing N uptake in *Vigna unguiculata* (L) Verdc.



1. Control 2. AM fungi 3. AM fungi+Rhizobium 4. AM fungi+PSB 5 .AM fungi+ *Rhizobium*+ PSB

Nodule number and biomass has been shown to increase significantly in several studies due to coinoculation of both microsymbionts (Saxena *et al.*, 1997; Zhao *et al.*, 1997). Like all symbiotic parameters, yield of legumes co-inoculated with AM and rhizobia has been reported to increase significantly when compared to un inoculated or inoculated with either microsymbiont (Corbera and Hernandez, 1997). Legumes, plant species of great agronomical and ecological interest, are able to establish beneficial symbiotic relationships with two types of soil-borne microorganisms: N_2-fixing bacteria and mycorrhizal fungi. Like most of the major plant families, legume plants also form associations with arbuscular mycorrhizal (AM) fungi (Barea *et al.*, 2004). Data with these literature support that in the light of present finding, have clearly demonstrated that when legumes symbiose with both rhizobia, AM-fungi and other beneficial microorganisms, plant growth, yield, and nitrogen nutrition are generally much greater than plants inoculated either with rhizobia or AM fungi alone or PSB alone (Antunes and Goss 2005). Coinoculation of AM fungi with other beneficial microorganisms can provide plants with a more balanced nutrition and improved absorption of nitrogen, phosphorus and other nutrients, and improve plant growth and yield compared to single inoculation (Lakshman, 2011). These findings are in agreement with that of Aysan and Demir (2009), Askar and Rashad (2010) and Xiurong *et al.*, (2011). It is well known that AM fungi can improve the nutrient status of their host plants (Smith and Read, 2008). It is also thought that the plant–*rhizobium* system benefits from the presence of AM fungi because the mycorrhizae ameliorate not only P deficiency but also any other nutrient deficiencies that might be limiting to *rhizobium*.

CONCLUSION

Most of the interaction studies between AM fungi and beneficial microorganisms suggest a synergistic effect on growth and yield of plants. The present study have clearly shown that the combined application of beneficial microorganisms like AMF+PSB+*Rhizobium* played a significant role in improving the growth response and nutrient uptake of *Vigna unguiculata* (L) Verdc. seedlings. Therefore, their use as biofertilizers for agriculture improvement has been beneficial to numerous researchers.

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Table 1: Effect of AM fungi and other microorganisms in *Vigna unguiculata*

Treatments	Plant height (cms)	DWS (g)	DWR (g)	Spore number	No. of nodules	% root colonization
			15 days			
Control	25.3±0.03e	0.15±0.00e	0.23±0.00e	0.00±0.00e	22.3±0.33e	0.00±0.00e
AM Fungi	28.3±0.03d	0.19±0.00d	0.15±0.00d	48.6±0.33d	25.3±0.33d	48.3±0.33d
AM fungi+ *Rhizobium*	36.5±0.03b	025±0.00b	0.21±0.00b	58.3±0.33b	33.3±0.33b	55.6±0.33b
AM fungi+ PSB	31.2±0.03c	0.21±0.00c	0.18±0.00c	52.2±0.33c	29.0±0.57c	50.3±0.33c
AM fungi+ *Rhizobium*+ PSB	39.3±0.03a	0.34±0.00a	0.26±0.00a	61.0±0.33a	38.3±0.33a	64.9±0.33a
			30 days			
Control	29.4±0.03e	0.20±0.00e	0.04±0.00e	0.00±0.00e	26.3±0.33e	0.00±0.00e
AM Fungi	33.6±0.03d	0.28±0.00d	0.28±0.00d	57.6±0.33d	32.0±0.57d	58.6±0.33d
AM fungi+ *Rhizobium*	41.4±0.03b	0.36±0.00b	0.35±0.00b	68.9±0.33b	40.0±0.57b	65.4±0.33b
AM fungi+ PSB	38.8±0.03c	0.31±0.00c	0.30±0.00c	64.5±0.33c	36.3±0.33c	60.3±0.33c
AM fungi+ *Rhizobium*+ PSB	46.7±0.03a	0.42±0.00a	0.41±0.00a	75.2±0.33a	45.6±0.33a	75.8±0.33a
			45 days			
Control	38.2±0.03e	0.34±0.00e	0.08±0.00a	0.00±0.00e	36.3±0.33e	0.00±0.00e
AM Fungi	45.6±0.03d	0.40±0.00d	0.33±0.00d	64.3±0.33d	42.5±0.33d	65.6±0.33d
AM fungi+ *Rhizobium*	56.4±0.03b	0.48±0.00b	0.41±0.00b	76.3±0.33b	46.6±0.33b	71.0±0.57b
AM fungi+ PSB	50.1±0.03c	0.46±0.00c	0.42±0.00c	71.3±0.33c	44.3±0.33c	68.6±0.33c
AM fungi+ *Rhizobium*+ PSB	66.7±0.03a	0.55±0.00a	0.45±0.00a	85.3±0.33a	50.6±0.33a	81.6±0.33a

Means sharing letter in common are not significantly different according to Duncan's test P <0.05

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