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Research Article

The inoculation effect of Phosphate Solubilizing Bacteria and AM Fungi on growth and Nitrogen uptake in *Vigna cylindrica (L.) Skeel.*, (L.) Skeels

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Abstract

The influence of a phosphate solubilizing bacteria (*Bacillus polymyxa*) and arbuscular mycorrhizal fungus (*Rhizophagus fasciculatus*) on growth and phosphorus uptake of *Vigna cylindrica* (L.) Skeel., were studied under green house conditions using both sterilized and unsterilized garden soil. AM Fungi and P-solubilizing bacteria inoculated to sterilized soil produced significantly higher growth, biomass, and increase in nodule number and N uptake in root. Moderate or lower growth response was recorded among the plants grown in unsterilized soil with either PSB or AM Fungi inoculation. And thus, a synergistic effect was recorded with increased plant dry matter, nodule number and N uptake in the plants inoculated in sterilized soil as compared to the plants inoculated in unsterilized soil.

INTRODUCTION

Soil is composed of diverse array of microorganism; most of them are beneficial to plants and interact with arbuscular mycorrhizal fungi. Crop production in agricultural system is treated as ecological component. The unity of ecological web of life is fragile and agricultural practice is not possible without some disturbance. Mycorrhizal hyphae are still relatively little known strands of this web; they do not yet figure prominently in the literature and discussions of agro- ecology. Plants can uptake immobile nutrient elements, for example, phosphorus (P) in soil through mycorrhiza, the symbiosis between mycorrhizal fungi and plant roots. There is always a considerable increase in the mycorrhizal plants in assessment to non-mycorrhizal plants (Lakshman *et al.*, 2001). Many studies showed that mycorrhizal fungi could enhance crops and uptake of phosphorus (Li *et al*., 2006; Antunes *et al*., 2007; Xiao Tong *et al.*, 2010). AM fungi are important plant-growth-promoting organisms, as they not only get better nutrition for their hosts but also protect the hosts from various pathogens by that they give the resistance against them to survive under adverse conditions (Aruna and Lakshman, 2007; Shwetha *et al.*, 2013; Lakshman, 2014; Maya *et al*., 2015; Chaitra *et al*., 2016). Recent studies have revealed that nodulation by indigenous rhizobia is greatly improved by AM Fungi (Hayman, 1982). Root exudates in mycorrhizal legumes may act as chemotactic attractants to rhizobia (Abbot and Robson, 1977; Jyoti and Lakshman, 2017). Recent researches on plant nutrition through PSB and AM fungi have amply demonstrated that these organisms play an important role in uptake of nutrient from the marginal soils. Research in the last two decades has established that dual inoculation of phosphate solubilizing bacteria and AM fungi stimulates plant growth.

Phosphate solubilizing bacteria solubilize insoluble P and help plants to absorb and translocate more soluble phosphate (Azcon-Aguliar *et al.*, 1986). No research work was directed on *Vigna cylindrica* (L.) Skeel., so far. Hence, the present study investigated the efficiency of phosphate solubilizing bacteria and VA-mycorrhizal fungi on the growth and P uptake of butterfly pea plants.

MATERIALS AND METHODS

Source of AM Fungal inoculum:

The Green house experiments were conducted in the Post Graduate Department of Botany, Karnataka University, Dharwad-580003 India. *Bacillus polymyxa*, a phosphate solubilizing bacteria, was procured from the Department of Agricultural Microbiology U.A.S. Dharwad-580003 India. The AM fungus *Rhizophagus fasciculatus* was multiplied with Sudan grass (*Sorghum vulgare* var. *sudaneese*) as a host plant. Soil based AM fungal inoculum was established and maintained in pot culture.

Seedlings were raised in earthen pots containing 4 Kg of sterilized and unsterilized garden soil in separate pots. Each experimental pot measuring 20×25cm in diameter. The soil separately used to the pot experiments were a sandy loam with a pH of 6.7, EC 0.13 mmhas/cm, organic carbon 0.38%, available N 199 kg/ha, available K 204 kg/ha and available P 4.6 kg/ha. One week old seedlings of *Vigna cylindrica* (L.) Skeel., were inoculated with PSB (0.5mg) and *Rhizophagus fasciculatus* (AM) inoculum (10g), around the root system. 10g of AM inoculum contains colonized chopped root bits (5g) and 5g soil of the host plants which consisted of spores (approximately 250/50g soil). The combinations of treatments were given below

1. Un-inoculated control
2. *Bacillus polymyxa*
3. *Rhizophagus fasciculatus*
4. *B. polymyxa* + *Rhizophagus fasciculatus*

Growth parameters:

The following observations were recorded on 60 days old seeding grown in green house. Plant height, dry weight of shoot and root, nodule number was recorded. Dry weight of root and shoot was determined after drying at 70^0C for 48 hrs under hot air oven.

Estimation of Arbuscular Mycorrhizal spores:

AM fungal spores were recovered from the rhizosphere soil of *Vigna cylindrica* (L.) Skeel., of both sterile and non sterile experimental pots, by adopting wet sieving and decanting method described by (Gerdemann and Nicolson, 1963). Mycorrhizal spore number / 50g of rhizospheric soil were estimated by using the procedures described by (Gionvannetti and Mossae, 1980) were recorded for all inoculated *Vigna cylindrica* (L.) Skeel.

Root Colonization

Per cent root colonization of *Vigna cylindrica* (L.) Skeel., was evaluated microscopically followed by clearing of roots in 10% KOH solution and staining with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970). The following formula was used to calculate the root colonization (Giovannetti and Mosse, 1980).

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

Total nitrogen content was determined by the Microkjeldahl method (Bremmer, 1960).

RESULTS AND DISCUSSION

The data in terms of plant height, dry matter and P uptake recorded at 60 days of plant growth in both sterilized and unsterilized soils are presented in Tables 1 and 2. *Vigna cylindrica* (L.) Skeel., plant biomass, percent of AM Fungi colonization, spore population was greatly improved by the inoculation of PSB and AM Fungi. This kind of variation has been observed in both inoculated and un-inoculated plants with respect to sterilized soil. The inoculation of *Rhizophagus fasciculatus* with PSB on growth parameters increased significantly over the unioculated plants. After 60 days, plants inoculated with AMF+PSB shows maximum plant height (56.8 cm), shoot dry weight (17.9 g), root dry weight (2.9g), spore number (178.5/50gsoil), number of nodules per plant (12.3) and per cent root colonization (72.9%) compared to non-inoculated plants or single inoculations of AMF or PSB in sterilized soil on the contrary values were not appreciable, of those plants inoculated with AMF+PSB resulted plant height (35.4 cm), dry weight of shoot (10.9 g) and root (0.98), spore number (112/50g soil), number of nodules

(8.2/plant), per cent root colonization (48.6) in plants maintained in experimental pots of unsterilized soil conditions.

The bacteria *Bacillus polymyxa* in combination with *Rhizophagus fasciculatus* brought a significant increase in plant height, per cent of AM Fungal colonization, spore population and N uptake in sterilized soil than unsterilized soils (Fig.1 and Fig. 2) The response was higher due to combined inoculation and it indicates that, the synergistic effect of two organisms shows better nutrient uptake and stability for the plan growth and development. The results indicated that, the dual inoculation could be used for better growth and increased number of root nodules in *Vigna cylindrica* (L.) Skeel., under greenhouse conditions. Interaction between AM fungi and certain rhizospheric microorganisms results in synergistic effect leading to the growth and enhancement of various plant growth parameters (Bagyaraj, 1984). Similar interactions between PSB and AM Fungi observed in the present study are in confirmation with results of earlier workers (Barea *et al*., 1975; Sattar and Gaur, 1989; Lakshman, 1996; 2014; Jyoti and Lakshman, 2017) on lavender, maize and lentils. The present study concludes that PSB under specific conditions act as phosphate solubilizer and made it available for the plants and AM fungus provided mobilizes nutrients to plants, which improved plant nutrient uptake and growth. The dual inoculated plants in sterile soil showed maximum biomass yield in terms of the above mentioned parameters over the un-inoculated (control) plants. AM fungi transverse soil with their extraradical mycelium (Swetha *et al*., 2013). This helps in the transport of P to the host roots. As absorbance P helps in the energy spending process of Nitrogen fixation in root nodules.

It is well known that AM fungi can improve the nutrient status of their host plants (Smith and Read, 2006). Co-inoculation of AM fungi with beneficial microorganisms like PSB can provide plants with balanced nutrition and improved absorption of phosphorus and other nutrients, and improve plant growth and yield compared to single inoculation (Lakshman, 2011). The stimulation of microbial activity by AM fungi has been postulated in recent years. Because of the microbial activity of this region is influenced by different organic matter released by the AM fungal colonized roots (Mukerji and Chamola, 2003; Maya *et al*., 2015). The results of present experiments are similar to that of earlier workers (Aysan and Demir, 2009; Askar and Rashad, 2010; Shwetha *et al.*, 2013 and Mahshad Hamidi, 2016).

Table 1: Effect of PSB and AM Fungi inoculation on growth, dry matter, nodule number and P uptake of 60 days old *Vigna cylindrica* (L.) Skeel., in sterilized garden soil.

Treatments	Plants height (cm)	Shoot dry weight (g/pl.)	Root Dry weight (g/plant)	% Root colonization	Spore count/50 g soil	Nodule number/ plant	N uptake in shoot (ppm)
Un-inoculated (control)	17.2a	2.8b	0.68b	-	-	0.91a	29c
PSB	35.1c	10.4a	0.89b	12.1a	30.4c	5.1b	48b
AM Fungi	39.9b	13.6d	1.9d	49.7e	128.8b	8.4c	91a
PSB+AMF	56.8g	17.9b	2.9e	72.9a	178.5c	12.3d	112c

Table 2: Effect of PSB and AM Fungi inoculation on growth, dry matter, nodule number and P uptake of 60 days old *Vigna cylindrica* (L.) Skeel., in unsterilized garden soil.

Treatments	Plants height (cm)	Shoot dry weight (g/pl.)	Root Dry weight (g/plant)	% Root colonization	Spore count/50 g soil	Nodule number/ plant	N uptake in shoot (ppm)
Un-inoculated (control)	9.6a	0.98b	0.46a	-	-	0.7b	27b
PSB	28.7b	4.8a	0.86b	10.3b	28.2b	3.8a	81c
AM Fungi	32.4ab	5.8b	0.87b	34.2d	87.0e	4.9c	86b
PSB+ AMF	35.4a	10.9e	0.98b	48.6g	112d	8.2c	102a

Fig. 1: The effect of PSB and *Rhizophagus fasciculatus* inoculation on Nitrogen uptake in sterile and unsterile soil at 60 days old *Vigna cylindrica* (L.) Skeel.

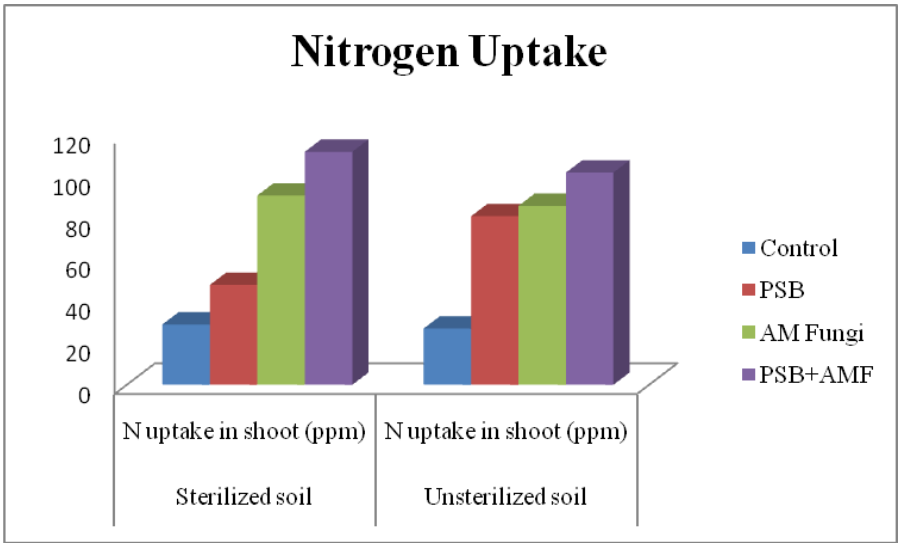
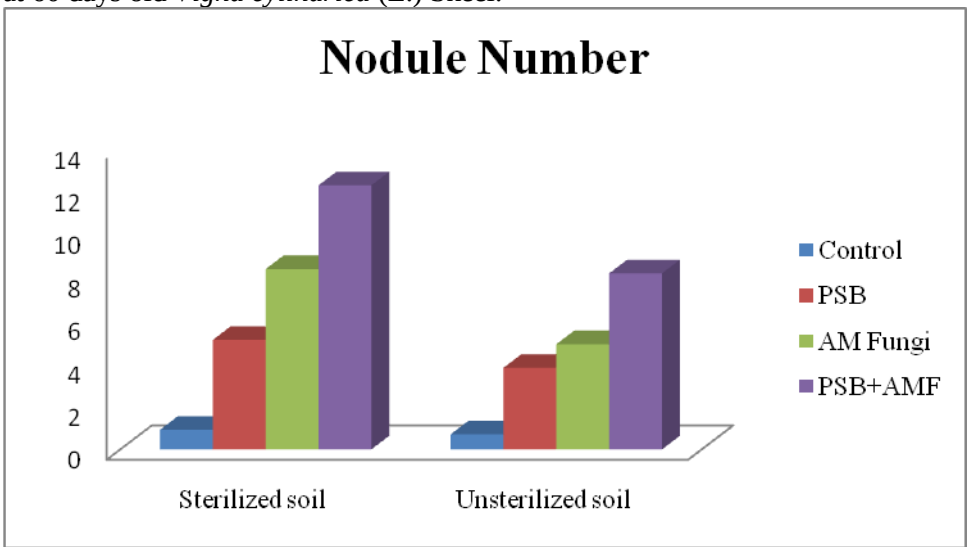


Fig. 2: The effect of PSB and *Rhizophagus fasciculatus* inoculation on Nodule number in sterile and unsterile soil at 60 days old *Vigna cylindrica* (L.) Skeel.



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