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● *RESEARCH ARTICLE* ●

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Effect of bioinoculants on *Phyllanthus reticulatus* poir. a medicinal plant raised through stem cutting

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ABSTRACT

The effect of AM fungus, PSB and *Azotobacter* was evaluated on *Phyllanthus reticulates* Poir. plants grown on sterilized soil. Plant aerial biomass phosphorus content in shoots were evaluated upon 60 day old harvested plants. Positive mycorrhizal colonization was recorded of the inoculated with *Glomus fasciculatum*. Although lower root colonization with higher P and N concentration was observed in plants treated with *Bacillus polymyxa*. Higher values of aerial biomass and plant height of the plants were seen in the treatments of co-inoculated with three organisms. The results confirms the synergistic effects between AM fungus, phosphate solubilizing bacteria (*Bacillus polymyxa*) a nitrogen fixer (*Azotobacter*) was most beneficial to plants raised through stem cuttings of *Phyllantus reticulates* Poir.

Key words : *Glomus fasciculatum*, *Bacillus polymyxa*, *Azotobacter* and *Phyllanthus reticulates* Poir

INTRODUCTION

Mycorrhizas constitute a symbiotic association between the roots of a wide variety of facultative host plants and this obligate symbiotic fungi belonging to the phylum Glomeromycota, class Glomeromycetes (Schiifler *et al.,* 2001). Arbuscular mycorrhiza form universal symbiosis which can be established with over 80% of plant species, including most of agricultural crops as well as herbaceous and scrublands species in natural ecosystems (Barea *et al.,* 2005). In this association, the fungus receives part of the syntheses produced by the plant (Sanders and Tinker, 1971) and increases the root absorption area by means of the extension of the extra radical mycelium (Barea, 1991; Tarafdar and Kumar, 1996), thus allowing nutrient absorption, especially in soils with low fertility.

Most agricultural crops are potential host plants for arbuscular mycorrhizal (AM) fungi. AM fungi increase the exploitation of the soil volume by the hyphal network, which increases the active absorption surface and spread beyond the phosphate depletion zone (Martin *et al.,* 2001). Mycorrhizal hyphae have a higher affinity for phosphate as expressed in the Michaelis-Menten equation by a lower Km value and absorb P at lower solution concentrations than roots do (Lange Ness and Vlek, 2000).

The combined inoculation of an arbuscular mycorrhiza-forming fungi and a phosphorus-solubilizing microorganism has demonstrated a better uptake both of native P from the soil and of the P coming from the phosphoric rock (Cabello *et al.,* 2005).

The aim of the present study was to evaluate the effect of AM fungus *Glomus fasciculatum*, a phosphate solubilizer *Bacillus polymyxa* and *Azotobacter* on growth of *Phyllanthus reticulatus* Poir. to study their interaction in potted green house conditions.

MATERIALS AND METHODS

Eight cm stem cutting of *Phyllanthus reticulatus* Poir. was taken from a old healthy plants grown in sterile soil with P deficiency. Each stem cutting was surface-sterilized with sodium hypochlorite (10% v/v) for 10 min and thoroughly rinsed with sterilized water. Earthen pots measuring 20×15 cm were filled with 5 kg sandy loam soil of (1:1) ratio. The chemical composition of the used soil shown in (Table 1).

The AMF strain used was *Glomus fasciculatum.* Inoculum consisted of rhizospheric soil from *Sorghum vulgare* L. plant pot culture that contained spores (10 g dry soil), mycelia and colonized root fragments were inoculated.

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The phosphorus-solubilizing microorganism (PSM) was the bacteria *Bacillus polymyxa* and *Azotobacters* cultured cellular mass prepared in a semi solid states paste comprising 8×10^5 cells and pasted at the end stem cuttings of planting end.

Table 1 : Garden soil characters used for pot experiments

Properties	Available
Texture	Sandy loom
pH	6.8
EC (ds/m)	3.4
Available P	5.0 ppm
Available N	0.13 %
Available K	13.5

Each treatment was replicated five times. These pots were placed in a greenhouse at 24 ± 1^0C day/ night 20 ± 1^0C. Plants were watered on alternate day and they were harvested once in a 60 days. Leaves and stems were used to determine aerial plant biomass through dry weight at 70^0C until constant weight. P content in leaves was estimated according to (Jackson, 1973). The root system of *P. reticulatus* Poir. was cleared and stained (Phillips and Hayman, 1970), and the colonization percentage was calculated according to procedure of Giovannetti and Mosse (1980). For spore extraction 100g of soil was subjected to wet sieving and decanting method (Gerdemann and Nicolson, 1963). The microbial inoculation effect was calculated according to Bagyaraj (1992) as below mentioned formula.

$$\text{Microbial inoculation effect (MIE)} = \frac{\text{DW inoculated plant - DW non-inoculated plant}}{\text{DW inoculated plant*}} \times 100$$

RESULTS AND DISCUSSION

Microscopic observations of stained roots showed mycorrhizal presence in AMF-inoculated treatments and where mycorrhizal absence, in pots that did not receive mycorrhizal inoculum. The AMF spore number in 100g dry soil was significantly different treatment as when inoculated with *G. fasciculatum*.

The highest percentage of colonized root was observed in the treatment inoculated with *G. fasciculatum* and *Azotobacter* without PSB. This treatment differed significantly from that inoculated only with *G. fascilatum* either or without PSB shown in (Table 1 and Fig. 1).

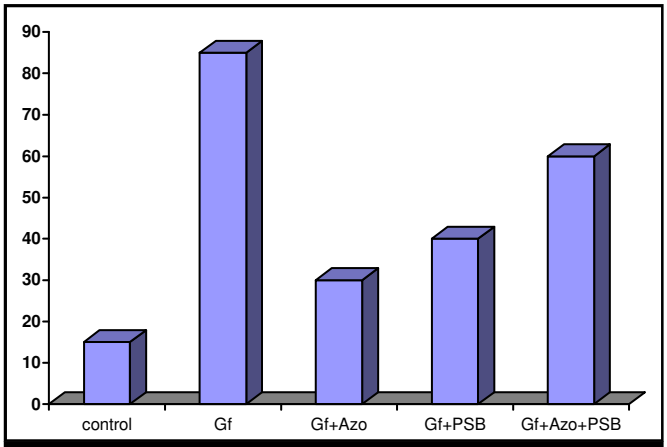

Fig. 1 : Showing per cent of root colonization of *Phyanthus reticulates* Poir. with different bioinoculants for 60 days

Values are the means of five replicates. Means in the same column followed by the same letter do not significantly differ between treatments according to ANOVA and LSD test ($P < 0.05$), respectively. The highest dry weight of root was observed in the inoculated plants compared to control, showing significant differences in relation with treatment of *Azotobacter* and *Glomus fasciculatum* or phosphate solubilizing bacteria (*Bacillus polymyxa*) and inoculated with *G. fasciculatum* with or with Azo+PSB, respectively. Values of P content in aerial biomass from treatments 3 and 4 with PSB were significantly different from those in which plants grew without mycorrhiza inoculation. Higher sporulation rate of *G. fasciculatum* in treatment 2 without *Azotobacter*

Table 2 : Effect of AM fungs, *Azotobacters* and phosphate solubilizer PSB on growth, dry weight of shoot, root and P content of shoot of *Phyllanthus reticulates* Poir. At 60 days

	Plant height (cm)	Shoot dry weight (g)	% of root (g) colonization	Dry weight P (µ/gplant root (g) content)	
Control (1)	32.3 a	0.5 ab	-	0.1 c	2800 a
Gf (2)	30.6 a	0.4 a	41.5b	0.2 a	3500 a
Gf (3)+Azo	42.3 b	0.9 e	69.3c	0.2 abc	3500 a
Gf (4)+PSB	35.8 ab	0.6 be	43.8a	0.2 ab	4700 b
Gf(5) + Azo + PSB	35.9 ab	0.7 cd	48.7d	0.3 be	5000 b
LSD P<0.05	43.4 b	0.8 d	0.07a	0.2 abc	4400 b

or PSB with a low nutritional state of the host plant, reflected in the growth parameters evaluated and the low content of phosphorus in the shoot biomass. It has been observed that under these conditions of nutritional stress, carbohydrate flow from the plant to the fungus decreases and, as a consequence, sporulation is stimulated (Lakshman, 2009). Although the differences in height and biomass production of plants inoculated with *G. fasiculatum* were not significant as compared to controls, a smaller size of inoculated plants was evident (Table 1). These results agree with those observed (Cardoso *et al.,* 1986; Lakshman, 1996) and could be explained as a competence between the mycorrhizal fungus and the plant for carbohydrates (So and Smith, 1988). However, it was observed that the co-inoculation with *G. fasciculatum* and *Azotobacter* improved *phyllantus* biomass path in the treatments.

In the treatment where plants were inoculated with both microorganisms and that were not inoculated with *G. fasciculatum* of shoot biomass was significantly higher than in the other treatments; this additionally supports the synergic effect of *G. fasciculatum* and *Azotobacter* which turned out to be beneficial for plant growth.

Plants with AM fungi increase the exploitation of the soil volume by the hyphal network, which increases the active absorption surface shown in (Fig.2). This increase in soil volume explored by arbuscular mycorrhizal fungi is evident by a lower production of radical biomass (Sieverding, 1991; Martin *et al.,* 2001). P-solubilizing microorganisms dissolve non-available forms of this nutrient by excretion of organic acids and quelant substances (Kucey *et al.,* 1989; Kapoor, 1995).

It may be concluded that soil cultures with nutritional deficit can be improved by mycobization with arbuscular mycorrhiza-forming fungi and P-solubilizing *Bacillus polymyxa*, which would allow plants to obtain P in insoluble forms such as phosphoric rock (soils with undissolved phosphate). However, more studies are necessary in order to evaluate the potential of co-inoculation and the application of *Azotobacter* under different agroclimatic conditions deserves special analysis.

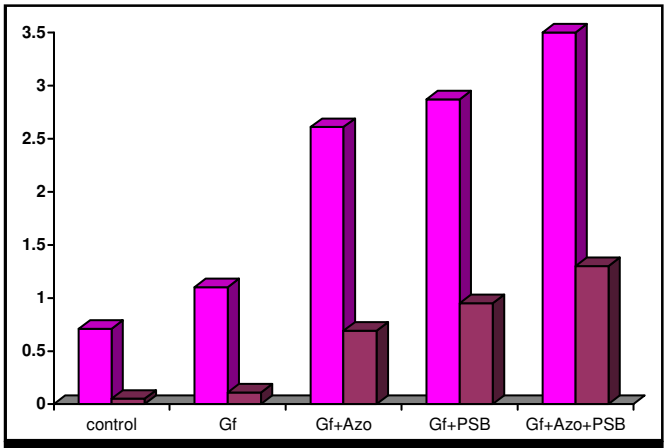

Fig. 2 : Showing dry wt of shoot of *Phyanthus reticulatus* Poir. and P content in shoot with different bioinoculants for 60 days

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