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Effect of Interactions of Three Growth-promoting Microorganisms on VAM Colonization, Spore Density, Plant Growth and Nutrient Accumulation in Tomato *(Lycopersicon esculentum)* Seedlings

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ABSTRAK

Kajian dibuat terhadap interaksi Azospirillum brasilense *dan* Bacillus megaterium *var.* phospharicum *dan* Glomus fasciculatum *dalam rizosfera tanaman tomato. Tidak terdapat perbezaan yang signifikan dalam parameter pertumbuhan tanaman antara rawatan-rawatan inokulat - VAM. Tanaman-tanaman yang dinokulat dengan fosfobakteria jelas mempunyai tunas yang lebih panjang yang mana bersamaan dengan VAM dan cantuman VAM + fosfobakteria yang lain. Cuma* Azospirullum *atau fostobakteria sahaja menambahkan biojisim tanaman dibandingkan dengan kawalan tak inokulat. Indeks VAM jelas menurun dengan penambahan fosfobakteria. Pemekatan nutrien tisu tidak berbeza antara rawatan.*

ABSTRACT

Interactions of Azospirillum brasilense *and* Bacillus megaterium *var.* phosphaticum *and* Glomus fasciculatum *in the rhizosphere of tomato plants were studied. There was no significant difference in plant growth parameters between VAM-inoculated treatments. Plants inoculated with the phosphobacteria had significantly higher shoot length, which was equivalent to VAM and other VAM + phosphobacteria combinations.* Azospirullum *or phosphobacteria alone increased plant biomass compared with the uninoculated control. VAM index was significantly reduced with the addition of phosphobacteria. There was no difference in tissue nutrient concentrations between treatments.*

INTRODUCTION

Interactions of growth-promoting microbial populations in the rhizosphere of VA-mycorrhizal plants have been studied by many workers (Barea *et al.* 1983; Pacovsky and Fuller 1985; Linderman 1988; Baas 1990). Subba Rao *et al.* (1985) reported that the synergistic interactions of VAM and *Azospirillum brasilense* significantly increased dry matter production and grain yield of barley. Response of plants to colonization by mycorrhizas depends on many biotic and environmental factors. Plant-available P is considered to influence the degree of mycorrhizal symbiosis (Bethlenfalvay *et al.* 1982). Among the many soil microorganisms known to solubilize unavailable forms of P, phosphobacteria have been used as bacterial fertilizer (Bagyaraj 1984). These bacteria survive for a longer period in the rhizosphere of mycorrhizal roots (Linderman 1988). Hence this trial aimed to study the interactions of VAM fungus with *Azospirillum* and phosphobacteria in rhizosphere soils of tomato seedlings and their effect on plant growth, tissue nutrient concentration, VAM colonization and spore density.

MATERIALS AND METHODS

The soil used was a nutrient deficient (N 225, P 22.5, K 780, Zn 0.20 and Cu 0.78 kg/ha^{-1}) alluvial deposit of sandy loam with pH 7.2 and EC 0.2 milli S/cm^{-1} from the Bharathiar University

Campus, Coimbatore. A mixture of equal parts of soil and sand autoclaved at 121°C and 15lb/inch^2 (1 h each on three consecutive days, followed by 1 week incubation at room temperature) was used to fill 30 x 12 cm polyethylene bags (about 3 kg per bag). As bacteria require an organic substratum for initial establishment in the soil (Lynch 1983; Subba Rao 1993), 50 g of autoclaved, (121°C, 15 lb/inch^2) dried cowdung was added to the topsoil in each bag.

A stock culture of *Glomus fasciculatum* was used as VAM inoculum, since it is known that this species is most effective in enhancing growth and P uptake (Sulochana *et al.* 1989; Sivaprasad *et al.* 1992). It was maintained in a pot culture of 90-day-old maize. Fresh cultures of *Azospirillum brasilense* and phosphobacteria, *Bacillus megaterium* var. *phosphaticum* (obtained from the Tamil Nadu Agricultural University, Coimbatore) were used as bacterial inocula. Ten grams of VAM inoculum soil, containing approx. 644 spores (64 spores/g dry soil) along with lyphae and infected root fragments/10 g charcoal base containing about 10^9 bacterial cells, (1 g charcoal base containing 10^8 bacterial cells) were placed as a thin layer about 2 cm below the soil surface in the bags. The control bags received autoclaved inocula. The treatments used were: (i) VAM-free (control), (ii) *Azospirillum,* (iii) phosphobacteria, (iv) VAM, (v) VAM + *Azospirillum,* (vii) VAM+ *Azospirillum* + phosphobacteria. Seeds of tomato *(Lycopersicon esculentum* Mill.) cv. Co 1 were sown in all the bags at the rate of 10 seeds/bag^{-1}. The bags were kept in a greenhouse, watered regularly and the seedlings were thinned on the 5th day after emergence (DAE) to maintain one seedling per bag. Each treatment was replicated four times.

At 60 DAE, the plants were harvested and growth parameters such as shoot and root length, leaf area, biomass, tissue nutrient (N, P, K, Zn and Cu) concentrations, VAM colonization index (VAMI) and spore density were determined. Leaf area was measured using a leaf area meter. Plant biomass was recorded after drying at 60°C for 12 h. Determination of VAMI was done after staining the root samples following the method of Phillips and Hayman (1970) and using the scoring method of Edathil *et al.* (1994). Spore density was assessed using the modified wet-sieving and decanting method (Gerdemann and Nicolson 1963) and expressed as the number of spores per gram of dry soil. Tissue nutrient concentration was determined following the standard methods of Jackson (1973).

RESULTS

There was no significant difference in the growth parameters of tomato seedlings between VAM treatments. Phosphobacteria-inoculated seedlings exhibited the highest shoot length (73 cm), which was equivalent to VAM (67 cm) and VAM + phosphobacteria (64 cm) combinations. Seedlings inoculated with *Azospirillum* or phosphobacteria alone had higher biomass than the uninoculated control. Leaf area and root length were more or less equal in all treatments (Table 1).

TABLE 1
Effect of interactions of microorganisms in the rhizosphere on the growth of tomato plants

Treatment	Leaf area (cm^2)	Shoot length (cm)	Root length (cm)	Biomass (g)
VAM-free (control)	23.05^{a}	61.00^{bc}	46.75^{ab}	2.48^{d}
Azospirillum	17.77^{a}	60.25^{bc}	33.25^{b}	4.23^{a}
Phosphobacteria	22.22^{a}	73.00^{a}	43.75^{ab}	4.12^{a}
VAM	20.81^{a}	67.00^{ab}	42.00^{ab}	3.87^{ab}
VAM + *Azospirillum*	19.32^{a}	60.75^{bc}	40.25^{bc}	3.01^{bd}
VAM + Phosphobacteria	18.20^{a}	64.75^{ab}	46.75^{ab}	3.05^{bcd}
VAM + *Azospirillum* + Phosphobacteria	23.15^{a}	64.50^{ab}	58.75^{a}	3.39^{abd}

Values are mean of four replications.
Values with the same letter are not significantly different P> 0.05 according to Duncan's new multiple range test.

The VAM and VAM + *Azospirillum*-inoculated plants registered higher VAMI than phosphobacteria-inoculated treatments. However, spore density was equal in all VAM treatments (Table 2). The accumulation of N, K, Zn and Cu in plant tissue was equal in all treatments. In the case of P accumulation, there was no regular trend (Table 3).

TABLE 2
Effect of interactions of microorganisms in the rhizosphere of tomato plants on VAM colonization and spore density

Treatment	VAMI (%)	Spore Density (individuals/g^{-1} dry soil)
VAM-free (control)	-	-
Azospirillum	-	-
Phosphobacteria	-	-
VAM	62.25[a]	12.61[a]
VAM + *Azospirillum*	69.67[a]	11.34[a]
VAM + Phosphobacteria	49.10[bc]	11.16[a]
VAM + *Azospirillum* + Phosphobacteria	42.51[ab]	9.23[a]

Values are means of four replications
Values with the same letter are not significantly different at P>0.05 according to Duncan's new multiple range test.

TABLE 3
Effect of interactions of microorganisms in the rhizosphere on tissue nutrient concentrations in tomato plants

Treatment	N (%)	P (%)	K (%)	Zn (%)	Cu (%)
VAM-free (control)	1.82[a]	0.14[b]	4.3[a]	0.011[a]	0.001[a]
Azospirillum	1.99[a]	0.15[ab]	4.2[a]	0.01[a]	0.0013[a]
Phosphobacteria	2.10[a]	0.16[a]	3.6[a]	0.01[a]	0.0012[a]
VAM	1.67[a]	0.14[b]	4.1[a]	0.01[a]	0.001[a]
VAM + *Azospirillum*	1.74[a]	0.16[a]	4.4[a]	0.01[a]	0.0011[a]
VAM + Phosphobacteria	1.81[a]	0.12[c]	4.1[a]	0.01[a]	0.0011[a]
VAM + *Azospirillum* + Phosphobacteria	2.01[a]	0.17[a]	4.2[a]	0.01[a]	0.0014[a]

Values are means of four replications
Values with the same letter are not significantly different at P>0.05 according to Duncan's new multiple range test.

DISCUSSION

The enhancement of plant growth with the addition of VAM fungi (Nicolson 1960; Koske *et al.* 1975; Tinker 1975, 1978; Menge *et al.* 1978; Koske 1981; Abbott and Robson 1982), *Azospirillum* (Barea *et al.* 1983; Pacovsky and Fuller 1985; Palanisami 1985; Subba Rao *et al.* 1985) and phosphobacterium (Graeves and

Webley 1965; Bagyaraj 1984; Meyer and Linderman 1986) has been well documented. Abbott and Robson (1982) reported that VAM fungi would manifest their performance to a greater extent in low nutrient soils. As sufficient nutrients are available in soil, the test plants could directly absorb the nutrients rather than depending on VAM fungi or other rhizosphere microorganisms. Futhermore, the addition of organic manure will result in increased soil microbial population releasing organic acids such as lactic acid, all of which have chelating properties which will ultimately promote P solubilization (Sperber 1958a, 1958b; Louw and Webley 1959; Duff *et al.* 1963; Banik and Dey 1982). If nutrient availability, especially phosporus, is high, the host plant may show a negative growth response to VAM fungi and VAM colonization can be reduced (Hayman *et al.* 1975; Johnson 1976; Sparling and Tinker 1978; Koide 1991). Data in Table 2 substantiate this observation because addition of phosphobacteria significantly reduced VAM index in tomato plants. The VAMI was significantly higher in VAM and VAM + *Azospirillum* treatments.

Higher shoot length was observed in the treatment with phosphobacteria or its combination with VAM fungus (Table 1). Shoot elongation may be a function of the excretion of certain growth promoting substances by the bacteria, because researchers have proved that the growth promotion by B. megaterium is mainly due to their excretion of growth-promoting hormones and vitamins (Banik and Dey 1982; Meyer and Linderman 1986).

Tien *et al.* (1979) reported the production of plant hormones by *Azospirillum.* The plant hormones present in bacterial cultures may improve the formation and development of VA mycorrhiza (Azcon *et al.* 1978).

The unaffected nature of VAMF spore density in the rhizosphere of tomato seedlings co-inoculated with either Azospirillum or phosphobacteria probably indicates the positive interactions among these growth-promoting micro-organisms (Table 2).

Since there is no difference in the tissue concentrations between the treatments (Table 3), it may not serve as an indication of the beneficial microbial interactions in a nutrient-rich (organic manure amended) soil with regard to nutrient accumulation.

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