

Amalgamation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth and yield of *Solanum lycopersicum* L.

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Abstract

In this research work the combination effect of arbuscular mycorrhizal (AM) fungus (*Glomus fasciculatum*) and Phosphobacteria (*Bacillus megaterium*) inoculation on % root colonization, AM fungal spore quantity, and Phosphobacterial populations in tomato (var PLR 2); *Solanum lycopersicum* L. rhizosphere soils was investigated in a pot culture experiment at different levels of phosphorus. A total of ten treatments were set up, Among them, *G. fasciculatum* and *B. megaterium* inoculated *S. lycopersicum* L. (tomato) recorded higher values than uninoculated plants. The root colonization percentage (84.52) and AM fungal spore number (129 100 g⁻¹ soil) in the rhizosphere soil were the highest at 75 per cent phosphorous level followed by 50 per cent phosphorous (71.28, 124.00 spores of 100 g⁻¹ soil) equally. The highest number of phosphobacterial populations (11.33 × 10⁶ Cfu) was recorded by the co-inoculation of *G. fasciculatum* and *B. megaterium* at 75 % phosphorous levels.

Keywords: AM Fungi, Phosphobacteria, *Solanum lycopersicum* L. Root colonization

INTRODUCTION

A significant impact of globalization on horticulture has been an increasing demand for quality improvement and the wider adoption of quality standards for fruit, vegetable, and salad commodities^{1,2,3,4,5,6,7}. *S. lycopersicum* L. (Tomato) is a major horticultural crop with an estimated global production of over 120 million metric tons. Growing demands for quality improvement and greater acceptance of quality standards for fruit, vegetable, and salad commodities have been a key influence of globalization on horticulture. *S. lycopersicum* L. (Tomatoes) is a prominent horticultural crop with a global production estimated at over 120 million metric tonnes⁸. Plants⁹, natural products^{10,11,12,13,14,15,16} and plant parts^{17,18,19,20,21,22,23,24,25} like Tomatoes have a high nutritional value due to their composition, which includes minerals such as potassium, calcium, salt, and iron, as well as soluble fiber²⁶. 92.7 percent water, 4% carbs, 1.4 percent protein, 1.3 percent fiber, 0.3 percent fats, 0.3 percent minerals, and a negligible amount of vitamin A. *Solanum melongena* L. varieties have a broad range of fruit forms and colours, ranging from oval or egg-shaped to long club-shaped, with white, yellow, green, purple pigmentation, and practically black pigmentation.

India, following China, is the world's second-largest tomato grower, with 14 million tonnes produced from 711.3 hectares. Farmers in India utilize massive amounts of chemical fertilizers for crop production, particularly for vegetables, resulting in soil degradation and groundwater contamination, posing health risks²⁷. To reduce environmental contamination, particularly soil pollution, most experts propose using biofertilizers in combination with inorganic fertilizers in a sustainable manner to preserve soil health, productivity, disease resistance, and yield growth²⁸ (Lekberg and Koids 2005).

The AM fungus differs with host ranges, according to Dwivedi²⁹. Despite their widespread presence, they revealed that every taxonomic group of plants and the list of species not infected is likely devoid of microorganisms such as bacteria, fungi, and actinomycetes that may aid in crop yields by assisting in the solubilization of insoluble phosphorus and encouraging plant growth by providing hormone levels, vitamins, and other growth-promoting substances. Phosphate Solubilizing Bacteria (PSB)³⁰ can hydrolyze organic and inorganic phosphorus from insoluble compounds, and PSB generates phosphatases similar to phytases that can efficiently hydrolyze organic forms of phosphate compounds³¹.

MATERIALS AND METHODS

ISOLATION AND SCREENING OF AM FUNGI

Tomato rhizosphere soil samples were collected from twenty different locations in the Cuddalore District of Tamilnadu^{32,33}. Four different AM fungal species viz., *Glomus fasciculatum*, *G. mosseae*, *Gigaspora margarita*, and *Acaulospora laevis* were isolated, characterized, and identified under a stereo zoom microscope according to Gerdemann and Trappe^{34,30,35,36,37,38,39}.

Isolated AM fungi are Root colonization % and AM fungal spore counts were used to assess efficiency in soil⁴⁰. The roots of tomatoes were invaded by all four AM fungus species. The degree of root infection and colonization, on the other hand, differed greatly amongst them. *Glomus fasciculatum* had the best reaction in terms of root colonization by AM fungi in soils, followed by *G. mosseae*, *Gigaspora margarita*, and *A. laevis*.

ENUMERATION OF PHOSPHOBACTERIA

To use the serial dilution plate technique, phosphobacteria were counted in the rhizosphere soils of several tomato-growing farms^{41,42,43,44}. The soil samples were serially diluted to a concentration of 10⁻⁴. Sperber's hydroxy apatite media was used to plate one ml of aliquots from the last dilution. At 28°C, the plates were incubated for up to two weeks. The clear zone bacterial colonies were counted and represented as CFU g⁻¹ of oven-dried soil.

TREATMENT DETAILS

T₁ - Control

T₂ -RDF (Recommended dose fertilizer)

T₃ - *Glomus fasciculatum*

T₄ - *Bacillus megaterium*

T₅ -75% of P+ *G. fasciculatum*

T₆ -75% of P + *B. megaterium*

T₇ -75% of P + *G. fasciculatum* + *B. megaterium*

T₈ -50 % of P+ *G. fasciculatum*

T₉ -50 % of P + *B. megaterium*

T₁₀ -50 % of P + *G. fasciculatum* + *B. megaterium*

AM FUNGAL COLONIZATION IN TOMATO ROOTS

The technique was used to determine the proportion of mycorrhizal colonization of the roots⁴⁵. The roots were gently cleaned with tap water. The roots were cleaned and cut into one-centimeter lengths before being immersed in a 10% KOH solution to remove the host cytoplasm and nuclei for stain penetration. Then it was autoclaved for about 20 minutes at 15 lbs/sq. inch pressure. The root parts were then removed and cleaned three times with tap water, or until no brown colour was seen in the rinsed water. For appropriate staining, the roots were acidified with 2% hydrochloric acid for 3-4 minutes. Root bits were stained with 0.05 percent trypan blue in lactophenol solution and boiled for 10 minutes after the acid was drained out without being rinsed with water. Under a compound microscope, these root fragments were inspected. In each replication, fifty root segments were utilized to calculate the percentage of AM fungus colonization.

$$\text{Per cent root colonization} = \frac{\text{Number of root bits with infection}}{\text{Total number of root bits examined}} \times 100$$

SURVEY FOR THE OCCURRENCE OF AM FUNGAL SPORES SURVEY FOR THE OCCURRENCE OF AM FUNGAL SPORES

Wet sieving and decanting procedure was used to determine the number of AM fungus spores. One hundred grams of rhizosphere soil samples were obtained from the tomato rhizosphere and thoroughly mixed in one liter of tap water for a few seconds to settle down the larger particles. To remove large pieces of organic matter, the suspension was decanted using a coarse dirt sieve (500-800 m sieve). The liquid that went through the sieve was separated and agitated to resuspend all of the particles. The suspension was decanted through a sieve (38-250 m sieve) fine enough to retain the desired spores. To ensure that all colloidal items moved through the sieve, the material that remained on the sieve was rinsed with a stream of water. The small amount of debris that remained in the petridish was transferred to a shallow layer of water and inspected with a stereo zoom microscope. The amount of spores in each soil sample was counted and represented as spores per 100 g of soil.

RESULTS AND DISCUSSION

The percentage of inoculation tomato root infection increased as the plants became older. At 130 DAT, the maximum root infection AM fungal spore and PSB population was found. At 130 DAT, the effect of *G. fasciculatum* injection on percent root colonization and AM fungal spore population was measured, and the findings are shown in Table 1 and Figure 1. The AM fungal root colonization and AM fungal spore number increased with an increase in plant age. In all the treatments, *G. fasciculatum* and *Bacillus megaterium* inoculated tomato recorded higher values than uninoculated plants. The root colonization percentage (84.52)

and AM fungal spore number (129.00, 100 g⁻¹ soil) in the rhizosphere soil were the highest in 75 per cent phosphorous level followed by 50 per cent phosphorous (71.28, 124.00 spores 100 g⁻¹ soil) respectively. From this study, it was observed that the addition of low levels of phosphorous increased the mycorrhizal colonization and AM fungal spore number in PLR 2 tomatoes.

Table 1. Co-inoculation effect of *G. fasciculatum* and *B. megaterium* on the per cent root colonization and spore population in the rhizosphere soil of tomato at different levels of phosphorus

S. No.	Treatments	AMF root colonization (%)			AMF spore numbers (100 g ⁻¹ soil)		
		45 DAT	90 DAT	130 DAT	45 DAT	90 DAT	130 DAT
1.	T ₁ : Control	25.01	44.71	52.53	48	58	70
2.	T ₂ : RDF	25.98	48.91	56.79	72	77	98
3.	T ₃ : <i>Glomus fasciculatum</i>	25.93	47.56	56.68	69	74	92
4.	T ₄ : <i>Bacillus megaterium</i>	25.53	46.75	56.13	62	71	91
5.	T ₅ : 75% of P + <i>G. fasciculatum</i>	30.23	51.31	62.17	92	110	122
6.	T ₆ : 75% of P + <i>B. megaterium</i>	29.17	51.17	69.00	70	101	94
7.	T ₇ : 75% of P + <i>G. fasciculatum</i> + <i>B. megaterium</i>	33.18	55.32	84.52	106	113	129
8.	T ₈ : 50% of P + <i>G. fasciculatum</i>	27.18	49.17	70.20	81	90	124
9.	T ₉ : 50% of P + <i>B. megaterium</i>	26.28	48.17	60.12	77	82	93
10.	T ₁₀ : 50% of P + <i>G. fasciculatum</i> + <i>B. megaterium</i>	27.75	50.49	71.28	83	97	124

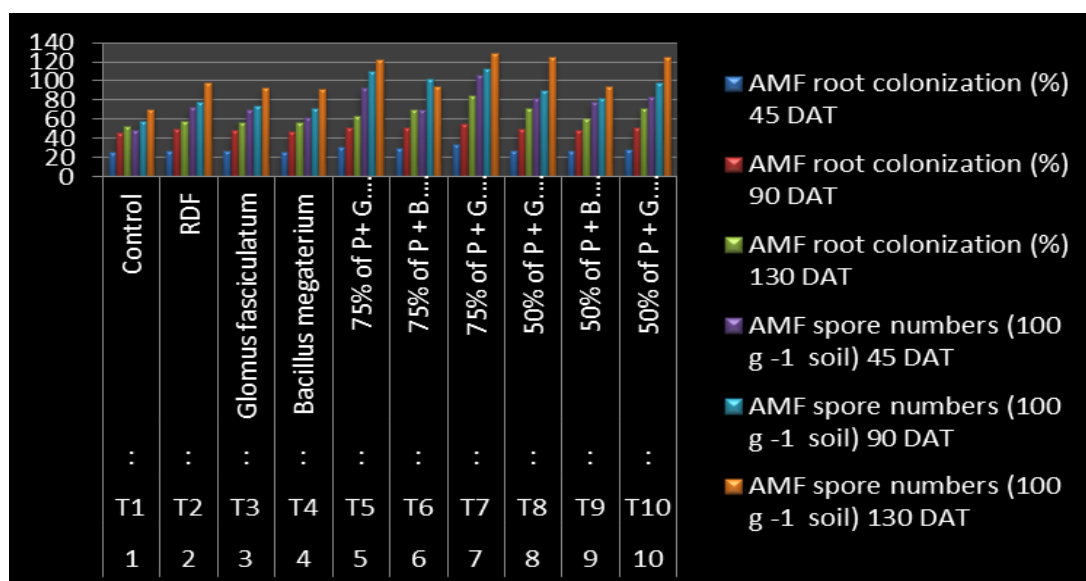


Figure 1. Co-inoculation effect of *G. fasciculatum* and *B. megaterium* on the per cent Root colonization and spore population in the rhizosphere soil of tomato at different levels of phosphorus

The rhizosphere soil phosphobacterial population was estimated at 45, 90, and 130 DAT, and the results are presented in Table 2. In all the periods, inoculation of *G. fasciculatum* and *B. megaterium* both single and combined at different phosphorous levels increased the rhizosphere soil phosphobacterial population compared to uninoculated control. Among the single inoculation, *B. megaterium* inoculated treatment, recorded more

number of phosphobacterial population (10.33×10^6 cfu) followed by *G. fasciculatum* (8.00×10^6 cfu). The highest number of phosphobacterial population (9.66×10^6 cfu) was recorded by the co-inoculation of *G. fasciculatum* and *B.megaterium* at 75per cent phosphorous levels. The addition of low levels of phosphorous with co-inoculation of *G. fasciculatum* and *B.megaterium* highly enhanced the survival of phosphate solubilizers in the rhizosphere soils of tomatoes.

Table 2. Co-inoculation effect of *G. fasciculatum* and *B.megaterium* on phosphobacterial population in the rhizosphere soil of tomato at different levels of phosphorus

S.No.	Treatments	Phosphobacterial populations ($\times 10^6$ CFU g^{-1} oven-dry soil)		
		45 DAT	90 DAT	130 DAT
1.	T ₁ : Control	5.33	6.66	7.33
2.	T ₂ : RDF	6.33	7.33	8.00
3.	T ₃ : <i>Glomus fasciculatum</i>	6.66	8.00	8.01
4.	T ₄ : <i>Bacillus megaterium</i>	9.00	10.33	9.33
5.	T ₅ : 75% of P+ <i>G. fasciculatum</i>	9.66	10.00	8.33
6.	T ₆ : 75% of P + <i>B. megaterium</i>	9.33	9.66	11.00
7.	T ₇ : 75% of P + <i>G. fasciculatum</i> + <i>B. megaterium</i>	8.66	10.66	11.33
8.	T ₈ : 50% of P + <i>G. fasciculatum</i>	8.00	8.66	9.50
9.	T ₉ : 50% of P + <i>B. megaterium</i>	8.66	9.00	10.20
10.	T ₁₀ : 50% of P + <i>G. fasciculatum</i> + <i>B. megaterium</i>	9.00	10.00	10.66

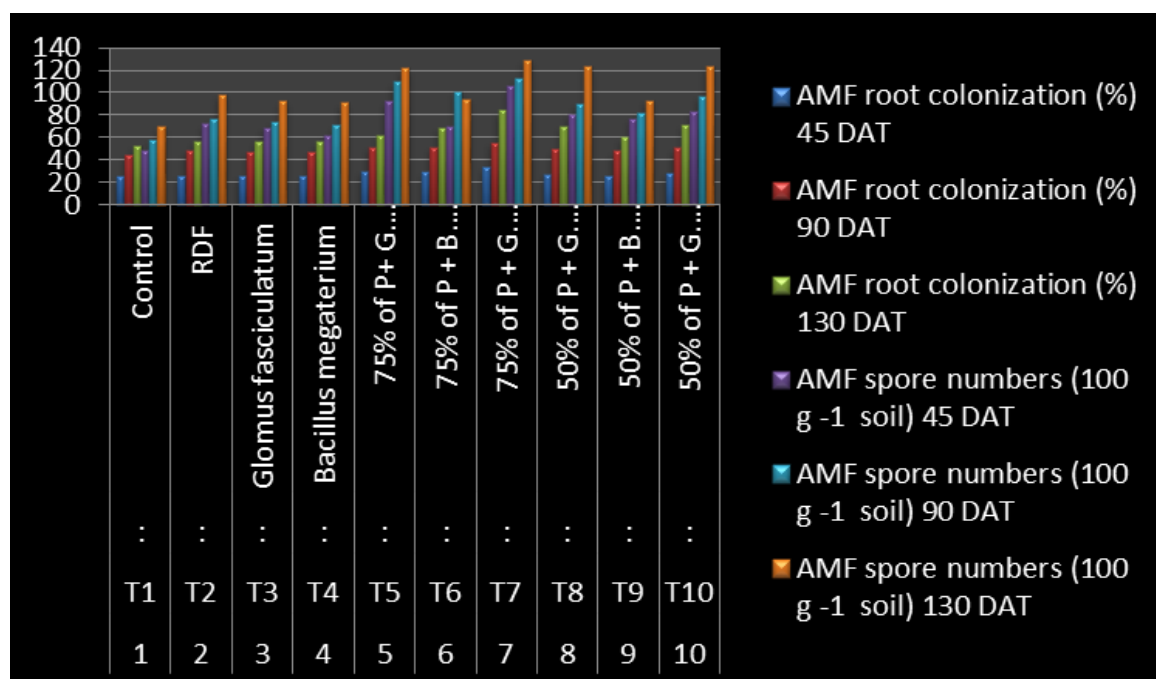


Figure 2. Co-inoculation effect of *G. fasciculatum* and *B.megaterium* on phosphobacterial population in the rhizosphere soil of tomato at different levels of phosphorus

The synergistic effect of *G. fasciculatum* and *Pseudomonas striata* on the growth, nutrient uptake, and total dry weight of neem seedlings were found to be superior over individual inoculation at different stages of Neem seedlings^{46,47}. The interaction of phosphate solubilizing bacteria and AM fungi on tomato growth, soil microbial activity, and production of organic acids in non-sterile soil containing hydroxyapatite and glucose was examined. The P concentration was greatest in all treatments and total N and P uptake in plants were higher in treated ones compared to control⁴⁸.

A tripartite symbiosis between *Azospirillum* sp., *Pseudomonas striata*, and *G. fasciculatum* enhanced the growth of rhizosphere microflora of cotton. Similar interaction occurred between P solubilization and AM fungi⁴⁹. The rhizosphere population of AM fungi and phosphate solubilizing bacteria were determined from

soil samples collected from mixed and monocropping coffee and cardamom. The population of phosphorous solubilizing bacteria and fungi was higher in coffee and cardamom, respectively in both cropping systems⁵⁰. The interaction between mineral phosphate solubilizing bacterium and AM fungus and *Azotobacter* at different levels of fertilizers increases the mycorrhizal colonization when all the three biofertilizers were added at 75 per cent of the recommended dose of NPK on sweet basil *Ocimum basilicum*. The interactive efficacy of phosphobacteria *Bacillus megaterium* and arbuscular mycorrhizae inoculated at different soil types were analyzed. Among the treatments, dual inoculation of AM fungi and phosphobacteria gave the most satisfactory outcome in *Amaranthus tritris*⁵¹.

CONCLUSION

In the present study the maximum root colonization percentage and spore numbers and Phosphobacterial population in the rhizosphere soils of tomatoes at different levels of phosphorus. The maximum root colonization percentage and spore numbers were recorded in treatment 7 (84.52), (129.00), and the minimum in control (52.53), (70.00) which was closely followed by treatment 10 (71.28), (124.00). The maximum Phosphobacterial population in treatment 7 (11.33) and the minimum in control (7.33) was closely followed by treatment 6 (11.00).

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CONFLICTS OF INTEREST

There is no conflict of interest

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