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Impact of Phosphate Solubilizing Bacteria and Arbuscular Mycorrhiza Inoculation on the Density of PSB, AM Fungal Spore Count, and Root Colonization Percentage in Chilli Rhizosphere Soils

C. Vignesh^{ID} and K. Sivakumar^{ID}*

Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, India

*Corresponding author

ABSTRACT

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In the rhizosphere soils of chilli var PLR 2, an inoculation experiment involving *Glomus fasciculatum* and *Bacillus megaterium* phosphobacteria. A study was carried out to investigate the effects of inoculation on root colonization, AM fungal spore count, and phosphobacterial population at varying phosphorus levels. Plants that were inoculated with *Bacillus megaterium* and *G. fasciculatum* showed greater values than plants that were not infected in all treatments. The rhizosphere soil had the highest root colonization percentage (84.32) and AM fungal spore quantity (127, 109 spores per 100 g of soil) at a 75% phosphorus level, which was followed by a 50% phosphorus level (73.40, 126.00 spores per 100 g of soil), respectively. The maximum phosphobacterial content was obtained by co-inoculating *G. fasciculatum* and *B. megaterium* at a phosphorus level of 75%.

Introduction

The family Solanaceae includes the chilli (*Capsicum sp.*), which has its origins in South Central America. It is a widely used spice or vegetable that is prickly and has green fruits. Chilli comes in a variety of tastes and levels of intensity. As compared to other vegetables, chillies are recognized for having a greater concentration of total soluble phenolics and vitamin C (Marinova *et al.*, 2005; Anil Kumar *et al.*, 2009).

Chilli is a popular vegetable crop these days, used to taste, scent, and color food (Zhuang *et al.*, 2012). Shape, size, color, flavor, and heat intensity which can range from fiery to sweet, fruity, earthy, smoky, and floral—are some of the qualities that set chillies apart from other

fruits and vegetables. The flavor of chillies is also significantly influenced by their variety and maturity stage (Kanner *et al.*, 2006; Sanatombi & Sharma, 2008).

According to empirical studies, capsaicinoids, the alkaloid molecule that gives *capsicum annum* its spicy flavor, are solely produced by this one crop. Because of their beneficial effects on the nervous system, capsaicinoids are significant in the pharmaceutical sector (Hayman & Kam, 2008). Additionally high in vitamins A, C, and P that protect against heart disease are chillies. One hundred grams of edible capsicum has 1.3 g of protein and 24 kcal of calories. Improving the production of chillies is crucial because of the world's expanding population and rising demand.

Additionally, capsaicin may reduce caloric intake. In a research of 24 people who regularly eat chilli, it was discovered that consuming capsaicin before to a meal reduced caloric intake. Only those who did not regularly consume chilli were shown to have significantly lower levels of malnutrition and caloric intake in another study. But it's unlikely that chilli peppers by themselves will have a significant impact. Additionally, with time, a tolerance to capsaicin's effects may develop, reducing its effectiveness.

Materials and Methods

Isolation and screening of AM fungi

Soil samples were gathered from the rhizosphere of chillies in twenty distinct places within the Tamil Nadu district of Dharmapuri. *Glomus fasciculatum*, *Glomus mosseae*, *Gigaspora margarita*, and *Acaulospora laevis* are four particular species of arbuscular mycorrhizal (AM) fungi that were disconnected, characterized, and distinguished beneath a stereo zoom magnifying lens utilizing the method described by [Gerdemann and Trappe \(1974\)](#).

The rate of roots colonized and the number of AM parasitic spores in the soil were measured to screen the effectiveness of the isolated AM fungus. The entire four-species AM fungus colonized the roots of the chilli plants. Nonetheless, there were notable differences in each other's levels of colonization and root infection. The highest AM fungus root colonization was observed in *Glomus fasciculatum*, followed by *Gigaspora margarita*, *Glomus mosseae*, and *Acaulospora laevis*.

Quantification of Phosphobacteria

The strategy of serial dilution plate method was used to count phosphobacteria from the rhizosphere soils of different chilli farms ([Sperber, 1958](#)). A 10^{-4} dilution was achieved by diluting the soil samples. One ml of samples from the final dilution was plateable using Sperber's hydroxyapatite media. Following this, the plates were then maintained in an incubator set at $28 \pm 2^\circ\text{C}$ for two weeks. The number of bacterial colonies in the clear zones was measured and reported as colony-forming units per milligram of soil that had been dried in the oven.

Treatment details

T₁ - Control

T₂ -RDF

T₃ -*Bacillus megaterium*

T₄ -*G. fasciculatum*

T₅ -75% of P+B. *megaterium*

T₆ -75% of P + *G. fasciculatum*

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

T₈ -50 % of P+B. *megaterium*

T₉ -50 % of P + *G. fasciculatum*

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

Colonization of AM fungi in the roots of chilliplants

Using the method described by [Phillips and Hayman \(1970\)](#), the proportion of mycorrhizal colonization in the roots was recorded. After a gentle rinse under the faucet, the roots were chopped into one-centimeter sections. Following that, the host cytoplasm and nuclei were cleared for improved dye penetration by immersing these root sections in a 10% KOH solution.

Then the root pieces were autoclaved for about 20 minutes at a pressure of 15 pounds per square inch. Three times, or until the rinsed water was no longer brown, the root parts were removed from the autoclave and cleaned them with tap water. It took about three to four minutes to properly stain after acidification with a 2% hydrochloric acid solution. And without washing the surface, the acid was then poured off.

$$\text{Percentage of roots infected} = \frac{\text{Number of root bits infected}}{\text{Total number of root bits examined}} \times 100$$

Determine the occurrence of AM fungal spores

The population of AM fungal spores was determined using the wet sieving and decanting method developed by [Gerdemann and Nicolson in 1963](#). One hundred

grams of rhizosphere soil samples were thoroughly combined with one liter of tap water. After a little time for the larger particles to settle, the mixture was filtered through a coarse soil-sieve (500-800 µm sieves) to remove the majority of the organic material.

To resuspend every particle, the liquid from the sieve was gathered individually and stirred. Subsequently, the suspension was poured through a finer (38-250 µm) screen, to collect the spores that were needed. In order to ensure that all colloidal materials have been processed, water was used to wash the remaining material on the sieve. With a stereo zoom microscope, the small pieces of debris was moved to a Petri plate with a shallow water layer and studied. Every 100 grams of soil had one hundred grams of spores, which were counted and expressed.

Results and Discussion

Growing older plants resulted in a higher percentage of inoculation chilli root infection. After 130 days (DAT), the maximum numbers of phosphorus-solubilizing bacteria (PSB), AM fungal spore density, and root colonization were noted. At 130 days after treatment, the effect of introducing *Glomus fasciculatum* on root colonization and AM fungal spore population was examined (Table 1). Growing older plants produced higher levels of both root colonization and AM fungus spores. Chilli plants were inoculated with *Bacillus megaterium* and *G. fasciculatum* showed greater values than plants were not inoculated in all treatment combinations.

In the rhizosphere soil, the 75% phosphorus level showed the greatest root colonization percentage (84.32) and AM fungal spore levels (127.00 spores per 100 g of soil), which were followed by the 50% phosphorus level (73.40 root colonization and 126.00 spores per 100 g of soil).

On three specific dates (45, 90, and 130 days after transplantation or DAT), the phosphobacterial population in the rhizosphere soil was measured. The findings are shown in Table 2. Comparing the phosphobacteria population to the control group without inoculation, there was always an increase in all time periods when *G. fasciculatum* and *B. megaterium* were present, either separately or in combination, at varying phosphorus

levels. Among the single inoculation, *B. megaterium* inoculated treatment, recorded more number of phosphobacterial population (11.35×10^6 cfu) followed by *G. fasciculatum* (8.98×10^6 cfu).

The highest number of phosphobacterial population (11.63×10^6 cfu) was recorded by the co- inoculation of *G. fasciculatum* and *B. megaterium* at 75 per cent phosphorous levels. The presence of *G. fasciculatum* and *B. megaterium* in the rhizosphere soils of chilli plants, combined with small amount of phosphorus significantly boosted the survival of phosphate solubilizers.

The growth, nutrient uptake, and total dry weight of neem seedlings were found to be more positively impacted by the combined inoculation of *G. fasciculatum* and *Pseudomonas striata* than by each inoculation alone at different stages of development, according to research conducted by [Karthikeyan in 1994](#) and [Karthikeyan et al., in 1995](#). We looked at how phosphate-solubilizing bacteria and AM fungus interacted with non-sterile soil that contained glucose and hydroxyapatite to produce organic acids, grow chillies, and stimulate soil microbial activity. [Kim et al., \(1997\)](#) discovered that the treatments with the most P had the highest N and P uptake in plants compared to the control group.

The growth of the rhizosphere microflora in cotton was improved by a tripartite symbiosis involving *Azospirillum sp.*, *Pseudomonas striata*, and *G. fasciculatum* ([Prathiba et al., 1994](#)). AM fungus and P solubilization reacted similarly ([Gurumurthy and Sreenivasa, 2000](#)). [Korikanthimathi et al., \(2000\)](#) found that cardamom had a higher population of phosphorous-solubilizing bacteria while coffee had more fungi in both cropping systems.

Adding all three biofertilizers at 75% of the authorized NPK dose enhances mycorrhizal colonization on sweet basil due to the interaction of the mineral phosphate-solubilizing bacterium, AM fungus, and Azotobacter at varying fertilizer levels ([Ajimuddin, 2002](#)). An analysis was conducted to determine the combined effectiveness of inoculating various soil types with phosphobacteria *Bacillus megaterium* and arbuscular mycorrhizae. Dual inoculation of phosphobacteria and AM fungus produced the best results among the treatments for *Amaranthus tritris* ([Rajashri and Sivapriya, 2004](#)).

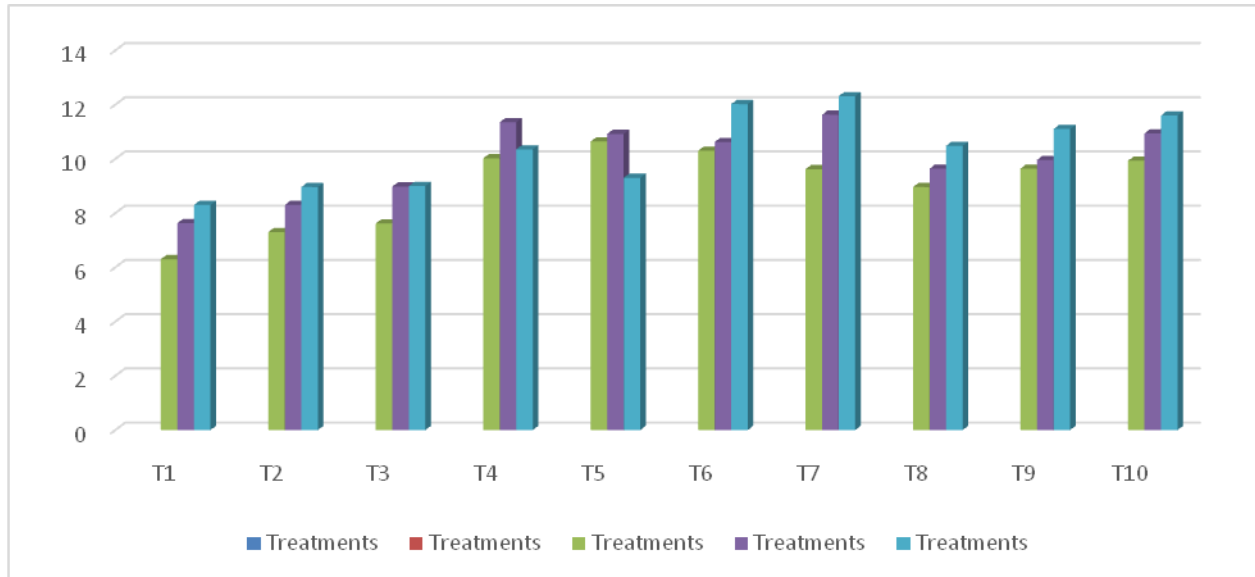
Table.1 Co-inoculation impact of *G. fasciculatum* and *B. megaterium* on root colonization percentage and spore count in chilli rhizosphere soil under varying phosphorus level

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	26.06	46.61	54.15	50	56	72
2.	T ₂	:	RDF	26.82	50.05	58.65	76	76	96
3.	T ₃	:	<i>B. megaterium</i>	26.78	49.87	58.56	72	72	90
4.	T ₄	:	<i>Glomus fasciculatum</i>	26.45	48.85	58.15	67	69	89
5.	T ₅	:	75% of P+ <i>B. megaterium</i>	29.20	53.65	63.23	94	108	120
6.	T ₆	:	75% of P + <i>Glomus fasciculatum</i>	27.80	53.46	70.03	73	99	92
7.	T ₇	:	75% of P + <i>B. megaterium</i> + <i>Glomus fasciculatum</i>	32.15	57.72	84.32	109	111	127
8.	T ₈	:	50% of P + <i>B. megaterium</i>	28.02	50.02	72.35	84	88	126
9.	T ₉	:	50% of P + <i>Glomus fasciculatum</i>	27.12	49.24	62.45	82	80	91
10.	T ₁₀	:	50% of P + <i>B. megaterium</i> + <i>Glomus fasciculatum</i>	27.68	51.45	73.40	85	95	126

Table.2 Effect of co-inoculation with *G. fasciculatum* and *B. megaterium* on phosphobacterial population in chilli rhizosphere soil at varying phosphorus levels.

S.No.	Treatments			Phosphobacterial population (×10 ⁶ cfu g ⁻¹ oven dry soil)		
				45 DAT	90 DAT	130 DAT
1.	T ₁	:	Control	6.30	7.63	8.30
2.	T ₂	:	RDF	7.30	8.30	8.96
3.	T ₃	:	<i>Glomus fasciculatum</i>	7.62	8.98	9.00
4.	T ₄	:	<i>Bacillus megaterium</i>	10.02	11.35	10.35
5.	T ₅	:	75% of P+ <i>G. fasciculatum</i>	10.64	10.92	9.30
6.	T ₆	:	75% of P + <i>B. megaterium</i>	10.30	10.62	12.02
7.	T ₇	:	75% of P + <i>G. fasciculatum</i> + <i>B. megaterium</i>	9.62	11.63	12.31
8.	T ₈	:	50% of P + <i>G. fasciculatum</i>	8.96	9.64	10.48
9.	T ₉	:	50% of P + <i>B. megaterium</i>	9.64	9.96	11.10
10.	T ₁₀	:	50% of P + <i>G. fasciculatum</i> + <i>B. megaterium</i>	9.93	10.94	11.60

Figure.1 Effect of co-inoculation with *G. fasciculatum* and *B. megaterium* on phosphobacterial population in chilli rhizosphere soil at varying phosphorus levels



T₁ :Control ; T₂ :RDF ; 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*.

In this research, we looked at the phosphobacterial population, spore counts, and root colonization % in the rhizosphere soils of chilli plants under varying phosphorus concentrating.

The control group had the lowest data (root colonization: 54.15%, spore numbers: 72.00), while Treatment 7 showing the highest root colonization percentage (84.32%) and spore numbers (127.00). Values for Treatment 10 had average values (73.40% for root colonization and 126.00 for spore counts).

The phosphobacterial population in treatment 7 was found to be the greatest at 11.52, followed by that in treatment 6 at 11.06, and the lowest at 7.64 in the control group.

Author Contributions

C. Vignesh: Investigation, formal analysis, writing—original draft. K. Sivakumar: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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