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Effect of AM Fungi and Phosphobacteria on the Growth and Yield of Tomato Crop (*Lycopersicon esculentum*)

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A B S T R A C T

The enhanced effect of arbuscular mycorrhizal fungus (AM fungi) and phosphate-solubilizing bacteria (PSB) on the normal soil of tomato (*Lycopersicon esculentum)* grown in pots was explored. Pot experiments were carried out on tomato inoculated with AM fungi (*Glomus fasciculatum*) and PSB (*Bacillus megaterium var phosphaticum*). Dual inoculation of AM fungi and PSB showed significantly higher variation in plant growth parameters, plant height, number of branches of tomato, number of fruits per plant, fruit volume, and fruit girth, than other treatments. The percentage of mycorrhizal root colonization of plants co-inoculated with AM fungi and PSB was higher than those plants inoculated with AM fungi alone. It is concluded that AM fungi inoculation with PSB application could synergistically increase the yield of tomatoes.

***Key words*:** AM fungi, Phosphobacteria, *Bacillus megaterium,* Tomato

Phosphorus is one of the three major essential nutrients for plant growth; the other two are nitrogen (N), and potassium (K). In circumstances of phosphorus deficiency, phosphate solubilizing microorganisms could play an important role in an eco-friendly and environmentally sustainable manner [1-3]. Phosphorus compounds in Indian soils are mostly inorganic that are mainly locked by $Ca_3(PO_4)_2$ (tricalcium phosphate). The PSM dissolving $Ca_3(PO_4)_2$ appears to imply Indian agriculture [4]. Phosphorus is one of the major essential macronutrients required for the biological growth and development of plants. Phosphorous is associated with many vital functions and is responsible for several physiological and biochemical plant activities such as the utilization of sugar and starch, photosynthesis, and transporting of genetic traits. It supports the early formation of the root, growth of plants, and progress the fruits quality, grains, and, vegetables and is imperative to the formation of seed [5].

The wild species of tomato originated in South America, probably mainly in Peru and Ecuador, and is thought to have been domesticated in pre-Columbian Mexico. The tomato was a pioneer in prematurely 16th century Europe by the Spanish, the Spanish and Italians appear to have been the first Europeans to approve of it as food [6]. Plant [7-17] and plant part [18-25] contain Vitamin C, 95% Water, 3.9 gm of Carbohydrates, 0.9 gm protein, 1.2 gm of fiber, 0.2 gm of Fat and it contains 2.6 mg of Lycopene which is a natural antioxidant [26-29]. Tomatoes are a perishable fruit with a mild taste, round-shaped, and are usually red in color sometimes they come in other colors too, from yellow to orange to purple.

Dwivedi [30] explained that AM fungi vary across host ranges. Although ubiquitous, showed that each taxonomic group of plants and the list of uninfected species is probably far from microorganisms such as bacteria, fungi, and actinomycetes that can help increase crop productivity by aiding in the solubilization of insoluble phosphorus stimulating plant growth by providing hormones, vitamins, and other growth-promoting substances. Phosphate-solubilizing bacteria (PSBs) can hydrolyze organic and inorganic phytase which effectively hydrolyze organic forms of phosphate compounds [31] phosphorus from insoluble compounds and PSBs produce phosphatases.

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MATERIALS AND METHODS

Plant materials

The experiment was conducted during the rabi season on tomato (*Lycopersicon esculentum)* grown in a greenhouse experiment of 2020-2022, Department of Agricultural Microbiology, Annamalai Nagar, Annamalai University, Chidambaram, Tamil Nadu. The experiment was conducted with an randomized block design (RBD) texture design consisting of 9 treatment combinations with 3 replicates and

was organized with the different treatments randomly assigned within each replicate.

Fungal inoculum

The mycorrhizal fungus was *Glomus fasciculatum*, the inoculum produced consists of a mixture of bedding material, spores, fragments, and pieces of infected root bits.

Assessment of arbuscular mycorrhizal root colonization

The study of mycorrhizal colonization with respect to chilli, Tomato, and tomato root from all places. The extent of colonization varies from different plants and sites. In tomato, it ranges from 43.04 to 82.04%, in chilli it is ranging from 38.88 to 73.34%, and in Tomato it is ranging from 31.10 to 81.95%.

Isolation and quantification of spores

Spores of AM fungi were isolated from rhizosphere soil by the wet sieving and decanting technique. Around (100 g) of individual rhizosphere soil sample- air-dried was disseminated in water (500 ml) in a beaker and the suspension was left untouched (15-20 min). Later then, decanted the suspension via the stack of sieves 180 µm and 38 µm (arranged in declining order of mesh dimension as of peak to bottom). The same process was repeated 2-3 times and the residue from each sieve was collected into Petri plates with little distilled water. Intact AM fungal spores were examined and counted under a stereomicroscope (Olympus OIC 1629) and identifications were made by observing diagnostic characteristics such as spore wall, color, size, and type of hyphal attachment according to [32] under a compound microscope (Nikon-Optiphot-2).

Taxonomic identification of the arbuscular mycorrhizal fungal species

The spores were recognized based on the diverse morphological description. The identification of arbuscular mycorrhizal fungal species is collected from the different rhizosphere soil. The arbuscular mycorrhizal fungi belong to mainly five genera viz. Acaulospora, Gigaspora, Glomus, Scelrocystis, and Scutellospora.

AM fungal inoculum preparation

Sorghum bicolor L. plants were used as the host plants for AM fungal inoculum preparation. Five prevailing native AM fungi such as *Glomus fasciculatum*, *Scutellospora heterogama*, *Acaulospora bireticulata*, *Glomus aggregatum*, *Gigaspora margarita*, and used for the production of inoculum. Glass funnels of 5 cm diameter were filled with sterilized soil sand (1:1) mixture. Spores were surface disinfected with Chloramine–T (2%) and 50–200 spores each were layered on the soil: sand mixture using funnel technique. Five seeds were sown on each funnel. Sorghum bicolor plants were transplanted from the funnel to pots after 20 days of germination. Small pots of 18 cm diameter x 15 cm height were filled with sterilized soil: sand (1:1) mixture. Test plants from the funnel were transplanted into pots. The pots were kept in the greenhouse (30±1°C) and watered regularly. The infectivity of *S. bicolor* roots by the AM fungi was checked at an interval of 15 days. After 3 months, the pot cultures were harvested by pruning *Sorhaum bicolor* plants to the soil level. The soil mass was removed from the pot and the mycorrhizal roots were chopped into small pieces. Inoculum impending of three AM fungal species was through MPN (Most Probable Number) technique.

Purification of AM fungi

To purify an isolated fungus, single spore isolation is needed. Even if the spores are morphologically identical, it often contains contaminants whose morphology is very similar. Successive pot Culture of such multi spore isolates would cause an unexpected outbreak of the contaminant. Furthermore, even If the culture contains only one species, it may be composed of genetically diverse populations. For such Genetic studies or population genetics, purification through single spore isolation is essential.

Mass multiplication of AMF spores for inoculation

The mass multiplication of native arbuscular mycorrhiza fungi with *Sorghum vulgare*, *Triticum aestivum*, *Zea mays*, and *Helianthus annus* as host plant.

Isolation of bacterial strain

The bacterial strain was isolated from the different rhizosphere soil [33] in and around Chidambaram. The soil samples were serially diluted up to 10^{-6} and spread plated on NBRIP (National botanical research institute's phosphate growth medium) which was developed for screening phosphate solubilizing microorganisms. The medium was incubated at 32°C for 3-5 days. The colonization was selected on the basis of phosphate solubilization as indicated by a clear halo zone around the bacterial colonies.

Composition of NBRIP medium

Glucose,10g;
Tricalcium phosphate $Ca_{3(}PO_{4)2}$,5g;
Di ammonium sulphate, 0.1g;
Magnesium chloride,5g;
Magnesium sulphate, 0.025g,
Potassium chloride,0.2g.
Agar, 15g
Distilled water, 100ml
pH,

Identification of bacterial strain

Bacterial genus-level identification was carried out by subjecting the bacterial isolate to cultural (oxygen requirement), morphological (colony morphology and pigmentation), microscopic (Gram staining), and biochemical (utilization of carbon sources and enzyme activity) tests followed by standard procedures.

Characterization of phosphobacteria

Phosphobacterial isolates were characterized by subjecting them to the following biochemical tests: Gram reaction, acid production, gas formation in glucose broth, hydrolysis of starch, hydrolysis of gelatin, Voges-Proskauer test, and utilization of citrate [34-36].

Screening the phosphobacterial isolates for their efficiency to solubilize different sources of phosphates

The different phosphor bacterial isolates were cultured in synthetic NBRIP broth. Then the broth was centrifuged at 3000 rpm for 10 min to harvest the log phase cells. The cell concentration was maintained at 1×10^{7} ml^{-1} by measuring the absorbance at 520 nm and used as standard inoculum.

Estimation of phosphate solubilization

The bacterial isolate was evaluated for its ability to solubilize $Ca_3(Po_4)_2$ in two different phosphate solubilizing media both qualitatively and quantitatively. Qualitatively the plates were incubated at 28±10°C for 12 days and observed regularly for the solubilization zone. Solubilization index (SI) was calculated according to the ratio of the total diameter (colony + halo zone) and colony diameter. Quantitative

estimation of tricalcium phosphate solubilization in broth was carried out at 280°C using an Erlenmeyer flask (250 ml) containing 100 ml of NBRIP broth inoculated with 1 ml of bacterial suspension (3.105 cells/ml); uninoculated control was used in each case.

Experimental design and biological treatments

To study the effects of AM fungi and PSB on tomatoes, we used a full factorial experiment design, with nine treatments. A pot culture testing of "Co-inoculation effect of AM fungi and Phosphate solubilizing bacteria on the growth and yield of tomato (*Lycopersicon esculentum*)

T_1: Control (No biofertilizers and chemical fertilizers)
T_2: RDF
T_3: *Glomus fasciculatum*
T4: *Bacillus megaterium*
T_5: 75% of P + *Glomus fasciculatum*
T_6: 75% of P + *Bacillus megaterium*
T_7: 75% of P + *Glomus fasciculatum* + *Bacillus megaterium*
T_8: 50% of P + *Glomus fasciculatum*
T_9: 50% of P + *Bacillus megaterium*

Cultivation conditions

Tomato seeds were surface sterilized in 2% of sodium hypochlorite for 3 min and then rinsed 5 times with distilled water. The seeds were germinated and grown in sterilized vermiculite trays. After 20 days after sowing, one seedling was transplanted into each pot and inoculated with 1ml of inoculum containing approximately 10^8 cells. The temperature in the greenhouse was maintained at 30±2°C with a relative humidity of 65% and a 16 hr photoperiod created by using supplemental lighting from high-pressure sodium lamps. Each treatment was replicated three times in a randomized block design and each treatment was comprised of 27 pots comprising 3 plants per pot. After 60 days, the plants were removed and the following morphological growth characteristics like plant height (cm), root length (cm), shoot dry weight (g), root dry weight (g), mycorrhizal colonization (%), and total phosphate were analyzed.

Plant analysis

Shoot length and root length

After 90 days, two plants of each control and experimental pot the plants were removed gently from the soil without disturbing the root system. The roots were washed with tap water. The shoot height was measured and expressed on a cm scale. The root length was also measured on a cm scale.

Dry weight of shoot and root

The plants were uprooted gently from the soil without disturbing the root. The roots were rinsed with tap water to remove the soil elements. The fresh shoot and root from each control and treatment were cut into pieces and kept in a hot air oven at 82°C for 24-72 hours. The dried samples are weighed in an electrical balance and then root and shoot dry weights are recorded (Table 1).

Fresh weight of root and shoot

The root and shoot of the plant were weighed in an electrical balance and the fresh weight of the roots and shoots were expressed in grams.

Mycorrhizal colonization (%)

The fresh root mass of two plants was used for determining AM fungi colonization. To assess AM fungi colonization, the roots of two plants were washed with 10% KOH and then stained with 0.05% trypan blue [37]. The percentage of roots colonized by AM fungi was estimated. The fungal roots were cut into small pieces of 1cm in length and 20 bits were examined per sample for their AM fungi colonization under a compound microscope (100X magnification). Positive counts for AM fungi colonization included the presence of vesicles or arbuscules within the roots.

The percentage of AM fungi colonization was calculated by using the following equation:

$$\text{Percentage of AMF colonization} = \frac{\text{Positive count of root segments}}{\text{No. of root segments observed}} \times 100\%$$

Table 1 Plant growth rate of potted single tomato plants

Treatment	Chlorophyll content	Root length (cm)	Shoot dry weight (g/plant^{-1})	Root dry weight (g/plant^{-1})	Mycorrhizal colonization (%)	Total phosphate (mg kg^{-1})
T_1	5.55	20.33	6.44	0.23	0	0.221
T_2	4.91	19.44	5,44	0.16	45.66	0.235
T_3	4.12	18.56	5,65	0.34	0	0.241
T_4	4.77	22.19	6.11	0.22	22.12	0.105
T_5	3.77	19.33	5.65	0.41	0	0.131
T_6	5.10	20.13	6.23	0.23	11.11	0.412
T_7	5.71	26.31	7.33	0.51	0	0.445
T_8	5.33	22.15	6.20	0.21	37.81	0.312
T_9	3.33	19.88	6.15	0,51	12.11	0.298

RESULTS AND DISCUSSION

Soil sample collection

After 90 days, according to Riley and Barber [38], the whole plants were removed from pots. The soil was obtained by gently shaking the roots and collected in a sterilized Petri plate. The rhizosphere soil was collected in another sterile Petri plate. One part of the soil sample was stored at 4°C for biological and biochemical analyses and another part of the soil sample was air-dried at room temperature for physical-chemical analysis.

Soil chemical analysis and enzymatic activity determination

The available phosphorus content in the soil was determined using the sodium bicarbonate-extractable phosphorus colorimetric method [39]. Phosphatase enzyme activity was determined according to the improved method [40], and phosphatase was represented by a phenol number of milligrams per gram of soil. Ten grams of dry soil from each sample were diluted with 50 ml of deionized water and measured by a pH meter.

Co-inoculation effect of G. fasciculatum and B. megaterium on the plant height of tomato at different graded levels of phosphorus

The effect of co-inoculation of *Glomus fasciculatum* and *Bacillus megaterium* on the plant height of tomatoes at grade levels of phosphorous was measured on 30, 60, and 90 DAT, and the results are presented in (Table 2). In general, all the treatments of inoculation with *Glomus fasciculatum* and *Bacillus megaterium* both single and co-inoculation at different phosphorus levels increased the plant growth compared to control. Among the single inoculation, *Glomus fasciculatum* recorded the maximum plant height of 98.77 cm on 90 DAT followed by *B. megaterium* (97.88 cm). It was also observed that all the graded levels of phosphorous with co-inoculation significantly increased the plant height. The maximum plant height of 113.09 cm was recorded by the co-inoculation of *Glomus fasciculatum* and *Bacillus megaterium* at 75 percent phosphorous levels. Interestingly, the inoculation effect in terms of increase in plant height was observed between 50 and 75 percent phosphorous levels were found to be on par with each other. The results indicated that a saving of 25 percent P is possible due to *Glomus fasciculatum* and *Bacillus megaterium* inoculation.

Table 2 Co-inoculation effect of *Glomus fasciculatum* and *Bacillus megaterium* on the plant height of tomato at different levels of phosphorus

Treatments		Plant height (cm)		
		30 DAT	60 DAT	90 DAT
T_1:	Control	40.03	70.13	92.34
T_2:	RDF	49.00	79.95	108.23
T_3:	*Glomus fasciculatum*	45.01	73.56	98.77
T_4:	*Bacillus megaterium*	44.00	77.98	97.88
T_5:	75% of P + *Glomus fasciculatum*	47.65	78.34	102.88
T_6:	75% of P + *Bacillus megaterium*	46.54	77.69	101.66
T_7:	75% of P + *Glomus fasciculatum* + *Bacillus megaterium*	53.66	85.98	113.91
T_8:	50% of P + *Glomus fasciculatum*	48.75	75.45	103.10
T_9:	50% of P + *Bacillus megaterium*	45.61	74.65	100.51
T_{10}:	50% of P + *Glomus fasciculatum* + *Bacillus megaterium*	51.61	81.66	119.51

Table 3 Co-inoculation effect of *Glomus fasciculatum* and *Bacillus megaterium* on the number of branches of Tomato at different levels of phosphorus

Treatments		Number of branches plant^{-1}		
		30 DAT	60 DAT	90 DAT
T_1:	Control	6.59	8.89	11.98
T_2:	RDF	10.23	12.98	15.61
T_3:	*Glomus fasciculatum*	7.99	10.13	14.13
T_4:	*Bacillus megaterium*	7.59	9.43	13.14
T_5:	75% of P + *Glomus fasciculatum*	10.01	12.23	16.43
T_6:	75% of P + *Bacillus megaterium*	9.56	11.57	15.31
T_7:	75% of P + *Glomus fasciculatum* + *Bacillus megaterium*	12.51	16.01	19.81
T_8:	50% of P + *Glomus fasciculatum*	8.98	10.56	13.91
T_9:	50% of P + *Bacillus megaterium*	8.43	11.01	13.01
T_{10}:	50% of P + *Glomus fasciculatum* + *Bacillus megaterium*	11.51	14.51	17.99

Co-inoculation effect of G. fasciculatum and B. megaterium on the number of branches of tomato at different levels of phosphorus

The number of branches of tomato as influenced by various treatments was recorded on 30, 60, and 90 DAT. The number of branches gradually increased up to 90 DAT both under inoculated and uninoculated conditions. In general, all the treatments of inoculation *G. fasciculatum* and *B. megaterium* both single and co-inoculation at different phosphorus levels increased the plant growth compared to the uninoculated control. The maximum number of branches per plant (19.81 branches plant^{-1}) was observed with co-inoculation of Glomus fasciculatum and *Bacillus megaterium* at 75 percent phosphorus levels followed by 50 percent phosphorus levels (17.99 branches plant^{-1}) (Table 3).

Table 4 Co-inoculation effect of *Glomus fasciculatum* and *Bacillus megaterium* on the number of fruits per plant of Tomato at different levels of phosphorus

Treatments		Number of fruit plant^{-1}		
		30 DAT	60 DAT	90 DAT
T_1:	Control	00	10.01	20.53
T_2:	RDF	00	16.59	28.69
T_3:	*Glomus fasciculatum*	00	12.59	23.99
T_4:	*Bacillus megaterium*	00	11.89	22.68
T_5:	75% of P + *Glomus fasciculatum*	00	15.69	28.01
T_6:	75% of P + *Bacillus megaterium*	00	13.98	26.98
T_7:	75% of P + *Glomus fasciculatum* + *Bacillus megaterium*	00	18.71	32.09
T_8:	50% of P + *Glomus fasciculatum*	00	13.91	25.61
T_9:	50% of P + *Bacillus megaterium*	00	12.33	25.13
T_{10}:	50% of P + *Glomus fasciculatum* + *Bacillus megaterium*	00	16.98	30.09

Co-inoculation effect of G. fasciculatum and B. megaterium on the number of fruits per plant of tomato at different levels of phosphorus

Several fruits of tomato in response to inoculation were observed at 30, 60, and 90 DAS, and the results are presented in (Table 4). In all the treatments, the inoculation effect of *Glomus fasciculatum* and *Bacillus megaterium* both single and combined at different phosphorus levels increased the number of fruits compared to the uninoculated control. The maximum number of fruits 32.09 plant^{-1} was recorded by the co-inoculation of *Glomus fasciculatum* and *Bacillus megaterium* at 75 percent phosphorus levels. Interestingly, it was also observed that the inoculation effect in terms of an increase in the number of fruits between 100 and 50 percent phosphorous levels was found to be in pair with each other.

Table 5 Co-inoculation effect of *Glomus fasciculatum* and *Bacillus megaterium* on the fruit volume and fruit girth of Tomato at different levels of phosphorus

	Treatments	Fruit volume (cc)	Fruit girth (cm)
T_1:	Control	41.69	3.23
T_2:	RDF	58.23	5.69
T_3:	*Glomus fasciculatum*	48.61	4.01
T_4:	*Bacillus megaterium*	45.43	3.76
T_5:	75% of P + *Glomus fasciculatum*	56.98	5.35
T_6:	75% of P + *Bacillus megaterium*	55.48	5.01
T_7:	75% of P + *Glomus fasciculatum* + *Bacillus megaterium*	65.91	6.43
T_8:	50% of P + *Glomus fasciculatum*	54.03	4.76
T_9:	50% of P + *Bacillus megaterium*	51.01	4.53
T_{10}:	50% of P + *Glomus fasciculatum* + *Bacillus megaterium*	60.59	6.03

Co-inoculation effect of G. fasciculatum and B. megaterium on the fruit volume and fruit girth of tomato at different levels of phosphorus

Fruit length and fruit girth of tomato in response to *Glomus fasciculatum* and *Bacillus megaterium* inoculation were observed at harvest and the results are presented in (Table 5). An increase in fruit volume and girth were observed both in inoculated and uninoculated control. The co-inoculated tomato at all the phosphorus levels recorded higher values than the single inoculation. The maximum fruit volume of 65.91 cc and girth of 6.43 cm was recorded in the co-inoculation of *Glomus fasciculatum* and *Bacillus megaterium* at 75 percent phosphorus levels.

CONCLUSION

This study confirms the dual inoculation of AM fungi and PSB increases the growth and yield of tomato. In non-inoculated plants.

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