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IMPROVING PHOSPHORUS USE THROUGH CO - INOCULATION OF AM FUNGIAND PHOSPHATE SOLUBILIZING BACTERIA IN TOMATO (*SOLANUM LYCOPERSICUM* L.)

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Abstract

Phosphorus is one of the major essential macronutrients required for biological growth and development of plants. AM fungi are obligatory symbiotic soil fungi which form a mutualistic symbiosis with the roots of plants. The AM fungi will mobilize available P by increasing the surface area of roots and greater exploration of soil volume by its hyphal network, leading to higher overall nutrient use efficiency and provide water and nutrients to plants. Bacteria form the largest group which bring about mineralization of organic phosphorus compounds and are known as 'Phosphate Solubilizing Bacteria'. The principal mechanism of mineral phosphate solubilization is the production of organic acids and acid phosphatases which play a major role in the mineralization of phosphorous in the soil. The PSB can enhance plant development and promote the yield of several agricultural important crops in different soils and climatic regions. The co-inoculation of AM fungi and PSB biofertilizer in tomato crop was studied with observation in AM fungi alone, PSB alone, AM fungi+ PSB, AM fungi+ PSB + 75% P_2O_5, 100% P_2O_5 treatment as compared to control. Use of AM fungi+ PSB biofertilizer to tomato crop in field is beneficial in improving Phosphorus uptake than AM fungi alone or chemical fertilizers.

***Keywords*:** AM fungi, Phosphate Solubilizing Bacteria.

Introduction

Phosphorus is one of the major macronutrients essential for biological growth and development of the plants. Soils contain substantial reserves of total phosphorus, however most of it remains relatively inert, and only less than 10% of soil phosphorus enter the plant-animal cycle (Kucey *et al.,* 1989). Phosphorus deficiency is widespread and phosphorus fertilizers are almost universally required to maintain crop production because when it is added to soil in the form of phosphatic fertilizers, only a small part of phosphorus is utilized by plants and the rest is converted into insoluble fixed forms (Rodriguez and Fraga, 1999).

When added to soils the soluble phosphates react with the constituents of soil and form compounds that are less soluble, depending upon the soil. Thus, in acid soils, the reaction products are aluminum and iron phosphates; in the predominantly calcareous soils, the reaction products are calcium phosphates. Different phosphatic fertilizers yield different reaction products. The formation of these reaction products depend on soil environment and the types of fertilizer material added (Sundara *et al.,* 2002).

It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Phosphate solubilizing microorganisms render these insoluble phosphates into soluble form. Phosphate solubilizing bacteria solubilize insoluble P by producing various organic acids. This available P is taken up by plants.

Nutrient absorption by AM fungi is due to external hyphae of the fungus proliferating beyond the nutrient depletion zone and reaching the source of nutrients (Sandhya *et al.,* 2013). Vesicular arbuscular mycorrhizae enhance the uptake, translocation and transfer of phosphate ions from the soil solution to the root cells (Harley and Smith 1983). The objectives of this study were to evaluate the synergistic effect of AM fungi and PSB on P uptake and tomato plant growth, and to examine the effect of dual inoculation with AM fungi and PSB on soil microbial activity in the rhizosphere of tomato.

Materials and Methods

Experimental Details

A field experiment was conducted at Department of Microbiology, Faculty of Agriculture, Annamalai University during rabi season of 2019 in black clayey soil. The experimental design employed was completely randomized design (CRD) with three replications. Treatments were absolute control, AM fungi culture and Phosphate solubilizing bacteria alone or in combination with or without 75% of recommended P_2O_5 dose as well as one treatment with sole application of 100% of recommended P_2O_5 dose (Ramamoorthy, Narasimham, and Dinesh 1967). The recommended N and K fertilizers were added in 75% and 100% doses of recommended P_2O_5 treatments.

AM fungi Inoculation

The AM fungiculture was prepared by raising the host crop such as maize until maturity in pots containing 7 kg sterilized soil + 2 kg FYM and 1 kg mother culture. The AM fungi inoculum is obtained by cutting all the roots of stock plants. After harvest, rhizosphere soil of pot as well as root biomass constituted the AM fungi culture. The inoculum produced consists of a mixture of bedding material, spores, pieces of hyphae and infected root pieces were used in the study. The spore count of AM fungi was 100 per 250 g air dried soil and54 mg of AM fungi were used per pot.

Root Infectivity Studies

After 8 weeks, the plants grown in pots were cut and the rhizosphere soil was carefully removed. The roots were washed under running tapwater.The cleaned roots were then chopped into small pieces and then subjected to fixation, cleaning, rinsing, and bleaching in potassium hydroxide following standard techniques for microscopic observations (Rajapakse and Miller 1992). In case AM fungi propagules established infection, the infected portion in rhizosphere showed the presence of AM fungi fructifications. On the basis of root length(s) that were infected and uninfected, the percentage of infection was worked out.

Isolation of PSB

PSB were isolated by serial dilution and spread plate method. One gram (1g) of soil sample was dispersed in 9 ml of autoclaved distilled water and was thoroughly shaken. 1 ml of the above solution was again transferred to 9ml of sterile distilled water to form 10^{-2}dilution. Similarly 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} serials were made for each soil sample. 0.1ml of each dilution was spread on Pikovskaya's agar medium (PVK) containing insoluble Tricalcium phosphate and incubated at 27-30^{o}C for 7 days. Colonies showing halo zones were picked and purified by 5 times subculture method on Pikovskaya's (PVK) agar medium for studying colony morphology.

Soil and Plant Chemical Analysis

Soil pH was measured by immersing a glass electrode in soil water suspensions (1:5, w/v) which were shaken in a rotary shaker for 20 min at 250 rpm., available N was measured by alkaline permanganate method (Subbiah and Asija, 1956), available P was found by Olsen's method (Olsen *et al*., 1954) and available K was determined by neutral normal ammonium acetate extraction method (AOAC 1970). Organic C was determined by rapid titration method (Walkley and Black 1934). Plant analysis was done by the standard procedures of Jackson (1967).

Results and Discussion

Root Infectivity and Root Biomass

Tomato plants inoculated with AM fungi have greater AM fungi infection in roots than their non inoculated plant (Table 1). AM fungi root colonization was improved with dual inoculation of AM fungi and PSB, indicating that there exists a synergistic interaction between AM fungi and PSB. Gryndler *et al*. (1990) have also reported similar results. Root weight at flowering also increased with individual application of AM fungi and PSB over the control. The sole application of 100% P_2O_5 recorded the maximum root weight, which was followed by the combined application of AM fungi and PSB along with 75% of the recommended P_2O_5 dose (Table 1). Thus, greater root biomass coupled with extensive AM fungi hyphal network in the soil–plant continuum can have great bearing on exploration of soil volume by the crop, leading to higher overall nutrient use efficiency (George *et al*., 1992).

Crop Productivity

AM fungi enhance the tomato crop yield over the control with significantly greater yields. The individual application of PSB also increased yield significantly compared to the control. The AM fungi induce many favorable changes in the crop rhizosphere by exudation or secretion of organic acids or chelating agents (Rovira 1969). These phenomena might have influenced the yields favorably. The PSB helped in the solubilization of insoluble phosphates, and encouraging the roots for better acquisition of nutrients and strengthening the root system. Application of AM fungi along with PSB and 75% of recommended P_2O_5 dose resulted in consistent and significant improvement in yield. This indicates that with the dual inoculation of AM fungi and PSB along with 75% P_2O_5, 25% of the fertilizer P dose can be saved without impairing the crop yield targets.

Protein Content

There was a significant increase in protein content over the control in the case of sole inoculation of AM fungi and PSB. The combined application of various AM fungi and PSB was additionally effective in enhancing protein content, indicating a synergistic interaction between AM fungi and PSB biofertilizers (Sreenivasa and Krishnaraj 1992). Results suggested that co-inoculation of AM fungi and PSB along with the 75% P_2O_5 dose increases 25% of the fertilizer P in tomato.

Nutrient Content and Uptake

Data presented in Table 2 reveal that nutrient (NPK) contents and their uptake in tomato were enhanced with the sole application of either AM fungi or PSB inoculation over control. However, combined application of AM fungi and PSB improved the NPK contents and their uptake in tomato. Dual inoculation of AM fungi along with PSB and 75% of the recommended P_2O_5 dose did not exceed sole application of 100% P_2O_5in these parameters, but the values tended to be close to each other, which indicates that these biofertilizers have some role in nutrient dynamics in the soil plant system (Narsian and Patel 2009).

Soil Fertility Status

Co-inoculation of AM fungi along with PSB resulted in significant reductions in available N, P, and K levels in soil after crop harvest in pot experiment (Table 3), mainly because of higher nutrient utilization (Table 2) and better crop growth (Table 1). The performance of AM fungi remained at par with control in terms of soil available P status. Dual inoculation of biofertilizers further depleted available N, P, and K because of the enhanced exploration of soil reserve nutrients. AM fungi mobilized soil nutrients thereby reducing soil nutrient status significantly over control. This may be ascribed to mobilization of soil N, P, and K from organic and inorganic complexes by the action of AM fungi due to its mycelial growth or release of organic acids in rhizosphere (Pare, Gregorich and Nelson, 1999). The PSB inoculation also followed the same trend. However, available P in soil under PSB treatment remained at par with the control despite a greater uptake, unlike AM fungi. PSB inoculation is useful in replenishing the available P status of soil (Suri *et al*., 2006a). Co-inoculation of PSB with AM fungi along with 75% recommended P_2O_5 dose remained statistically at par with the 100% P_2O_5 dose with respect to soil fertility status. Thus, this study indicates that dual inoculation of PSB and AM fungi along with 75% of the recommended P_2O_5 dose can increases 25% of the fertilizer P in tomato.

Conclusion

From this study, it can be inferred that application of AM fungi alone or with PSB with or with out inorganic P fertilization improved the nutrient uptake, crop productivity and protein content, while AM fungi in combination with PSB as well as 75% of recommended P_2O_5 dose helped in increases 25% of the fertilizer P in tomato.

Table 1 : Effects of co-inoculation of AM fungi, PSB, and applied P on AM fungi colonization, root weight, crop productivity and protein content

Treatments	Root colonization (%)	Root weight (g)	Yield (g/pot)	Protein content (%)
Control	9	3.25	28.08	8.19
AM fungialone	17	3.57	33.04	8.38
PSB alone	11	3.53	34.08	8.32
AM fungi+ PSB	21	3.69	35.11	8.44
AM fungi + PSB + 75% P_2O_5	31	6.88	117.04	9.38
100% P_2O_5	12	7.03	118.01	9.32
C.D.(P=0.05)	1.56	0.32	2.46	1.16
S. Ed.	0.74	0.16	1.23	0.58

Table 2 : Effect of co-inoculation of AM fungi, PSB, and applied P on nutrient content (NPK) and uptake in tomato

Treatments	Available nutrient status (mg kg^{-1} soil)		
	N	P_2O_5	K_2O
Control	35.9	1.8	115.8
AM fungi alone	34.1	1.8	114.5
PSB alone	31.1	1.8	112.3
AM fungi+ PSB	30.6	1.7	113.6
AM fungi+ PSB + 75% P_2O_5	28.8	1.7	106.1
100% P_2O_5	26.1	1.6	102.4
C.D.(P=0.05)	1.86	0.54	2.62
S. Ed.	0.93	0.27	1.31

Table 3 : Effect of co-inoculation of AM fungi, PSB, and applied P on available nutrient status (mg kg^{-1} soil) of soil

Treatments	Nutrient content (%)			Nutrient uptake (mg/pot)		
	N	P	K	N	P	K
Control	1.42	0.24	0.32	368.3	38.4	9.04
AM fungialone	1.43	0.24	0.32	410.7	42.8	68.4
PSB alone	1.44	0.26	0.34	453.9	50.9	79.4
AM fungi + PSB	1.45	0.25	0.34	443.3	47.3	76.0
AM fungi + PSB + 75% P_2O_5	1.59	0.36	0.44	631.7	276.5	364.7
100% P_2O_5	1.60	0.63	0.38	759.3	315.9	414.3
C.D. (P=0.05)	0.68	0.32	0.24	4.26	2.52	1.86
S. E.	0.34	0.16	0.12	2.13	1.26	0.93

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