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Synergistic effect between Phosphate Solubilising Bacteria and Arbuscular Mycorrhizal Fungi on Growth and P uptake in *zea mays*

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Abstract
Green house earthen pot experiments were undertaken by using sterilized sandy loam garden soils, with the inoculation of (AM fungi) and *Bacillus megaterium* (Phosphate Solubilising Bacterium). Plants inoculated with AM fungi and PSB in sterilized soil produced significantly higher growth, dry matter, increased per cent root colonization, chlorophyll content in leaves and Phosphorus uptake in shoot and root. Moderate or lower growth response was observed among the plants which were inoculated either PSB or AM fungus alone. On the contrary uninoculated plants in sterilized garden soil did not showed the improvement of plant growth and P uptake. A synergistic effect was recorded with increased plant dry matter, per cent root colonization and P uptake in *Zea mays.* Plants with inoculation both the inoculants in sterilized soil compare to unsterilized soil.

Keywords: *Zea mays*, *Bacillus megaterium*, *Gulomus fasciculatum*, Root colonization.

Introduction
The excessive use of chemical fertilizers in agriculture has resulted in several environmental problems like ozone layer depletion, poor soil health, due to the decline in natural microflora and acidification of water. To overcome these problems application of biofertilizers has been found effective. Generally the biofertilizers are beneficial microorganisms involve in breakdown of organic matter, Nitrogen fixation, and secretion of growth promoting substances. They also supply nutrients to the plants, control soil borne diseases and maintain the soil structure in cultivable fields.

Arbuscular mycorrhiza (AM) contain widespread symbiotic interactions that are commonly described as the result of co-evolution between fungi and plants, where both partners benefit from the reciprocal nutrient exchange (Bonfante and Genre, 2008) [6]. Arbuscular mycorrhiza symbiosis is witnessed in approximately 80% of vascular plant species in all major terrestrial biomass (Feddermann *et al.*, 2010; Smith *et al.*, 2010) [10, 34]. Dual inoculation with both microorganisms results in a tripartite mutualistic symbiosis and generally increases plant growth (Chalk *et al*., 2006) [8].

Phosphorus (P) is one of the major essential macronutrients limiting plant growth owing to its low bioavailability in soils (Feng *et al*., 2004) [11], and improving plant acquisition of P from soil is an obvious alternative to the management of those low P soils (Zhu *et al*., 2003) [37]. It is commonly known that arbuscular mycorrhizae (AM) provide a direct link between soil and roots. AM fungi help plants to capture water and nutrients (notably P) from the soil, and in return, the plant provides the fungus with relatively constant and direct access to carbohydrate (Smith and Read, 2008) [33], which are translocated from their source to root tissue and on to fungal partners. It is also generally accepted that AM fungi receive all their carbohydrate from host plant and that the association could create a sink demand for carbohydrate, which could result in a 4–20% drain of C from the host plant and could indirectly influence C storage in soils (Graham, 2000) [13].

Zea mays, is the major food source for humans and livestock in many parts of the world has a unique root system which is highly efficient not only in anchoring the plant to the soil but also in acquiring nutrients and water from the soil (Hochholdinger & Tuberosa, 2009) [14]. In addition, the root system of also possesses several morphological and metabolic traits that are essential for increased efficiency like adventitious roots, long and dense root hairs, basal root shallowness, root etiolating and cortical aerenchyma (Hochholdinger & Tuberosa, 2009) [14]. Several studies reported the mycorrhizal status and colonization patterns like Arum, Paris and intermediate type in (Muthukumar & Prakash 2009; Muthukumar & Tamilselvi 2010; Chandra

Gandhi *et al*., 2017) [24, 25, 9]. Further, genotypes exhibit variation in their responsiveness to mycorrhizal colonization (Kaeppler *et al.*, 2000) [16]. Recently, Wang *et al*., (2017) [36] in a long term experiment showed that increasing P fertilization in spite of reducing root colonization and community structure of AM fungi can still contribute substantially to P nutrition of plants.

Materials and methods

The Green house experiments were conducted in the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar. We had isolated the dominant AM fungi, composited rhizospheric soil samples were collected from the *Zea mays* growing places. This was done by digging by soil digger with a small amount of soil close to the plant roots up to 05-30 cm depth. Samples were kept in sterilized polythene bags with labelling and stored in refrigerator 4ºC for further processing. *Bacillus megaterium*, a Phosphate solubilising bacterium, was procured from the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar. The AM fungi was mass multiplied with (*Zea mays*) as a potential host plant. Soil based AM fungal inoculum was established and maintained in pot culture separately without contamination in polyhouse at Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar.

Seedlings were raised in earthen pots containing 4 Kg of sterilized and unsterilized garden soil. Each pot measuring 20 cm × 25 cm (length ×breath) the soil used for the experiment was a sandy loam with a pH; of 7.80, E.C; 0.75 mmhos/cm^2, organic carbon; 0.65%, available N; 107.45 kg/ha, available K; 14.40 kg/ha and available P; 129.30 kg/ha, annual temperature 28-340 C, one week old seedlings surrounding the rhizosphere of *Zea mays* was inoculated with PSB and AM fungus. PSB was isolated from the rhizosphere soils of *plants* growing in Annamalai University, Agriculture field. Using pikovskay medium at 20^{0}C of 48 hrs. There were 2×108 cells per ml of both cultures. All the treatments were followed as mentioned below;

1. Un-inoculated control
2. *Glomus fasciculatum*
3. *Bacillus megaterium*
4. *Glomus fasciculatum* + *Bacillus megaterium*

The following observations were recorded on two harvests 45 to 90 days plants were maintained in random designed with 4 replicates in green house. All the plants were watered on alternate day and once in 15 days 10 ml of minus P Hoagland nutrient solution was given to each experimental plant.

Plants growth parameters

Plant height, dry weight of shoot and number of leaves and nutrient uptake in shoots and roots were determined. The per cent of AM fungal colonization of roots were estimated according to (Philips and Hayman, 1970). The extra metrical chlamydospores were isolated and root samples were stained by adopting wet sieving and decanting technique outlined by (Gerdemann and Nicolson, 1963) [12]. Phosphorus content of shoots was estimated by vanadomolybdate phosphoric yellow colour method outlined by (Jackson, 1973) [15]. The leaves were collected for the analysis of Chlorophyll a and Chlorophyll b was estimated following the procedure (Arnon, 1949) [1], after 45 and 90 days of inoculation of AM Fungus and Phosphate solubiliser.

Results and Discussion

The soil of maize grown region was sandy clay with pH 6.7 and annual temperature 28-34^{0}C. Most of the plants show arbuscular mychorrhizal dependency of mean are range from 59.2 to 82.4% among the 80% of *maize* examined plant roots, and spores population was 201-300 spores /50g soil, with 33 different spores. The most dominant spores was mass multiplied for inoculation. Similarly, PSB was recovered from the rhizosphere of maize field. Inoculation both AM fungus *Glomus fasciculatum* and Phosphate solubilizing bacteria *Bacillus megaterium* significantly improved the plant height, root length, stem girth, shoot and root biomass yield was drastically increased from 45-90 days shown in (Tables 1-2) compare to single inoculation either AM fungus or Phosphate solubilizing bacteria. However, no increased shoot and root length, and biomass yield in uninoculated (control) plants. Per cent root colonization, spore number, seeds number per head, phosphorus content in shoot and root was absorbed in maize plants (Tables 3-5).

The chlorophyll content in leaves, showed the influence of AM fungus and phosphate solubilizer *Bacillus megaterium*, single inoculation with PSB does not influence much in enhancing chlorophyll content in leaves. But, AM fungus *Glomus fasciculatum* inoculated plants showed increase chlorophyll of both chlorophyll *a* and *b* compare to non inoculation (control) plants. There was significantly increased chlorophyll content in leaves of maize after the inoculation of both the bioinoculants of AM fungus and *Bacillus megaterium* (Tables 5-6), and. The percentage of root colonization or sporulation was higher with phosphate solubilizer and phosphorus absorber (AM fungus), as compare to PSB alone. Earlier studies indicated that PSB may reduce AM fungal spore populations, hyphal growth in the soil (Bagyaraj, 1984) [3, 4].

Similar results were obtained in the present study. However, significant plant growth, mychorrhizal development enhanced, dry matter production plant P uptake over the uninoculated plants. Recent researches on plant nutrition through PSB and AM fungi have amply demonstrated that these organisms play an important role in uptake of nutrient from the marginal soils. Research in the last three decades has established that dual inoculation of phosphate solubilising bacteria and AM fungi stimulates plant growth. Phosphate solubilising bacteria solubilise insoluble P and help to absorb and translocate more soluble phosphate (Azcon-Aguliar *et al*., 1986; Lakshman, 1996) [2, 23].

Soil microorganisms play an important role in increasing phosphorus availability to plants by dephosphorylating P-bearing organic compounds and bring about favourable changes in soil reaction and in the soil microenvironment leading to solubilisation of insoluble inorganic phosphate sources. Phosphate solubilising microorganisms belonging to genera Bacillus, pseudomonas, Citrobacter, Enterobacter and Serratia have been isolated in the recent years from the soil and rhizosphere of crop plants (Kim *et al*., 1997; Kim *et al*., 1998) [20].

The positive response of maize plants growth, per cent root colonization in P deficient soil supports, the previous findings with and wheat (Khan, 1972, 1975; Perry *et al*., 1987; Lakshman, 1996) [17, 18, 26, 23]. The increase in plant growth, biomass yield and number of grains (seeds) and P uptake in shoots and roots with dual inoculation suggesting the synergistic interactions among microbial inoculants (Filter and Garbage, 1994; Singh and Kapoor, 1999; Chaiharn and

Saisamorn, 2009; Swetha and Lakshman, 2013) [32, 7]. A similar, increase of plant growth, phosphorus uptake has been reported by several agricultural crops (Poonguzhali *et al*., 2008; Lakshman, 2009) [28]. Similarly, the dual inoculation with mycorrhiza and phosphate solubilizing bacterial species effective in increasing the Chlorophyll content, leading to enhanced growth in maize (Srihari and Srinivasa, 1992) [35]. In the present findings, it is clear that leading to a better awareness of the ways in which different types of beneficial microorganisms contribute to the development and enhancing the plant growth at different special scales. The effect of AM fungi on the initial growth and establishment of *Zea mays*. was studied on the bases of examined growth parameters. It may be concluded that microbial inoculated plants showed a significantly greater growth rate than the uninoculated plants, especially dual inoculation of with PSB (*Bacillus megaterium*) found to be superior in increasing the growth, biomass yield, Chlorophyll content increases and P uptake in Shoots and roots of *Zea mays*.

Table 1: Effect of AM fungus and PSB on plant height, root length, fresh and dry weight of plants in *Zea mays* for 45 days.

Treatments	Shoot length (cm)	root length (cm)	Shoot Girth (cm)	Fresh weight Shoot/g	Dry weight Shoot/g	Fresh weight Root/g	Dry weight Root (g)
(Control)	41.0	1.4	1.2	1.0	0.91	0.29	0.16
AMF	82.3	4.2	1.8	6.3	2.0	1.76	0.94
PSB	76.0	3.7	1.8	4.7	2.2	1.74	0.90
AMF+PSB	108.3	5.2	2.0	9.7	3.0	1.80	0.95

Table 2: Effect of AM fungus and PSB on plant height, root length, fresh and dry weight of plants in *Zea mays* for 90 days.

Treatments	Shoot length (cm)	root length (cm)	Shoot Girth (cm)	Fresh weight Shoot/g	Dry weight Shoot/g	Fresh weight Root/g	Dry weight Root (g)
(Control)	59.4	2.8	1.1	1.7	0.84	0.41	0.24
AMF	97.3	3.6	2.0	7.4	1.8	1.90	0.92
PSB	91.0	3.4	2.0	6.8	1.2	1.87	0.88
AMF+PSB	115.5	7.2	2.3	15.3	2.6	2.0	0.96

Table 3: Effect of AM fungus and PSB on per cent root colonization spore number and Number of seed yield, and P content in Shoot and root in *Zea mays* for 45days.

Treatments	% root colonization	AMF spore number/50g soil	Number of seeds/head (plant)	Percent P content	
				Shoot	Root
(Control)	12.0	24.2	NA	0.03	0.03
AMF	49.3	132.0	NA	0.10	0.06
PSB	-	46.0	NA	0.11	0.10
AMF+PSB	70.4	155	NA	0.12	0.10

Table 4: Effect of AM fungus and PSB on per cent root colonization spore number and Number of seed yield, and P content in Shoot and root in *Zea mays* for 90 days.

Treatments	% root colonization	AMF spore number/50g soil	Number of seeds/head (plant)	Percent P content	
				Shoot	Root
(Control)	17.2	31.1	79.2	0.03	0.03
AMF	62.6	149.0	157	0.14	0.9
PSB	-	46.0	145	0.17	0.10
AMF+PSB	75.2	167.0	201	0.20	0.10

Table 5: Effect of AM fungus and PSB on chlorophyll content in *Zea mays* pers. for 45 days.

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
(Control)	0.200	0.217	0.419
AMF	0.535	0.582	1.120
PSB	0.496	0.501	0.99
AMF+PSB	0.566	0.575	1.143

Table 6: Effect of AM fungus and PSB on chlorophyll content in *Zea mays* pers. for 90 days.

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
(Control)	0.216	0.233	0.451
AMF	0.557	0.590	1.149
PSB	0.505	0.512	1.019
AMF+PSB	0.613	0.600	1.215

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