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Research Paper

Interaction Studies Between Different AM Fungi and PSB on Growth, Chlorophyll Content and Lipids.

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Abstract

Current trends in agriculture are focussed on the reduction of pesticides and inorganic fertilizers. Force to search of alternative ways to improve a most sustainable agriculture. For which beneficial microorganism play an important role in the availability of soil phosphorus to plant roots and increasing phosphate mobilization in the soil. In the present investigation, Sunflower (*Helianthus annus* L.) was inoculated with four different AM fungi with phosphate solubilising bacteria with and without AM Fungi or PSB. The results revealed that there was significantly increased Plant height, shoot length, plant biomass, and number of seeds, % root colonization, and spore number, chlorophyll content in leaves and lipid content in seeds was higher in plants inoculated with *R. fasciculatus* with *Bacilus polymyxa*. It was followed by *Glomus macrocarpum* plus.

Bacillus polymyxa and *Glomus bhagyarajii* plus *Bacillus polymyxa* respectively as second and third bioinoculants. The enhancement of growth and biomass was not recorded in non-inoculated plants or control. And thus, AM fungal indigenous strains cope up with PSB in sunflower rhizosphere promoting synergistic interaction between AM fungi PSB. The importance's of beneficial microorganism with AM fungi have been discussed.

Keywords: *Helianthus annus* L., PSB (Phosphate Solubilising Bacteria), AM fungi, *Rhizophagus fasciculatus, Glomus macrocarpum, Glomus bhagyarajii*, plant biomass.

INTRODUCTION:

An increasing demand for low-input agriculture as resulted in a greater interest in soil microorganisms which are able to enhance plant nutrition and health and to improve soil quality. Modern agriculture, which is characterized by intensive cultivation methods, is totally dependent on regular input of numerous types of inorganic fertilizers. The long term effects of such massive fertilization of the environment are now a matter of serious concern. Current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizers forcing the search for alternative ways to improve a more sustainable agriculture. Therefore, there is need of development of eco-friendly technologies to escape from the adverse effects caused by the synthetic or chemical fertilizers used in the modern agricultural practices. One possible approach is to explore soil microbial diversity for beneficial microbes' soil environment. These microorganisms play an important role in effecting the in soil. The ability of soil microorganisms to convert insoluble forms of phosphorus to a soluble form is an important trait in plant growth-promoting bacteria (PGPB) for increasing plant yields [5, 7].

Arbuscular mycorrhizal fungi (AM) fungi produce beneficial and stable symbiosis with most plant communities. AM fungi are ubiquitous, important for terrestrial ecosystems and have a potential application. AM fungi an endomycorrhiza and has got its name from the distant fungal tree shaped, short lived structure that develops in plant root cells. AM fungi are found under all climates and in all ecosystems, regardless of the type of soil, vegetation or growing conditions. AM fungi can affect the water balance of both amply watered and droughty host plants and have been documented well on various agricultural and horticultural crop plants.

IMPORTANCE OF PHOSPHATE SOLUBILISING BACTERIA:

Plant-associated bacteria that are able to utilize/solubilise the phosphorus from the soil to make it available for the plant are referred as phosphate solubilising bacteria (PSB). These are also called as rhizobacteria that stimulate plant growth are usually referred to as plant growth promoting rhizobacteria (PGPR) [2]. Phosphorus solubilising bacteria play rote in P nutrition by enhancing its availability to plants released from inorganic and organic soil P pools by solubilization and mineralization.

Microorganism enhance the P availability to plants by mineralizing organic P in soil by solubilising precipitated phosphates [1,13,20]. These bacteria in presence of liable carbon serve as a sink for P by rapidly immobilizing it even in low P soils [5]. Hence in the view above constrains, the present study was undertaken to investigate the effect of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on the growth and biochemical contents of *Helianthus annuus,* L., under experimental conditions.

SOURCE OF INOCULUMS:

Four different AM fungal species were selected for the experiment namely *Rhizophagus fasciculatus, Glomus macrocorpum, Glomus Bagyarajii and Sclerocystils dussii.* The soil based inoculums containing chlamydospores and colonized roots, rhizosphere soil of sorghum (i.e. host plant used for mass multiplication of all AM fungal species) having mycelia was served as AM fungal inoculums. These inoculums were maintained at 4^0c in refrigerator of microbiology Laboratory, P.G. Department of Studies in Botany, Karnatak University, Dharwad. 580003, India.

Isolation of phosphate solubilising bacteria 10g rhizosphere soil was suspended in 100ml of distilled water. An aliquot (100ml) from decimal dilutions was inoculated on Pikcovskaya's medium (pH 7.2 incubated at 30^0c Colonies (cfu) of PSB were counted after 24 hours. The solubility of phosphate was observed as a zone of clearance with a diameter that was measured in millimeters and observations were noted in triplicates. Single colonies appearing on Pikcovskaya's agar plates were transferred in liquid nutrient broth and on agar slants and maintained at 4^0 C for further study.

EXPERIMENTAL SET UP:

Earthen pots measuring about 25 X 30cm cm diameter containing 4 kg growth media (sand : soil: Fym = 1:2:1 ratio v/v) were used for the experiment. The surface sterilized seeds (4 seeds per poot) were sowed in the pots containing growth media. After 15 days of seed germination four different AM fungal inoculums along with *Bacillus Polymyxa* were inoculated and the following treatments were maintained under greenhouse conditions.

1. Control
2. Inoculation with *Rhizophagus fasciculatus and Bacillus polymyxa*
3. Inoculation with *Glomus bagyarajii and Bacillus polymyxa*
4. Inoculation with *Glomus macrocarpum and Bacillus polymyxa*
5. Inoculation with *Sclerocystils dussii and Bacillus polymyxa*
6. Inoculation only with *Bacillus polymyxa*

AM fungi spores were recovered from the rhizosphere of *Helianthus annuus L.*, inoculated with different AM fungi, by adopting wet sleving and decanting method described [12]. One to five gram (105g) of *Helianthus annuus* L,. fine roots were collected and maintained in lactoglycerol solution. Roots were then cleared in 10% KOH and stained with 0.05% tryphan blue in lacto glycerol to reveal AM fungi structure. Stained roots were cut in to 1cm fragments and macerated on slides in a drop of lacto glycerol [10].

RESULTS

Helianthus anmus L., showed a significant positive response to each AM fungi and *Bacillus polymyxa* inoculation. Experimental results showed increased growth of plants inoculated with AM fungi and *Bacillus Polymyxa* compared to non-inoculated ones. But the rate of extent of increased growth was varied with each AM fungi inoculation. *H. annuus* L. had maximum growth with inoculation of AM fungus *Sclerocystis dussii* and *Bacillus polymyxa* compared to other treatments. It was observed that other four inoculated plants shows significantly increased plant height, root length, weight, over the non-inoculated plants. Next to the *Sclerocysis dussii* and *Bacillus polymyxa*, *Rhizophagus fasciculatus* with *Bacillus polymyxa* showed efficient strains to influence on growth. *Bacillus polymyxa* individually showed good result compared to *Glomus macrocarpum* and *Glomus bagyarajii* with *Bacillus polymyxa*, but it was significantly higher than the non *mycorrhizal* plants. Experiments results also revealed that almost 50% increase in plant growth response at 90 days after the observation recorded at 60 days.

H. annuus L. subjected for analysis of shoot and root fresh weight as well as dry weight of AM fungi with *Bacilus polymyxa* inoculated plants showed increased biomass production over control plants. The rate of extent of increase in biomass was varied with each AM fungi and *Bacilus polymyxa* influenced the increased value for biomass production, after inoculation of AM fungus *Sclerocysstic dussii with Bacillus polymyxa* when compared to other four inoculants. The second best AM fungus with *Bacillus polymyxa* was *Rhizophagus fasciculus.* The *Bacillus polimyxa* also showed significantly increased weight in fresh as well as dry weight over the other two inoculants. The least values for plant fresh weight as well dry weight recorded with *Glomus bagyarajiii but* it was significantly higher than that of non mycorrhizal plants.

H. *annuus* L. also showed increased in the sized of the head. The inoculation with *Sclerocystis dussii with bacillus polymyxa showed an* enormous growth in size of head and also in the yield. It was also found that other four inoculated plants have significantly increased in the size of head and the yield over the non-inoculated plants. The least value for the growth of head and yield was observed in *glomus bagyarajii* and *Glomus macrocarpum* with *Bacillus polymyxa* brought significantly higher than non mycorrhizal plants.

Helianthus annus L,. was inoculated with one bacterial species that is *Bacillus polymyxa* and four different AM fungi and were found to be positively mycorrhizal over the non-inoculated plants. Result showed that maximum percent of mycorrhizal colonization was recorded in the roots of *Helianthus annuus L*., inoculated with *Sclerocystis dussii and Bacillus polymyxa* when compared to other inoculants of AM fungi and non-inoculated plant. It was found that the lesser value for mycorrhical colonization with inoculation AM fungi *Glomus bagyarajii with Bacillus polymyxa* but it was significant over non inoculated plants. Results also indicate that increased number of AM fungal spores in the rhizosphere of *Helianthus annuus L.,* inoculated with four different AM fungi over the control plants, but it was varied with each fungal species.

Helianthus annuus L, with dual inoculation of *Sclerocystis dusuii* and *Bacillus polymyxa* showed maximum chlorophyll and lipid content over the non-inoculated plants. It was also observed that the second highest lipid and cholorphyll content was seen in plants inoculated with *Rhizophagus fasciculatus and bacillus palymyxa.* Similarly, the P uptake was also highest by the inoculation of AM fungi. The *Glomus macrocarpum and Glomus bagyarajii with Bacillus polymyxa* showed least content of chlorophyll and lipid but greater than the non-inoculated plants.

Table 1: Showing the effect of AM fungi on growth parameters of *Helianthus annuus* L. inoculated with four different AM fungi with *Bacillus polymyxa* at 60 DAS and 90 days.

Treatments	SL in cm	RL in cm	FWS in g	DWS in g	FWR in g	DWR in g	No of Seeds
60 days							
Control	72.0a	3.5a	4.431a	0.71a	0.261b	0.048a	NA
B.p	80.4d	6.2b	8.284c	1.342e	1.342c	0.112d	NA
R.f+B.p.	100.5c	7.2d	11.301b	1.529b	0.796ad	0.143g	NA
G.m+B.p	77.2e	5.6ab	9.492d	1.01c	0.295g	0.108e	NA
G.b+B.p	71.5g	5.2c	7.917e	1.138e	0.498e	0.180b	NA
S.d+B.p	109.2bc	8.2d	13.483c	2.712d	1.11d	0.176c	NA
90 days							
Control	82b	4.7a	7.56b	1.602a	0.429c	0.112a	98
B.p	93a	7.5b	11.374ab	2.013b	0.659d	0.156e	105
R.f+B.p.	107.5b	8.5d	17.185g	3.271d	1.178b	0.512g	151
G.m+B.p	82.5c	7.4e	14.54b	2.512c	0.727e	0.152c	131
G.b+B.p	78.5d	6.0ab	13.905c	2.502e	0.659c	0.196ab	117
S.d+B.p	119.5g	9.0g	20.005a	4.788g	1.755g	0.622b	197

Note : FWR-Fresh Weight of root, DWR-Dry Weight of Root, B.p-Bacillus polymyxa, R.f- *Rhizophagus fasciculatus*, G.m-*Glomus macrocarpum*, G.p-*Glomus bagyarajii*, S.d- *Sclerocystis dussii*, SL-Shoot length, RL-Root Length, SW-Shoot width.

Table 2: Showing the effect of AM fungi and PSB on biochemical parameters on *Helianthus annuas L.*,

Treatments	Percent mycorrhizal colonization		Spores number		Chlorophyll mg/g tissue	Lipid content mg/g seeds	P uptake mg/g tissue	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Control	12.63a	28.34a	1.173a	0.332a	1.173a	0.332a	0.05	0.05
B.p	-	-	1.425d	0.546g	1.425dc	0.546c	0.11	0.11
R.f+B.p.	35.15c	66.86b	1.27c	0.51b	1.27e	0.51b	0.14	0.17
G.m+B.p	29.59d	29.59e	1.25b	0.176a	1.25d	0.176d	0.12	0.11

G.b+B.p	22.32g	58.19ad	0.650bd	0.19c	0.65c	0.19e	0.12	0.12
S.d+B.p	39.53f	74.32c	1.400e	0.48e	1.4b	0.48g	0.13	0.12

Note : B.P= *Bacillus polymyxa, R.f=Rhizophagus fasciculatus, G.m-=Glomus macrocarpum, G.p=Glomus bagyarajii, S.d=Sclerocystis dussii.*

Fig 1: showing the effect of AM fungi on Chlorophyll and Lipid content of *Helianthus annuas* L., for 60 and 90 days.

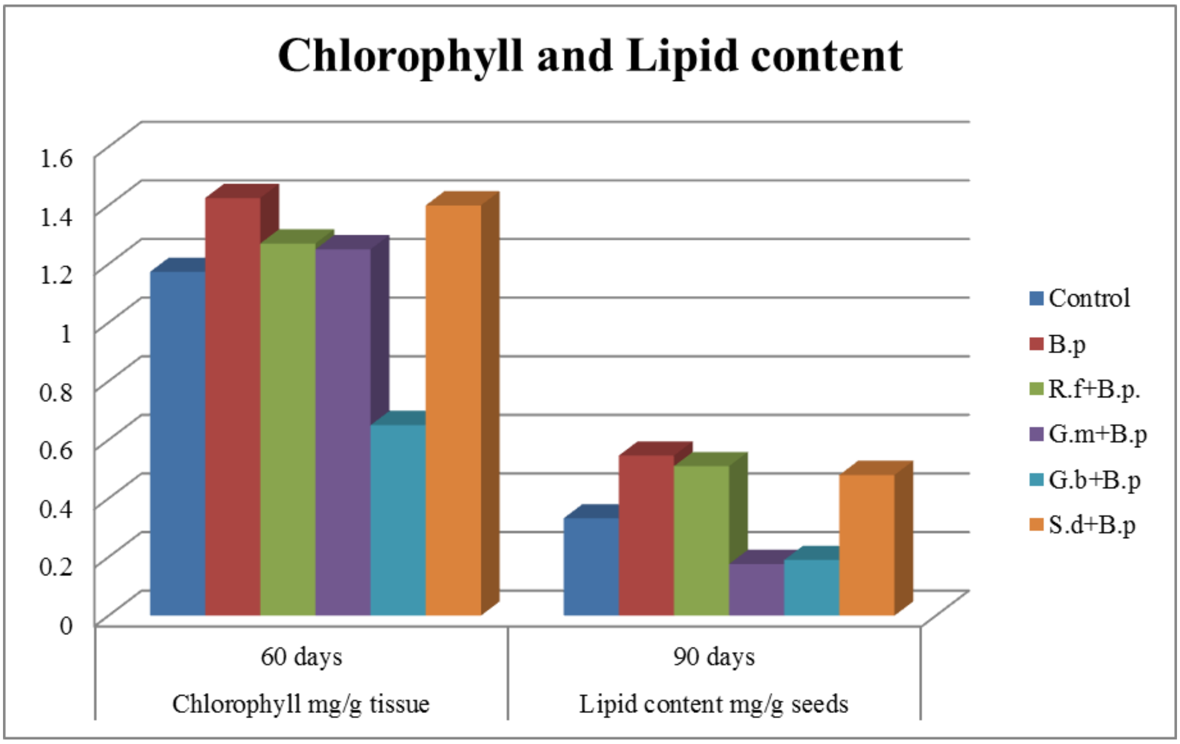
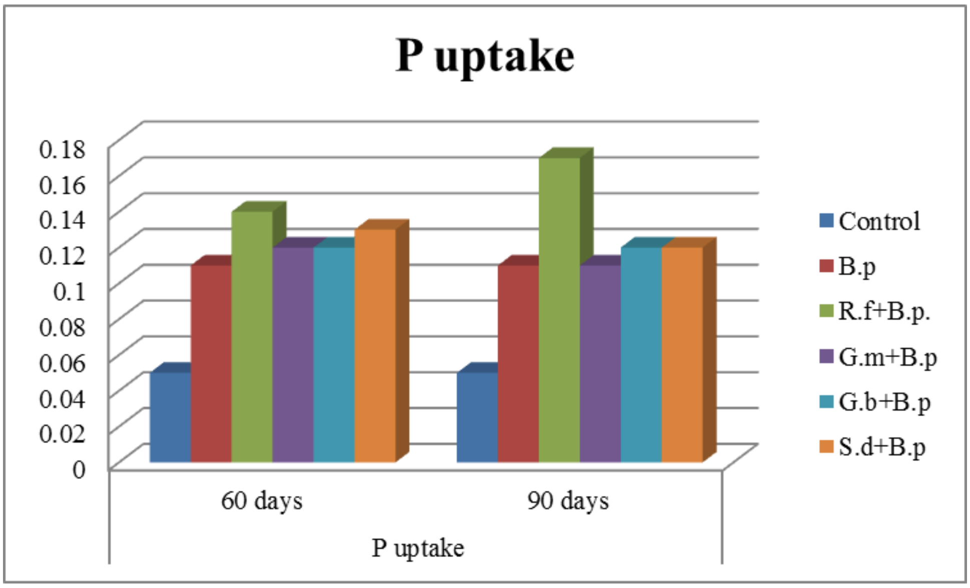


Fig 2: Showing the effect of AM fungi on P uptake of *Helianthus annuas* L., for 60 and 90 days.



DISCUSSION

The experimental results revealed that the sunflower inoculated with AM fungus and *Bacillus polymyxa* have showed synergy. The increased growth parameters were encountered with each treatment. The similar results were reported by many earlier researchers. The synergistic microbial interaction between AM fungi and phosphate solubilizing bacteria (PSB) in improving P supply to plant has been reported [9]. The increased plant growth was recorded for sunflower inoculated with

AM fungus and *Basillus polymyxa*. This indicated that microorganisms act synergistically when, they were inoculated simultaneously [7, 17].

The microbial inoculations to the sunflower plants have shown much significant results with dual inoculation over the single inoculation with *Basillus polymyxa* alone. Effects of these interactions may be exploited for the benefit of sustainable agriculture. A number of studies on the interaction of AM fungus with wide variety of soil microorganiss. The present findings are par with the results of [4, 6, 14, 16] exist under various agroclimatic conditions. The experimental results confirmed that, the dual inoculation of AM fungus and PSB yielded positive results over the remaining treatments. Since, both are beneficial for increasing growth and yield [3, 11]. Since the soil contains extremely rich pool of microbial entities with highly diversified and complex relationships, this characteristic of soil may sometimes contribute difficulty to reproduce similar results [14].

The mycorrhizal colonization was more in sunflower roots inoculated with AM fungus along with PSB over the control. The similar results were recorded by [18, 19] who have demonstrated that, the presence of *Trichoderma* enhanced mycorrhizal colonization but, the root colonization with dual inoculation of AM fungus *Glomus bagyarajii* and PSB, *Bacillus polymyxae* had shown lesser increased per cent root colonization over the other dual inoculations. These findings are inconsistence with reports of [8, 21] the interaction effect of AM fungi and phosphate solubilising soil micro-organisms on plant growth have been demonstrated thoroughly in this study. The earlier contributors found that combined inoculation of *G.mosseae and P.oxalicum* enhanced growth of *geranium* plants. The present experimental results strongly support these findings.

The experimental results revealed that, sunflower inoculated with AM fungus along with PSB have showed significant results with respect to mycorrhizal spore population in the rhizosphere. This indicated that, AM fungus cope up with PSB associated sunflower rhizosphere. Similar results were encountered by earlier workers. Since AM symbiosis is known to alter microbial population composition in the rhizosphere [15] testing the interaction of AM fungi and soil beneficial microbes is useful to understand the possible additive or synergistic effects.

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