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TRIPARTITE BIOINOCULANTS BENEFICIAL TO *PHASEOLUS AUREUS ROXB.* (GREEN GRAM) TO IMPROVE PLANT GROWTH BIOMASS PRODUCTION

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Abstract: Inoculation of beneficial microorganisms gains more important in recent days. Interactions between Arbuscular Mycorrhizal Fungus (*Rhizophagus fasciculatus*), Rhizobium and Phosphate Solubilizing Bacteria (PSB) *Bacillus polymyxa* have been studied on Green gram. In the present work, results revealed that single inoculation (AM Fungus) favours in the percent of colonization and spore number. But dual inoculation (Mycorrhiza+*Rhizobium* and Mycorrhiza+PSB) has improved the plant growth. However, plants inoculated with three bioinoculants such as AM Fungus, *Rhizobium* and Phosphate Solubilizing Bacteria (PSB) showed significantly increased tripartite biomass production of shoot and root. The nutrients like Nitrogen and Phosphorus also have been increased significantly over the control plants. It can be concluded that legume seeds need tripartite microbial inoculants before sowing to get maximum yield.

Keywords: Arbuscular Mycorrhizal Fungi (AMF), *Rhizobium*, Green house, Phosphate Solubilizing Bacteria, Percent root colonization, Biomass production.

INTRODUCTION

Legumes are the second most important group of food plants belonging to the family Leguminosae. They form an important and indispensable part of our daily diet and are commonly known as pulses. Leguminous plants are cultivated all over to the world. India has the distinction of being the world's single largest producers of pulses. Most of the legumes providing high protein grain and improving soil fertility. Leguminous plants form two types of symbiotic associations with microorganisms. One with *Rhizobium* species involved in nitrogen fixation, and other with arbuscular mycorrhizal fungi, concerned with the uptake of phosphorus and other nutrients (Schreiner and Bathlen Falvay, 1995). Rhizosphere is the specialized ecological niche and supports intense microbial activity extending several milimeters from the root system of growing plants. About 80% of the terrestrial plants are associated with AM fungi and are thus potential factors determining diversity in ecosystems (Giovannetti and Gianinazzi Pearson, 1994; Lakshman, 1996). The present investigation experiments were undertaken to improve the yield of Green gram at green house conditions.

MATERIALS AND METHODS

Collection of soil

Rhizospheric soil samples of Green gram plants were collected in polythin bags with proper labeling from various locations of Dharwad, where the plants are growing. The Geographical location of Dharwad is lies between (15˚ 28´ N 75˚ 1´ E) of Northwestern part of Karnataka state, in India. The Collection was made during the month of June 2013 and December 2013. Triplicate rhizosphere soil samples from 0 to 20 cm depth along with roots were brought from various locations of Dharwad. The soil collected samples were stored in clean sterile polythene bags and kept, in refrigerator at 4° C until use.

Isolation of Arbuscular Mycorrhizal (AM) spores in soil

Triplicates with fifty grams of rhizospheric soil samples were taken from experimental plant and assayed for AM spore count using wet sieving and decanting technique according to (Gerdemann and Nicolson, 1963).

$$\text{Percent of root colonization} = \frac{\text{No. of root bits showing colonization}}{\text{Total number of root bits observed.}} \times 100$$

RESULTS AND DISCUSSION

AMF spore number and per cent root colonization was recorded in Green gram plants inoculated with mycorrhiza. Plants inoculated with mycorrhiza (*Rhizophagus fasciculatus*), *Rhizobium japanicum* plus PSB showed maximum growth at 90 days (As shown in Table 1). Dual inoculation with *Rhizobium* plus mycorrhiza produced significantly higher N and P uptake in shoots of green gram. Single, double or triple inoculation further caused an improvement in the per cent colonization and maximum improvement in biomass production. The seed inoculation with *Bacillus polymyxa* with soil inoculation of AMF and *Rhizobium japanicum* brought a

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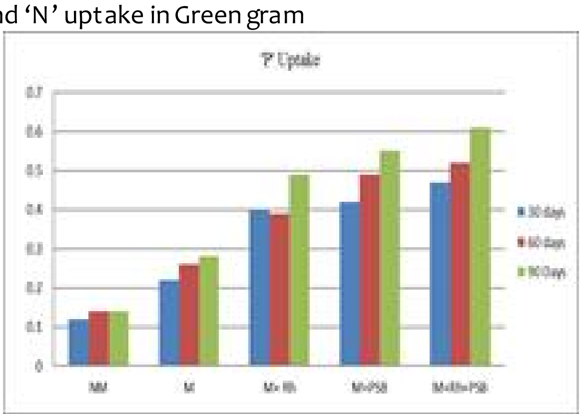
significant increase in shoot and root biomass and total uptake of Nitrogen and Phosphorus by plants (As Shown in Figure 1). The inference can be made that PSB (*Bacillus polymyxa*) have some impact on spore germiniation, AMF colonization and eventually spore production. These results considered AMF inoculation is needed with beneficial bacteria i.e., *Rhizobium* and phosphate solubilizing bacteria. Because dual inoculated plants were significantly showed enhanced growth when compared with the control plants. There the results are in consistent with (Bhagyraj *et al.*, 1979; Azcon and Rubio, 1990; Lakshman and Ratageri 2002) early workers contribution. Similar findings were recorded by (Young *et al.*, 1988; Behl *et al.*, 2007). It has been found that some bacteria present in the mycorrhizosphere can modify or alter the establishment of mycorrhizal sysmbiosis, which are also called as mycorrhizal helper bacteria (MHB) essential for plant community (Duponnois *et al.*, 1993). Inoculation of legumes with AM fungi can stimulate nodulation and nitrogen fixation (Rose, 1980). Recent investigations have brought light to instances where biological activities are markedly enhanced in two or three beneficial microorganisms association. The phosphate solubilizing microorganisms (PSM) interact well with AM fungi in P deficient soils (Lakshman *et al.*, 2005). The present investigation brings out clearly that AMF greatly assist nodulation and Nitrogen fixation with *Rhizobium* inoculation over single inoculation with only *Rhizobium*. Therefore tripartite interaction brings out an effective plant growth, biomass production N and P uptake in Green gram plants on the inoculation of AM fungi, *Rhizobium* and phosphate solubilizing bacteria (Lakshman, 2009 and 2013). It can be concluded that it is a common practice to grow nodulated plants in poor agricultural soil to increase their fertility and effective strains of *Rhizobia* are often used for treating seeds. The present study brings out that an indigenous AM fungi, PSB with *Rhizobium* tripartite system could contribute to the efficiency of such system, especially in phosphorus deficient soils.

Figure 1: Interaction between R. *fasciculatum*, *Rhizobium* and Phosphate solubilizing bacteria on 'P' and 'N' uptake in Green gram



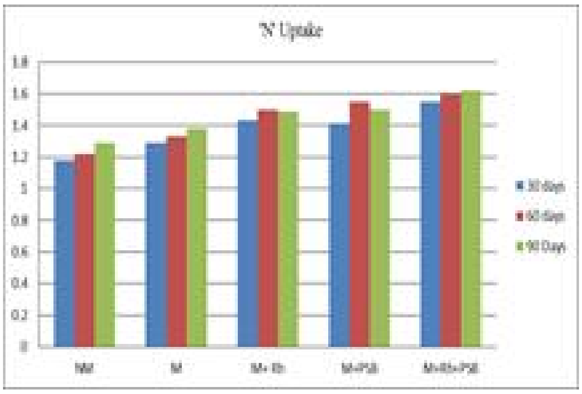


Table 1: Interaction between *Rhizophagus fasciculatus*, *Rhizobium*, Phosphate solubilizing bacteria on plant height, shoot and root dry weight, per cent root colonization and spore number for 90 days on Green gram.

Treatment	Plant Height (cm)	Dry weight of shoot (g)	Dry weight of root (g)	Percent of coloniza-tion	No. of spores/50 g of soil
30 days					
NM	21.40 e	0.54e	0.09e	00.00 e	000.00 e
M	43.96 d	1.15d	0.31d	65.80 d	211.00 d
M+ Rh	56.70 c	1.19c	0.37c	62.50 c	113.00 c
M+PSB	62.70 b	1.20b	0.40b	71.18 b	117.00 b
M+Rh+PSB	86.00 a	1.29a	0.46a	76.34 a	121.00 a
60 days					
NM	26.72 e	0.56e	0.16e	00.00 e	000.00 e
M	59.04 d	1.24d	0.37d	67.00 d	215.00 d
M+ Rh	78.85 c	1.26c	0.39c	70.00 c	122.00 c
M+PSB	85.72 b	1.31b	0.43b	76.00 b	131.00 b
M+Rh+PSB	97.31 a	1.35a	0.50a	85.00 a	142.00 a
90 Days					
NM	46.48 e	0.70e	0.20e	00.00 e	000.0 e
M	82.83 d	1.30d	0.39d	69.04 d	347.0 d
M+ Rh	94.87 c	1.33c	0.45c	69.38 c	352.0 c
M+PSB	103.22 b	1.38b	0.49b	75.80 b	354.0 b
M+Rh+PSB	125.23 a	1.44a	0.58a	62.20 a	361.0 a

NM-Non-Mycorrhizal; M-Mycorrhizal (*Rhizophagus fasciculatus*) Rh-*Rhizobium*; PSB- Phosphate solubilizing bacteria

*Mean values followed by the same letter within a column do not differ significantly at *P*= 0.05 by DMRT.

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REFERENCES

1. Azcon R, Rubio R, Interaction between different AMF and *Rhizobium* on growth and nutrition of *Medicago sativa.* Agr. Ecosy. Environ, 1990, 29, 5-9.

2. Bagyaraj DJ, Manjunath A, Patil RB, Interaction between an AMF and *Rhizobium* on *Soybean*, New Phytol. 1979, 82, 141-145.

3. Behl RK, Ruppel S, Kothe E, Narula N, Legume, *Azotobacter,* AMF interaction towards plant nutrition and growth. An gewandte Botanik. 2007, 81(2), 95-109.

4. Duponnois R, Garbaye J, Banchard D, Charin JL, The fungus specificity of mycorrhization helper bacteria (MHBs) used as an alternative to soil for inoculation of base root Douglas for planting stocks with *Laccaria laccata.* Plant soil, 1993, 157, 257-262.

5. Gerdemann JW, Nicolson TH, Spores of mycorrhizal Endogone species extracted from soil by wet-sieving and decanting, Trans. Brit. Mycol. Soc*, 1963,* 46, 235-244.

6. Giovannetti, Gianinazzi Pearson, Diversity of arbuscular mycorrhizal fungi, Mycol. *Res,* 1994, 98, 705-715.

7. Griffin DM, Ecology of soil fungi, Chapman Hall, London, 1972, 193.

8. Jackson ML . Soil chemical analysis. New Delhi; Prentice Hall of India (P) Ltd, 1973, 498.

9. Lakshman HC, VA mycorrhizal fungi occurrence in some important timber tree species of Kalaghatgi forest in Karnataka. J. Bihar. Bot. Soc, 1996, 5(1-2), 16-22.

10. Lakshman HC, Ratigeri RH, Improvement of *Dolichos lablab* with dual inoculation of AMF and Rhizobium, Eco. Planning Environ. 2002, 112, 79-84.

11. Lakshman HC, Ratigeri RH, Dual inoculation of VA-mycorrhizal and *Rhizobium* beneficial to Pithecollobium dulse Roxb., J. of Microbial World, 2005, 7(2), 163-168.

12. Lakshman HC, Growth response and nitrogen fixation of *Phaseolus lunatus* (Lima bean) with the inoculation of AM fungi and *Rhizobium, Asian Sciences*, 2009, 4 (1and2), 37-41.

13. Lakshman HC, Synergistic interaction among *Azotobacter, Pseudomonas* and AM fungi on two varieties of *Sesamum indicum* L. Communications in Soil Science and Plant Analysis, 2011, 42, 2122-2133.

14. Lakshman HC, Effect of AM fungi, *Azotobacter* and phosphate solubilizing bacteria in improvement of *Amaranthus paniculatus* L. a leafy vegetable, Research Journal of Biotechnology. 2013, 8(3), 36-39.

15. Phillips JM, Hayman DS, Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection, Trans. Brit. Mycol. Soc, 1970, 55, 158-161.

16. Rose SL, Mycorrhizal association of some nodulated nitrogen fixing plants*,* Can. J. Bot, 1980, 58, 1449-1454.

17. Schenck NC, Perez Y, Manual for the Identification of VA mycorrhizal fungi Gainesville, Florida; University of Florida, 1990, 249.

18. Schreiner RP, Bethlenfalvay GJ, Mycorrhizal interactions in sustainable agriculture, Critical Review in Biot, 1995, 15, 271-28.

19. Young CC, Juang TC, Chao CC, Effect of *Rhizobium* and AM fungi inoculation on nodulation, Nitrogen fixation and yield of soybean, Biology and Fertility soils, 1988, 6, 165-169.

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