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Comparative studies of Mycorrhizae and Rhizobium on *Phaseolus mungo* L. and *Phaseolus aconitifolius* Jacq.

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Abstract
The study was initiated to determine the influence of VAM fungi on a Rhizobium- legume interaction. The pot culture and Leonard jar experiment was conducted during rabbi season with seed of *Phaseolus mungo* L. and *Phaseolus aconitifolius* Jacq. The different inoculations of Rhizobium and Mycorrhizae were incorporated. During the period of experiment, samples were analyzed for nodule numbers, dry weight of nodules, fresh weight, dry weight of plant, yield of pods, and length of plant and percentage of Mycorrhizal colonization. The results revealed that dual inoculation of Rhizobium with VAM enhanced seed yield of black gram and Turkish gram.

Keywords: VAM, Rhizobium, *Phaseolus mungo* L, *Phaseolus aconitifolius* Jacq. *Glomus sps.* crop yield, Rhizobium-legume interaction, pot culture, Leonard-jar experiment

1. Introduction

Mycorrhizal fungi are vital component of natural ecosystem, being gifted to form symbiont with plant roots. AM fungi have mutualistic relationships with more than 80% of terrestrial plant species. Because of wide range of relationships with host plants, it becomes difficult to identify the species on the morphological bases as the spores are to be extracted from the soil. In spite of their abundance and wide range of relationship with plant species, AMF have shown low species diversity. (Prasad *et al* 2017) [14] AMF have high functional diversity because different combinations of host plants and AMF have different effects on the numerous aspects of symbiosis. In soil microbiology new area have received the attention over the hundred years of subject's history as the fixing symbiosis that exists between plants of Leguminosae and Rhizobial (Harris 1988) [10] and forming nodules on them. Biofertilizer provide a means to keep together two compatible partners. A producer of bio fertilizer's therefore must seek out revalent basic information such as soil type, crop rotation, allelopathic effects, and cultivars grown in the region and choose a suitable diazotroph that will have positive interaction with host genome etc.

Mycorrhizal fungi interact with a wide range of other soil organisms, in the root, in the rhizosphere and in the bulk soil. These interactions may be inhibitory or stimulatory; some are clearly competitive, others may be mutualistic. Effects can be seen at all stages of the mycorrhizal fungal life-cycle, from spore population dynamics (predation, dispersal and germination) through root colonization to external hyphal growth. Two areas that seem likely to be of particular importance to the functioning of the symbiosis are the role of bacteria in promoting mycorrhiza formation and of soil animals in grazing the external mycelium. Mycorrhizal fungi also modify the interactions of plants with other soil organisms, both pathogens, such as root-inhabiting nematodes and fungi, and mutualists, notably nitrogen-fixing bacteria (Fitter and Garbaye 2002) [9].

In this paper the effects of Mycorrhizae and rhizobium on the yield pattern of black gram and Turkish gram have been given. The soil bacteria such as rhizobia have ability to cause infection in root tissues of the compatible host plant of legume and start the formation of nitrogen fixing nodules. It is the site for symbiotic nitrogen fixation formed because of series of interactions between rhizobium and leguminous plants. Most rhizobium isolates can nodulate more than one host plant species, while several different bacterial species are often isolated from a single legume host plant (Cooper, 2007) [8].

Vesicular Arbuscular Mycorrhizal (VAM) fungi form a symbiotic relationship with the host by colonizing the roots. Associative effects of VAM with rhizobia have been well-documented. Legumes are generally nodulated by rhizobia but many legumes grow poorly and failed to nodulate even with the rhizobial inoculation in autoclaved soil unless they were inoculated with mycorrhiza also. The tripartite symbiosis between leguminous plants, rhizobium species and VAM fungi has been the subject of intensive research in recent years. A synergistic beneficial effect of dual inoculation with V AM fungi and rhizobium in growth and nutrition in legumes has been demonstrated by many workers. Generally VAM fungi are known to improve phosphate nutrition, which in turn enhances plant growth and nitrogen fixation. Leopald and Hofner reported the combined inoculation of clovers with rhizobium strains and Tunsia isolate of *Glomus etunicatum* and application of rock phosphate gave greatest shoot dry matter, yield increase and has variable effects on numbers of large nodules formed.

AM fungi on changes in key biochemical components of plants? Showed increase in chlorophyll, protein, total carbohydrates and ascorbic acid, starch, peroxidase and polyphenol oxidase levels when plants were grown with mycorrhiza. Alkaline and acid phosphatase analysis showed lowered levels with mycorrhiza thereby confirming their role in achieving symbiosis. Collaborated results confirmed the positive effect on biochemical and nutritive capacity of mycorrhizal plants (Shinde and Khanna 2014) [2].

A successful symbiosis and nitrogen fixation may be attained, if the conditions of Rhizobium inoculants remain optimized

(Zharan, 2001). The isolation and screening of highly effective and competitive strains from native rhizobial population to be used as inoculums could be much beneficial under field conditions (Chatel and Greenwood, 1973) [7]. Based on these potent reviews, the aim of the research was to examine *Glomus sps.* On a Rhizobium-legume interaction with and *Phaseolous mungo* L. and *Phaseolus aconitifolius* Jacq.

2. Materials and Methods

Collection, isolation, purification and authentication of Rhizobium root nodules were done by methods commended by Vincent (1970). Multiplication of rhizobial culture was done by "yeast extract mannitol technique. The four treatments involved in the study are; 1) Control 2) inoculation with Mycorrhizae 3 inoculation with Rhizobium 4) Inoculation with Mycorrhizae +Rhizobium.

2.1 Isoatlon and Identification of Rhizobium from nodules

Legume nodules generally arise as globose or flat elongate swelling, varying from a few mm. to a few cm. in length and few mm. in diameters.

The following methods are used to study the morphology and anatomy of the nodules of *Phaseolous mungo* L. and *Phaseolus aconitifolius* Jacq. Seeds of were sown in botanical garden, after three to four weeks, plants were uprooted for the study of nodules.

Authentication of isolates as rhizobia and mycorrhiza by plant infection tests. These includes 1) pot cultures 2) Leonard jar assemblies (Leonard, 1944 and Vincent 1970) Modified Leonard jar assemblies were set up using plastic saline bottles of 500 ml. capacity with it bottom and neatly ground is inverted in 250 ml. conical flask with suitable dimensions in such a way that the neck of the bottle snugly fits in it, and mouth is pluged with absorbent cotton. A cotton lamp wick was made to pass through the narrow end of the bottle in such way that part of it remained in upper half of sand substrate and half in nitrate free nutrient solution.

The wick is intended to maintain a steady supply of nutrient solution to the growing plant by the capillary action of the sand. The saline bottle is filled with washed and oven dried sterile river sand and the conical flask containing one-fourth strength Reading's nitrogen free nutrient solution (Rao 1981) which was also used to saturate the rooting medium. The top portion of assembly is covered with petridish half.

2.2 Collection off resting spores of VAM

The soil near the root systems of the garden planted plants as well as the local planted plants were collected and this soil was used for collection of resting spores of VAM. By wet sieving and floating technique the spores were collected.

Arbuscular mycorrhizal fungi can provide numerous benefits to their plant hosts, including improved nutrient uptake, drought resistance, and disease resistance. However, the symbiosis is not mutualistic in all circumstances and may often be parasitic, with a detrimental effect on plant growth. Rarely, some plant species can parasitise the fungi

Surface sterilized and pre-germinating seeds of *Phaseolous mungo* L. and *Phaseolus aconitifolius* Jacq. were selected and washed with sterilized water were sown on sand at 3 to 4 cm deep by removing petridish half, one ml of Rhizobial suspension and 12-15 spores of mycorrhizal fungus *Glomus sps* were added to two different plants and four sets were maintained like as:

1) Control
2) Rhizobium
3) Mycorrhiza
4) Rhizobium+ Mycorrhiza.

In control, the assembly contains only two-three pregerainated seeds there were no nodules or VAM spores.In Rhizobium, the assembly contains pregerminated seeds along with one rhizobial suspension. In mycorrhiza the assembly had 12-15 surface sterilized mycorrhizal spores of *Glomus sps* and in Rhizobium +Mycorrhiza the assembly contains one ml rhizobial suspension along with 12-15 VAM spores. Thinning was carried out after 5-7 days keeping 1-2 uniform plants per assembly. Plants were harvested at the end of 30 days and observations such as number and size of nodules, infection of VAM fungi, fresh and dry weight of roots and shoots, number of spores etc. were recorded.

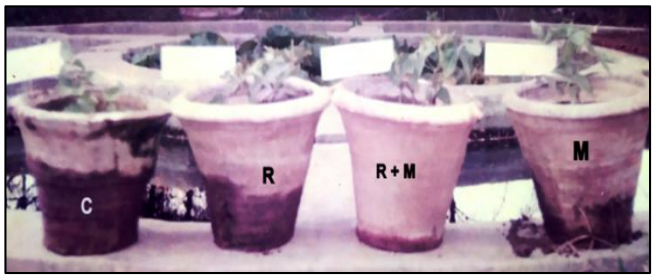

Fig 1: Pot Culture Experiment

Fig 2: Leonard jar assembly

3. Results and Discussion

The number of nodules shoots weight, root weight. The number of nodules were recorded higher where plants grown in pot culture than in Leonard jars. In the same way, the VAM spores were found higher in pot cultures than in Leonard jars.

Table 1: Authentication of Rhizobium and VAM in Pot Culture experiment

	Phaseolus mungo				*Phaseolus aconitifolius*			
	C	**R**	**M**	**R+M**	**C**	**R**	**M**	**R+M**
No. of nodules per plant	---	14	--	03	--	17	--	26
No. of spores per 10 gm soil	-	--	38	07	--	--	48	56
% of Mycorrhizal colonization	--	--	70	73	--	--	60	80
Shoot length in Cm.	15.2	22.3	20.30	25.3	12.1	21	27.19	30.20
Fresh wt. of plant(gm)	14.80	17.70	19.30	22.30	14.10	17.00	18.70	23.20
Dry wt. of plant(gm)	0.670	0.790	0.890	0.980	0.610	0.820	0.900	1.100
Root length in cm.	11.10	15.59	19.30	22.30	10	13.40	17.20	21.40
Wt. of pods in gms.	25	27.50	30	40.50	19	22.50	25.40	33.70

Table 2: Authentication of Rhizobium and VAM in Leonard Jar experiment

	Phaseolus mungo				*Phaseolus aconitifolius*			
	C	**R**	**M**	**R+M**	**C**	**R**	**M**	**R+M**
No. of nodules per plant	---	17	--	18	---	15	--	22
% of Mycorrhizal colonization	---	---	60	80	--	--	55	43
Shoot- length in cm.	14.2	19.00	21.00	23.00	11.60	20.10	23.00	26.26
Fresh wt. of plant(gm.)	0.900	1.000	1.100	1.250	0.633	0.700	1.110	1.367
Dry wt. of plant(gm.)	0.400	0.700	0.900	0.900	0.360	0.470	0.645	0.700
Root length in cm.	10.00	14.00	17.00	19.00	9.00	13.01	15.30	18.48
Fresh wt. of plant(gm.)	0.400	0.425	0.900	0.950	0.520	0.485	0.700	0.850
Dry wt. of plant(gm.)	0.250	0.280	0.475	0.600	0.300	0.287	0.435	0.500
Wt. of pods in gms.	15	24.13	28.35	40.51	13	15.60	22.16	33.10

In pot culture studies using sterilized soil for the growth of the four test plants, it was observed that the *Phaseolus aconitifolius* by VAM spores and Rhizobium showed the best symbiotic effectiveness as a result of inoculation not only the nodulation improve but there was also the corresponding increase percentage of mycorrhizal colonization (Table no-1).

In Table No. 1 it was observed that the fresh weight of plant was higher in dual inoculation i.e. Mycorrhizae and Rhizobium (23.20 gm) this was followed by only inoculation of VAM spores (18.70 gms) while only Rhizobium showed 17 gm. and un inoculated plants were 14.10 gm.

It was also observed that dry weight was higher in dual inoculation followed by only Mycorrhiza, Rhizobium and control. Their dry weights were 1.100, 0.900,0.820 and 0.610 gms respectively.

This has resulted in the development of pods, in dual inoculation the number of pods were 21, in mycorrhiza it was 14 in Rhizobium it was 11 and in control it was 8.

In pot culture studies using sterilized soil for the growth of the test plants, it was observed that the *Phaseolous mungo* inoculated by VAM spores and Rhizobium showed best symbiotic effectiveness as a result of inoculation not only the nodulation improve but there was also the corresponding increase in percentage of Mycorrhizal colonization. (Table 1)

It was observed that fresh weight of plant was higher in dual inoculation i.e. mycorrhiza and Rhizobium (22.30 gm.) this was followed by only inoculation of VAM spores (19.30 gm.) only Rhizobium showed 17.70 gm. and un inoculated plants were 14.80 gm.

It was also observed that dry weight was higher in dual inoculation followed by only mycorrhiza, Rhizobium and Control, their dry weights was 0.980 gm, 0.890 gm0.790 gm, and 0.670 Gms. respectively.

This has resulted in the development of pods, in dual weight of pods were 40.50, in mycorrhiza it was 30 gm, in rhizobium it was 27.50 gm. and in control it was 25gms.

In table no. 2 the shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight was observed higher in dual inoculation. The shoot length was 25 cm. in rhizobium and mycorrhiza combination fallowed by Mycorrhizae.

Root length was observed higher in dual inoculation followed by Mycorrhizae, Rhizobium and control. The dual inoculation with rhizobium and *Glomus sps* improved the nodulation, dry weight, Mycorrhizal colonization, (Manjunath et.al 1984.

The data indicates that in Leonard jar assemblies (Vincent, 1970) the better nodulation in *Phaseolous mungo* L. was on laterals and brownish pink. The number of nodule were 11 on rhizobium-inoculated plant, while in dual inoculation showed17nodules; on other hand percentage, mycorrhizal colonization was also higher in Mycorrhizae and mycorrhiza-rhizobium combination 70 and 80 percentage respectively. The plants inoculated either with rhizobium or AM fungi significantly increased the shoot length and root length, dry weight of shoot and root, total number of nodules and dry weight of nodules when compare to control. The dual inoculation of AM fungi and rhizobium showed maximum values in all the tested parameter than plants inoculated with individual endophytes (Table 1). Fewer nodules with increased biomass were formed in dual inoculated plants, compared to plants inoculated individually with rhizobium and AM fungi and un inoculated control plants. This contradicts other reports, where more nodules are reported on dual inoculation than non- mycorrhial plants.

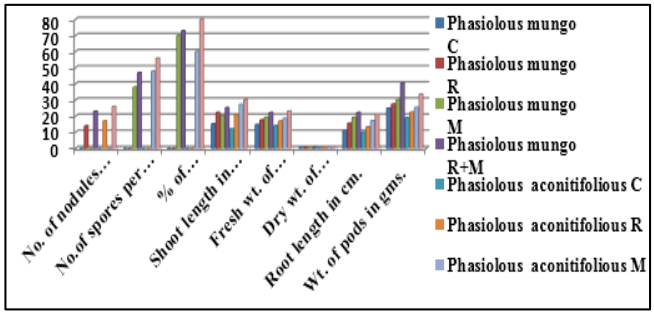

Fig 3: Rhizobium and VAM in Pot Culture experiment

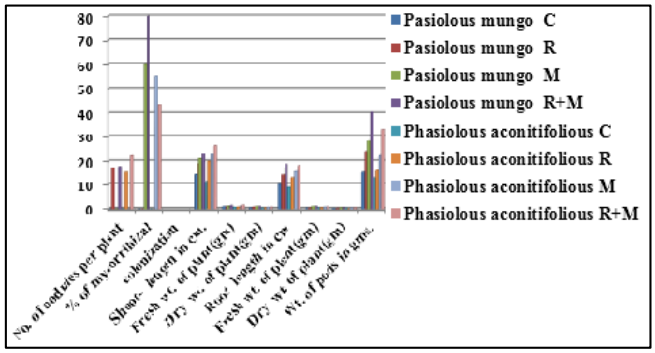

Fig 4: Comparison of Rhizobium and VAM in Leonard jar experiment

The results clearly indicate that the synergistic combination of rhizobium with *Glomus sps* showed the anticipating improvement in growth parameters as well as in the biomass when compared with single inoculation of rhizobium and *Glomus sps* alone. This is due to the ability of rhizobium for nitrogen fixation (Burris and Miller, 1941) as well as well-known effect of VAM fungal association on P- uptake (Grey and Gerdemann 1969). Based on present investigation and results obtained use of bio-fertilizers are highly recommended in agro-foresting ecosystem which is eco-friendly, cost-effective and alternative to synthetic fertilizers. Root nodules formed by Rhizobium sp. under field condition were larger than the nodules formed in the plants under pot condition. Higher nitrogen fixation by large size nodules may account for reduced development (Barker and Huisingh, 1970) [4], Results of the experiment confirms various reports on enhanced plant growth due to AM inoculation to medicinal plants (Mate and Saindanshiv, 2018) [13].

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