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"STUDIES ON LEGUME-RHIZOBIUM AND MYCORRIHIZAL INTERACTION WITHSPECIAL REFERENCE TO PHASEOLUSRADIATUSL. AND PHASEOLUS ACONITIFOLIUSJACQ."

Sunil A. Gosavi
Sathaye College, Dixit Road Vile-Parle (E) Mumbai.Maharashtra

Abstract: The study was initiated to determine the influence of VAM fungi on a Rhizobium- legume interaction. The pot culture experiment was conducted during rabbi season with seed of Phaseolu sradiatus.L.and PhaseolusaconitifoliusJacq.and Leonard jar experiment was conducted for Phaseolusa conitifolius.The different inoculations of Rhizobium and Mycorrihizawere incorporated. During the period of experiment, samples were analysed for nodule numbers, dry weight of nodules,freshweight, dry weight of plant, yield of pods, and length of plant and percentage of Mycorrihizalcolonization. The results revealed that dual inoculation of Rhizobium with VAM enhanced seed yield of chickpea and golden- green gram.

Keyword: PhaseolusradiatesL,PhaseolusaconitifoliusJacq.Glomusfasciculatum,VAM,Rhizobium, Crop yield, Rhizobium-legume interaction, Leonard-jar experiment.

INTRODUCTION:

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) and forming nodules on them.Biofertilizer provide a means to keep together two compatible partners. A producer of biofertilizer'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.

Mycorrihizais greatly defined as a composite structure formed by nutritional associations between fungal hyphae and roots of higher plant.

Frank who first noted the extensive of such a characters association in the root of Cupuliferous plant in 1885 coined the term "Mycorrhiza". Subsequently mycorrhizawas found to occur in roots of many other plants including both Gymnosperms and Angiosperms, and in certain Bryophytes and Angiosperms, and in certain Bryophytes and Pteridophytes. The mycorrhiza are vital for uptake and accumulation of ions from soil and translocation because of their high metabiolic rate and strategically diffuse distribution in the upper soil layers.

In this paper the effectsof Rhizobiumand VAM on the yield pattern of chickpea and green Golden 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 (Stougaard, 2000; Prell and Poole, 2006). 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).

Vesicular ArbuscularMycorrhizal (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 mycorrhizaalso. 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 improvephosphate nutrition, which in turn enhances plant growth and N2 fixation. Leopald and Hofner reported the combined inoculation of clovers with Rhizobium strains and Tunsia isolate of Glomusetunicatumand application of rock phosphate gave greatest shoot dry matter, yield increase and has variable effects on numbers of large nodules formed.

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). Based on these potent reviews, the aim of the research was to examine Glomusfasciculatumon a Rhizobium-legume interactionwithand Phaseolous radiates

L.andPhaseolusaconitifoliusJacq.

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) Rhizobium 3) inoculation with mycorrihiza 4)Inoculation with Mycorrihiza+Rhizobium.

Isolation 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 radiatusL.and Phaseolusa conitifolius Jacq.Seeds of Phaseolous radiatesL. and PhaseolusaconitifoliusJacq.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 intented 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.

Surface sterilized and pre-germinating seeds of Phaseolous radiatesL.andPhaseolusaconitifoliusJacq.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 Glomusfasiculatum 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 Rhizobiumthe assembly containspregerminatedseeds along with oneRhizobial suspension.In mycorrhiza the assembly had 12-15 surface sterilized mycorrhizal spores of Glomusfasiculatumand 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. wererecorded.

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.

In pot culture studies using sterilized soil for the growth of the four test plants, it was observed that the Phaseolusaconitifolius by VAM spores and Rhizobium showed the best symbiotic effectiveness as a result of inoculation not only the nodulation improvebutthere 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. mycorrihiza and rhizobium(12 gm) this was followed by only inoculation of VAM spores (9 gms) while only rhizobium showed 8.20 gm. and uninoculated plants were 9.20 gm.

It was also observed that dry weight was higher la dual inoculation followed by only mycorrhiza, Rhizobiumand Control.Their dry weights were 4.30, 3.00, 3.20, and 1 gms respectively.

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

In pot culture studies using sterilized soil for the growth of the three test plants, it was observed that the Phaseolous radiates 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 gm.) this was followed by only inoculation of VAM spores (15 gm.) only Rhizobium showed 8.5 gm. and uninoculated plants were 5 gm.

It was also observed that dry weight was higher in dual inoculation followed by only mycorrhiza, Rhizobium and Control, their dry weights was 6.350 gm, 2.750 gm, 5.200 gm, and 1.500 gms. respectively.

This has resulted in the development of pods, in dual inoculation,number of pods was 16, in mycorrhiza it was 12, in Rhizobium it was seven and in control it was 6.

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 mycorrihiza.

Root length was observed higher in dual inoculation followed by mycorrihiza, rhizobium and control.Thedual inoculation with Rhizobium and Glomusfaslculatumimproved the nodulation, dry weight, mycorrihizal colonization, (Manjunath et.al 1984). Phaseolous radiates L.andPhaseolusaconitifoliusJacq.

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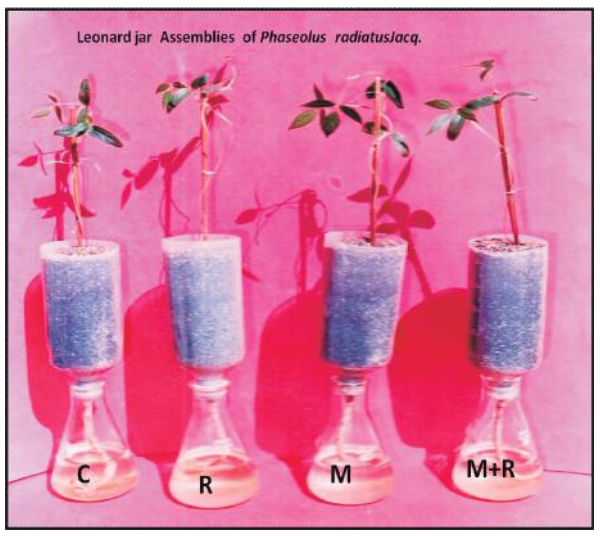
Table-1: AUTHENTICATION OF RHIZOBIUM AND VAM IN POT CULTURE EXPERIMEN

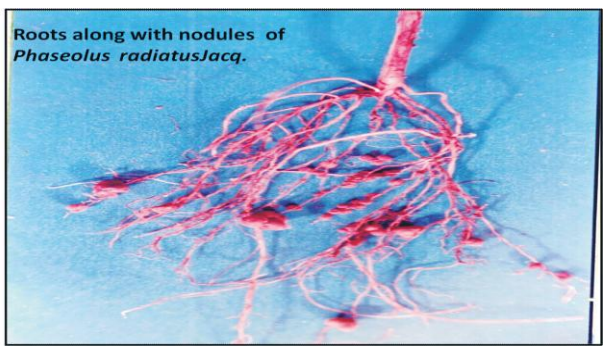
	Phasiolous radiates				*Phasiolousaconitifolious*			
	C	R	M	R+M	C	R	M	R+M
No.of nodules per plant	--	14	--	3	--	17	--	26
No.of spores per 10 gm soil	-	--	38	7	--	--	48	56
% of Mycorrihizal colonization	--	--	70	0	--	--	60	80
Fresh wt. of plant(gm)	4.80	7.70	9.30	12.30	4.10	7.00	8.70	13.20
Dry wt. of plant(gm)	0.650	0.750	0.890	0.990	0.610	0.820	0.900	1.100
No.of pods per plant	3	7	6	9	8	11	4	21

Table-2:AUTHENTICATION OF RHIZOBIUM AND VAM IN LEONARD JAR EXPERIMENT Phasiolous radiatesJacq

	Pasiolousradiates			
	C	R	M	R+M
No. of nodules per plant	---	17	--	18
% of mycorrihizal colonization	---	---	60	80
Shoot- Length in cm.	14.2	19.00	21.00	25.00
Fresh wt. of plant(gm)	0.900	1.000	1.100	1.250
Dry wt. of plant(gm)	0.400	0.700	0.900	0.900
Root Lengthincm	14.00	14.00	17.00	19.00
Fresh wt. of plant(gm)	0.400	0.425	0.900	0.950
Dry wt. of plant(gm)	0.250	0.280	0.475	0.600

The data indicates that in Leonard jar assemblies (Vincent, 1970) the better nodulation in Phaseolous radiates Jacq. 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 mycorrihiza andmycorrhiza-rhizobiumcombination 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 uninoculated control plants. This contradicts other reports, where more nodules are reported on dual inoculation than non- mycorrhial plants.





The results clearly indicate that the synergistic combination of Rhizobium with Glomusfasciculatumshowed the anticipating improvement in growth parameters as well as in the biomass when compared with single inoculation of Rhizobium and Glomusfasciculatumalone. 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 (Akhtar and Siddiqui, 2008). Higher nitrogen fixation by large size nodules may account for reduced development (Barker and Huisingh, 1970)

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