

DUAL INOCULATION EFFECT OF *RHIZOBIUM* (COW PEA MISCELLANY) AND VAM FUNGI ON GROWTH, NODULATION AND NITROGEN FIXATION IN *PROSOPIS CINERARIA*

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Of several dual combinations of rhizobial isolates (PC Rhz-5, PC Rhz-7, PC Rhz-8) and VAM fungi (*Gigaspora calospora*, *Glomus fasciculatum* and *Glomus mosseae*) tested, the seedlings inoculated with *Rhizobium* (PC Rhz-5) + *Glomus mosseae* as twin symbionts recorded maximum enhancement of all parameters tested (growth, nodulation and nitrogenase activity) in comparison to the seedlings inoculated with *Rhizobium* alone. Among single inoculated seedlings, the seedling inoculated with rhizobial isolate PC Rhz-5 recorded highest values of all the parameters recorded. The highest value of mycorrhizal root colonization was found in seedlings inoculated with *Glomus mosseae*.

Keywords : Enhancement; Growth; Nodulation; *Prosopis cineraria*; *Rhizobium*.

Introduction

Legumes can form two types of symbiotic associations with microorganisms, one with *Rhizobium* sp. involved in fixation of atmospheric nitrogen, the other with endomycorrhiza that forms arbuscles and vesicles, concerned with the uptake of phosphorus and other nutrients¹. A lot of work has been done on dual inoculation of *Rhizobium* and endomycorrhiza to improve the functioning of dinitrogen fixation and yield of leguminous crop plants²⁻⁷. Literature on similar aspects with regard to tree legumes which are very important source of fodder and timber has been scanty. Therefore an attempt has been made to study influence of *Rhizobium* and endomycorrhiza on growth, nodulation and N₂-fixation in *Prosopis cineraria*.

Materials and Methods

The surface sterilized seeds of *Prosopis cineraria* inoculated with the symbiotically three most efficient isolates of *Rhizobium* i.e. I-PC Rhz-5, II-PC Rhz-7 and III- PC Rhz-8 (grown to 1 O.D at 600 nm in yeast extract mannitol broth containing 8x10⁸, 5x10⁸ and 3x10⁸ cell/ml, respectively) were sown in pots filled with sterilized soil (2 kg each). The endomycorrhizal spores of the three most preferred VAM fungi by the host (I-*Gigaspora calospora*, II-*Glomus fasciculatum* and III-*Glomus mosseae* preferred by *Prosopis cineraria*) as

mycorrhizal inoculum to provide 250 spores per 50 g soil was also added in each pot. Soil with no microbial addition served as control. Three seedlings were maintained in each of the five replicates for each treatments. The pot trial was carried out for 5 months. The following treatments were used (1) uninoculated controls, (2) *Rhizobium* I (PC Rhz-5) alone, (3) *Rhizobium* II (PC Rhz-7) alone, (4) *Rhizobium* III (PC Rhz-8) alone, (5) VAM I (*Gigaspora calospora*) alone (6) VAM II (*Glomus fasciculatum*) alone (7) VAM III (*Glomus mosseae*) alone and (8) *Rhizobium* + VAM fungi combinations. The three most efficient isolates of *Rhizobium* (PC Rhz-5, PC Rhz-7 and PC Rhz-8) each combined with three VAM fungi (*Gigaspora calospora*, *Glomus fasciculatum* and *Glomus mosseae*) to form nine different dual combinations of the rhizobial isolates and VAM fungi.

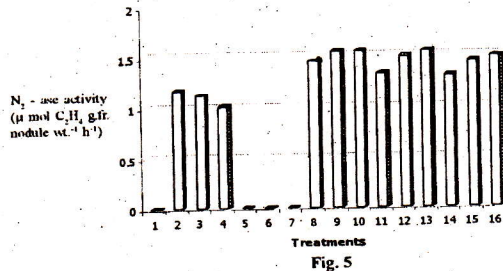
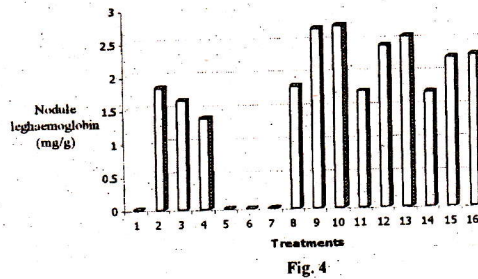
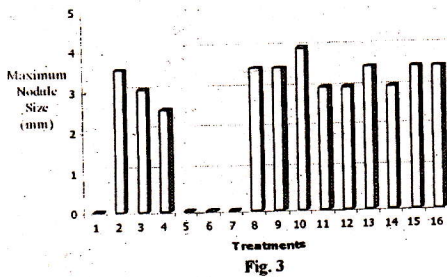
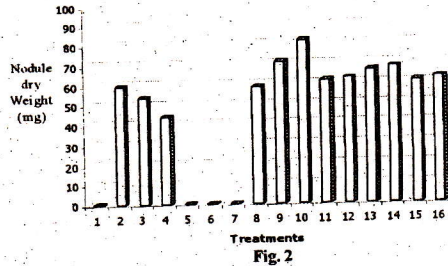
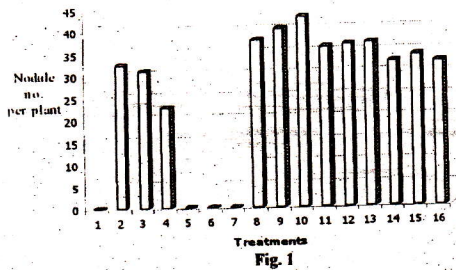
Following parameters were selected for the study : shoot length, root length, shoot and root dry wt., total plant protein⁸, total chlorophyll⁹, total nitrogen¹⁰, phosphorus content¹¹, total soluble sugar¹², nodule number, nodule dry wt., maximum nodule size, nodular leghaemoglobin¹³, nitrogenase activity¹⁴ of root nodules and VAM colonization by root (%).

Results and Discussion

The study reveals that (Table-1) dual

Table 1. Effect of Single (*Rhizobium* and VA mycorrhiza individually) and dual (*Rhizobium* + VA mycorrhiza combined) inoculation on growth and VAM root colonization of *Prosopis cineraria* (Linn.) Druce. (Values are mean \pm Standard Deviation of 15 replicates)

Treatments	Shoot length (cm)	Shoot dry weight (g)	Root Length (cm)	Root weight (g)	Total plant protein (mg/g)	Total plant chlorophyll (mg/L)	Total plant sugars (mg/g)	N-content (%) (dry weight)	P-content (%) (dry weight)	VAM Root colonization (%)
Uminoculated control	16.3 \pm 4.3	0.73 \pm 0.06	32.2 \pm 2.5	1.38 \pm 0.14	100.00 \pm 1.36	0.97 \pm 0.00	36.33 \pm 1.01	1.21 \pm 0.02	0.10 \pm 0.00	Zero
PC Rhz-5	22.9 \pm 2.4	0.39 \pm 0.08	50.3 \pm 9.2	2.13 \pm 0.16	151.79 \pm 1.11	1.21 \pm 0.01	56.56 \pm 1.06	1.58 \pm 0.01	0.23 \pm 0.00	Zero
PC Rhz-7	19.8 \pm 3.2	0.85 \pm 0.04	48.3 \pm 7.6	1.99 \pm 0.20	145.54 \pm 1.56	1.17 \pm 0.04	53.67 \pm 1.17	1.56 \pm 0.03	0.23 \pm 0.01	Zero
PC Rhz-8	19.4 \pm 2.3	0.84 \pm 0.03	45.4 \pm 6.4	1.96 \pm 0.14	139.73 \pm 1.56	1.14 \pm 0.07	51.67 \pm 1.08	1.52 \pm 0.02	0.22 \pm 0.01	Zero
<i>Gigaspora calospora</i>	17.4 \pm 3.1	0.69 \pm 0.11	32.8 \pm 2.8	1.73 \pm 0.12	129.46 \pm 1.51	1.13 \pm 0.02	48.33 \pm 0.93	1.33 \pm 0.03	0.23 \pm 0.00	25.3 \pm 4.1
<i>Glomus fasciculatum</i>	17.0 \pm 5.1	0.74 \pm 0.09	35.3 \pm 3.9	1.71 \pm 0.11	135.71 \pm 1.33	1.15 \pm 0.01	48.56 \pm 0.07	1.33 \pm 0.01	0.24 \pm 0.01	26.8 \pm 3.2
<i>Glomus mosseae</i>	18.6 \pm 1.6	0.81 \pm 0.04	36.4 \pm 5.1	1.92 \pm 0.04	136.61 \pm 1.45	1.17 \pm 0.03	49.67 \pm 1.04	1.49 \pm 0.01	0.29 \pm 0.00	28.5 \pm 5.2
PC Rhz-5 + <i>Gigaspora calospora</i>	26.2 \pm 4.2	1.44 \pm 0.06	58.1 \pm 2.02	2.83 \pm 0.08	158.04 \pm 1.73	1.22 \pm 0.01	59.66 \pm 0.86	1.60 \pm 0.08	0.46 \pm 0.00	25.5 \pm 3.1
PC Rhz-5 + <i>Glomus fasciculatum</i>	38.6 \pm 2.9	1.62 \pm 0.07	58.9 \pm 6.7	2.88 \pm 0.09	168.75 \pm 1.76	1.26 \pm 0.02	62.78 \pm 1.34	1.68 \pm 0.08	0.50 \pm 0.00	35.8 \pm 4.3
PC Rhz-5 + <i>Glomus mosseae</i>	34.7 \pm 1.5	1.65 \pm 0.11	66.5 \pm 4.8	3.28 \pm 0.06	181.25 \pm 1.58	1.58 \pm 0.03	96.33 \pm 1.26	1.73 \pm 0.04	0.51 \pm 0.01	39.1 \pm 4.1
PC Rhz-7 + <i>Gigaspora calospora</i>	25.1 \pm 1.2	0.95 \pm 0.01	49.1 \pm 2.1	2.37 \pm 0.06	151.79 \pm 1.38	1.38 \pm 0.06	61.56 \pm 1.23	1.43 \pm 0.06	0.33 \pm 0.01	25.4 \pm 4.3
PC Rhz-7 + <i>Glomus fasciculatum</i>	26.1 \pm 1.9	1.34 \pm 0.02	54.0 \pm 5.1	2.39 \pm 0.04	158.04 \pm 1.57	1.42 \pm 0.08	62.78 \pm 1.46	1.64 \pm 0.05	0.46 \pm 0.01	32.3 \pm 3.2
PC Rhz-7 + <i>Glomus mosseae</i>	29.5 \pm 5.2	1.49 \pm 0.10	58.8 \pm 8.2	2.84 \pm 0.13	174.11 \pm 1.39	1.56 \pm 0.07	93.22 \pm 1.67	1.67 \pm 0.04	0.42 \pm 0.01	35.5 \pm 4.1
PC Rhz-8 + <i>Gigaspora calospora</i>	21.6 \pm 1.1	0.93 \pm 0.07	53.3 \pm 1.4	2.22 \pm 0.06	145.11 \pm 1.74	1.36 \pm 0.02	73.33 \pm 1.36	1.59 \pm 0.07	0.29 \pm 0.01	24.9 \pm 5.2
PC Rhz-8 + <i>Glomus fasciculatum</i>	23.8 \pm 1.3	0.94 \pm 0.04	54.2 \pm 3.5	2.26 \pm 0.14	151.79 \pm 1.05	1.41 \pm 0.04	85.66 \pm 1.48	1.60 \pm 0.07	0.34 \pm 0.01	31.7 \pm 5.3
PC Rhz-8 + <i>Glomus mosseae</i>	24.8 \pm 1.3	1.15 \pm 0.06	63.9 \pm 5.9	2.31 \pm 0.04	158.04 \pm 1.93	1.55 \pm 0.03	91.01 \pm 1.53	1.61 \pm 0.04	0.31 \pm 0.01	31.9 \pm 4.2



Effect of single (*Rhizobium* and VA mycorrhiza individually) and dual (*Rhizobium* + VA mycorrhiza combined) inoculation on nodulation (nodule number, nodule dry weight, maximum nodule size and nodule leghaemoglobin Fig. 1-4) and nitrogen fixation (nitrogenase activity Fig. 5) in *Prosopis cineraria*.

Treatments : 1. Uninoculated control, 2. PC Rhz-5, 3. PC Rhz-7, 4. PC Rhz-8, 5. *Gigaspora calospora*, 6. *Glomus fasciculatum*, 7. *Glomus mosseae*, 8. PC Rhz-5 + *Gigaspora calospora*, 9. PC Rhz-5 + *Glomus fasciculatum*, 10. PC Rhz-5 + *Glomus mosseae*, 11. PC Rhz-7 + *Gigaspora calospora*, 12. PC Rhz-7 + *Glomus fasciculatum*, 13. PC Rhz-7 + *Glomus mosseae*, 14. PC Rhz-8 + *Gigaspora calospora*, 15. PC Rhz-8 + *Glomus fasciculatum*, 16. PC Rhz-8 + *Glomus mosseae*.

inoculated plants performed better showing enhanced values of all the parameters in comparison to single inoculated plants. Of several dual combinations of rhizobial isolate and VAM fungi tested, the seedlings inoculated with dual combination of rhizobial isolate, PC Rhz-5 + *Glomus mosseae* recorded maximum enhancement of growth (in terms of shoot length, shoot dry wt, root length, root dry wt.) and in the values of total plant protein, total chlorophyll, total sugar, total nitrogen and total phosphorus contents as compared to the seedling inoculated with *Rhizobium* PC Rhz-5 isolate alone.

Nodulation and total leghaemoglobin content were greatly influenced by dual inoculation in test plants (Fig. 1-4). Maximum enhancement of nodulation in terms of nodule number (42.89), nodule dry wt (81.75 mg/g), maximum nodule size (4.0 mm) and total leghaemoglobin content (2.74 mg/g) was found in seedlings inoculated with *Rhizobium* PC Rhz-5 + *Glomus mosseae* as compared to other dual inoculated plants, while seedlings inoculated with isolate PC Rhz-5 of *Rhizobium* alone recorded maximum nodule number (32.22), nodule dry wt (59.13 mg), nodule size (3.5 mm) and total leghaemoglobin content (1.83 mg/g). Plants inoculated with VAM and uninoculated control did not bear nodule (Fig. 5).

Nitrogen fixation in terms of nitrogenase activity by nodules was also influenced by dual inoculation of *Rhizobium* and VAM fungi. The maximum nitrogenase activity (ARA) of nodulated roots (Fig. 5) was recorded $1.55 \mu \text{mol C}_2\text{H}_4 \text{ g fresh nodule wt}^{-1} \text{h}^{-1}$ in seedlings inoculated with dual combination of *Rhizobium* (PC Rhz-5 + *Glomus mosseae*) in comparison to the nitrogenase activity of $1.16 \mu \text{mol C}_2\text{H}_4 \text{ g fresh nodule wt}^{-1} \text{h}^{-1}$ in the seedlings inoculated with *Rhizobium* PC Rhz-5 alone, showing 33.62% enhancement of nitrogenase activity.

Maximum percentage VAM root

colonization (Table 1) was recorded to be 39.1% in seedlings inoculated with dual combination of *Rhizobium* (PC Rhz-5) + *Glomus mosseae* followed by other dual combinations. Among single inoculated seedlings the highest value recorded was 28.5% for *Glomus mosseae* inoculated seedlings.

The result from the experiment clearly indicated that *Rhizobium* isolate PC Rhz-5 and *Glomus mosseae* formed an effective dual combination resulting in highest values of all the parameters studied. The percentage VAM root colonization enhanced further when VAM was associated with *Rhizobium*. These results were also supported by other workers^{3,15-17}. The uptake of major nutrient element N and P showed higher values in plants with dual inoculations in comparison to single inoculation and uninoculated control. This is in conformity with the findings of Mosse *et al.*¹⁸, Manjunath *et al.*¹⁶ and Hayman¹⁹.

The dual inoculation considerably stimulated (enhanced) root nodulation, leghaemoglobin content and nitrogen fixation (nitrogenase activity of nodules) than plants inoculated with *Rhizobium* alone²⁰⁻²². This suggested that effective VAM fungi such as the species used with increased uptake of N and particularly P played a major role in nodulation and nitrogen fixation^{1,3,17,23,24}. The positive correlation between increased mycorrhizal infection and enhanced total N and P contents, nodule dry wt and nitrogenase activity in *Prosopis cineraria* further attest to these observations²⁵.

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