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Role of AM Fungi and Rhizobial Inoculation for Reclamation of Phosphorus Deficient Soil

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Abstract: This study was conducted to enrich the soil nutrients and plant growth using *Glomus fasciculatum* and *Rhizobium* inoculation along with input of N and P fertilizers. The entire study was designed in low nutrients available soil collected from Marikundu (Theni District, Tamilnadu). The supplemented nitrogen with *Rhizobium* and *Glomus fasciculatum* showed increased biomass (0.439 g plant^{-1}), total nitrogen content (38.13 mg N g^{-1} dry plant) and phosphorus content (5.16 mg P g^{-1} dry plant) in dual inoculation at 200 mgN kg^{-1} of soil). Similarly, supplemental phosphorus in combination with dual inoculation significantly increased the biomass, total nitrogen content and phosphorus content followed by *Rhizobium* and *Glomus fasciculatum* inoculated plants. The nitrogen content was found to be higher in all dose rates of dual inoculated plants, specifically higher at 50 mg P kg^{-1} of soil (22.83 mg N g^{-1} dry plant). Dual inoculation with *Rhizobium* and *Glomus fasciculatum* increased the nodule nitrogenase activity (36-213%), dry matter yield (156-279%), total nitrogen content (12-159%) and total chlorophyll content of leaves of *Acacia mellifera* (125-395%) compared to the uninoculated control or single inoculation with either *Rhizobium* or *Glomus fasciculatum* alone. This study considered the impact of triple interaction involving *Rhizobium* AcM05, *Glomus fasciculatum* and *Acacia mellifera* on soil nutrients, soil enzymes and microbial dynamics.

Key words: *Glomus fasciculatum, Rhizobium, Acacia mellifera*, tree legumes, soil enzymes

INTRODUCTION

In general, the *Acacia* tree is capable of providing man most of his domestic and industrial needs like food and other products (Nuruddin and Pangalin, 2007; Maslin and McDonald, 2004). This tree is also of medicinal importance as it is used for treating stomachache, pneumonia, malaria and syphilis (Ene *et al*., 2008). The plants can be used for apiculture and in gum production. The bees will be attracted very easily to the scent and it is being widely used in Kenya, for producing good quality Acacia's honey (Raouf *et al*., 2003; Goyal *et al*., 2011). This also acts as soil binder, for shade providing, prevention of soil erosion and to fix nitrogen and improve soil fertility. All this will certainly contribute to sustainability and improving the living standards in the dry land community (Goel and Behl, 2005). Investigation has shown that supplemental dose of nitrogen and phosphorus with minimum level (50 mg kg^{-1} of soil) along with tripartite association enhances the sufficient plant growth as well as improves the soil fertility (Roy *et al*., 2008).

BNF in legume -Rhizobium association demands a steady supply of ATP, it is need to synthesize. Interestingly, majority of the root systems of the legumes are naturally infected with Arbuscular Mycorrhizal (AM) fungi which augments efficient uptake of phosphorus from the soil. In legume plants the importance of AMF-symbiosis has been attributed to high phosphorus requirements and enhanced phosphorus uptake. That AM fungi mediated 'P' mobilization has been shown to improve soil fertility in response of P-availability in the soils of the tropics (Dodd, 2000). Mycorrhizal fungi also improve the absorption of nitrogen from ammonical (NH_4^+-N) mineral fertilizers, transporting it to the host plant (Ames *et al*., 1983; Johanson *et al*., 1993; Nasr *et al*., 2006). Soil microorganisms produce quite a number of extra cellular enzymes to decompose the complex organic matter before it is absorbed as a source of energy (Kathiresan and Selvam, 2006; Richard *et al*., 2007). Seasonal variations in enzymes activities in forest soils are seen to bear correlation with the counts of fungi and bacteria. Though much has been explored on the interactions of rhizobia for grain legumes and for some tree legumes, little is known as the response of tree legumes. Thus, the present study discussed with inoculation of tree legumes species with *Rhizobium* and AM fungus enhanced plant growth by providing a balanced nutrient supply due to their beneficial association with root system of the host plant.

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MATERIALS AND METHODS

Experiment set up: Experiment was set in the department of Microbiology during first week September 2010 (Karpagam University). The soil used in this study was collected from Marikundu Village, Theni Dt, Tamil Nadu.

Supplementation of inorganic fertilizers: An inorganic fertilizer like ammonium chloride and super phosphate was given as supplementary nutrients to *Acacia mellifera*. Calculated quantities of these fertilizers at 0, 50, 150 and 200 mg kg^{-1} of soil of the respective plant nutrients were mixed with pot soil before sowing the seeds.

One gram of soil based inoculum containing 180-200 spores and sporocarps of *Glomus fasciculatum* was spread over the lower layer of sterile soil (1.5 kg). Then 1 kg of sterile soil was layered over the inoculum. Seeds of *Acacia mellifera* obtained from the Oddukkum Seed Centre, Nallampatti, Tamilnadu were surface sterilized with 0.1% $HgCl_2$ and sown in earthen pots containing garden soil and sand (2:1 ratio w/w). Plant growth conditions and *Rhizobium* (Cowpea miscellany isolated from *Acacia mellifera*) inoculation were as described by Rajagopalan and Raju (1972). The plants were watered with sterile tap water and harvested at 45 DAI (Day after Inoculation).

The percent colonization was measured by the gridline intersect method (Giovannetti and Mosse, 1980). Dry matter yield (plant materials dried to constant weight), total nitrogen content by microkjeldahl method (Umbriet *et al*., 1972), total chlorophyll content (Arnon, 1949) and nodule nitrogenase activity by acetylene reduction technique (Stewart *et al*., 1968) were determined.

Total bacterial and fungal population by dilution technique, isolation of AM spores by wet sieving and decanting method (Gerdemann and Nicolson, 1963), determination of nitrogen, phosphorus and potassium content (Jackson, 1973), assay of amylase, chitinase (Skujins, 1976) phosphatase (Tabatabai and Bremner, 1969), protease (Nannipieri *et al*., 1980) in the soil were carried out. The data was subjected to statistical analysis (significant level at $p<0.05$) by using Costat package for one-way ANOVA and Student Newman Kauls test (Appendix).

RESULTS AND DISCUSSION

Supplemental nitrogen and dual inoculation on biomass, total nitrogen and phosphorus content in *Acacia mellifera*: Of the four treatments with different dosage of nitrogen (0, 50, 150, 200%) showed dual inoculation with *Rhizobium* (AcM05) and *Glomus fasciculatum* better biomass. As the nitrogen supplement was increased biomass was also increased (0.439 g/plant) at 200% nitrogen supplementation. In treatment with *Rhizobium* and *Glomus fasciculatum* individually showed increased weight of biomass in compared to the control (Fig. 1). In treatment with *Rhizobium* and *Glomus fasciculatum* showed maximum nitrogen content. This also showed better nitrogen content as the supplemented dosage of nitrogen increased (Fig. 2). When phosphorus content was examined treatment of dual inoculation with *Glomus fasciculatum* and *Rhizobium* gave more phosphorus availability in all differential dosages of supplement nitrogen. In treatment, single inoculation with *Rhizobium* and *Glomus fasciculatum* inoculation the phosphorus content was more than the control but less with the dual inoculation (Fig. 3).

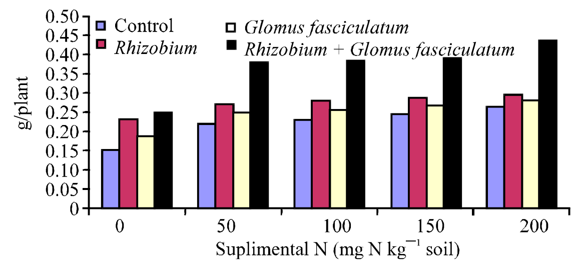

Fig. 1: Supplemental nitrogen and dual inoculation on biomass accumulation in *Acacia mellifera*

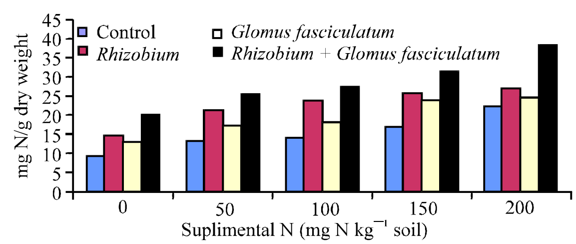

Fig. 2: Supplemental nitrogen and dual inoculation on total nitrogen content in *Acacia mellifera*

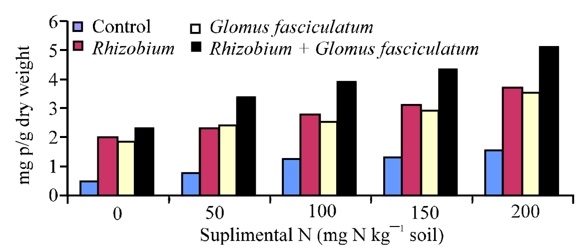

Fig. 3: Supplemental nitrogen and dual inoculation on total phosphorus content in *Acacia mellifera*

Supplement nitrogen in combination with dual inoculation increased the plant biomass, total nitrogen and phosphorus over the single inoculation with either cowpea *Rhizobium* alone or *Glomus fasciculatum* alone (Fig. 1-3). Similar synergistic interaction between *Rhizobium* and AM fungi and supplemental nitrogen leading to greater plant biomass accumulation and plant growth have been reported in several instances (Hoque and Satter, 1989; Singh, 1990; Tham and Tham, 2007; Aminifard *et al*., 2010). There are several possible explanations for this synergistic effect, one such explanation is that the mycorrhizal plants have got the capacity to uptake more phosphorus than non-mycorrhizal plants. However, Azcon and Barea (1992) have reported that improved plant growth in alfalfa is the result of enhanced N-acquisition by AM infected plants by mechanisms additional to P-mediated enhancement of nitrogen fixation. External hyphae of AM fungi can take up NH_4^+ or No_3^- from the soil and transfer it to the plant (Ames *et al*., 1983; Johnson *et al*., 1992). Thus it is clear that dual inoculation, improved the nitrogen nutrition of *Acacia mellifera*, in addition to nitrogen fixation.

Supplemental phosphorus and dual inoculation on biomass, total nitrogen and phosphorus content in *Acacia mellifera*: A substantial increase in biomass accumulation was found in dual inoculated plants, followed by *Rhizobium* inoculated plants and *Glomus fasciculatum* inoculated plants. With increasing concentrations of supplemental phosphorus, a progressive increase in plant biomass accumulation was observed in the control and in the inoculated plants. Further, the additive effect was much pronounced in dual inoculated plants in comparison with cowpea *Rhizobium* inoculated plants or *Glomus fasciculatum* inoculated plants (Fig. 4).

The total nitrogen content was a function of supplemental phosphorus in all the treatments. Maximum level of total nitrogen accumulation was found at a concentration of 200 mg P kg^{-1} soil in all the treatments. Further, the additive effect of inoculation and supplemental phosphorus was significant in the dual inoculated plants as compared to either the *Glomus fasciculatum* inoculated plants or the cowpea *Rhizobium* inoculated plants (Fig. 5).

A progressive increase in total phosphorus accumulation was observed with increasing concentrations of supplemental phosphorus in all the treatments. Moreover, the maximum level of total phosphorus accumulation was noticed in plants treated with higher level of phosphorus (200 mg p kg^{-1} soil) in either inoculated or uninoculated plants (Fig. 6).

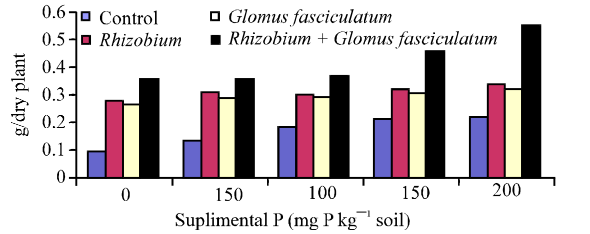

Fig. 4: Supplemental phosphorus and dual inoculation on biomass accumulation in *Acacia mellifera*

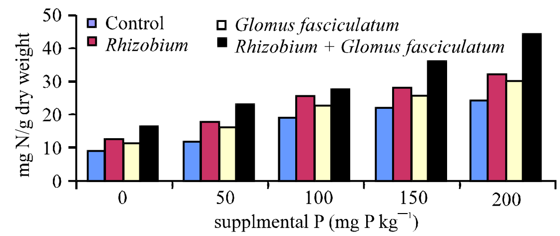

Fig. 5: Supplemental phosphorus and dual inoculation on total nitrogen in *Acacia mellifera*

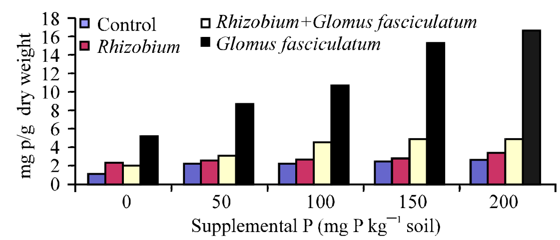

Fig. 6: Supplemental phosphorus and dual inoculation on total phosphorus in *Acacia mellifera*

Phosphorus is an important element required for rapid growth of plants (Hayman, 1986; Koide, 1991; Saimbhi *et al*., 2002). This study demonstrated that a progressive increase in plant biomass, total nitrogen and phosphorus content of *Acacia mellifera* upon supplemental phosphorus nutrition. Further, dual inoculation caused an increase in dry matter yield, total nitrogen and phosphorus content (Fig. 4-6) in comparison with either cowpea *Rhizobium* or *G. fasciculatum* inoculation. Beneficial effects of dual inoculation in the presence of inorganic fertilizers have been studied in several forest tree species (Reddy *et al*., 1990; Khan and Uniyal, 1999; Dwivedi *et al*., 2003). Colonna *et al*. (1991) have studied the phosphorus effect in dual inoculated *Acacia senegal* and found an increase in leaf dry matter, total N and P content. The result presented here revealed that supplemental inorganic nutrients in the form of N or P play a significant role in plant growth.

Impact of dual inoculation on *Acacia mellifera* grown in nutrient poor soil: The entire study was designed in unfertile soil collected from Marikundu (Theni district). arbuscular mycorrhizal fungi for the phosphorus and *Rhizobium* for nitrogen which obviated the necessity especially for the low nutrient status soils live laetrile soils for successful pulses grain yield (Gupta and Rahangdale, 1999), dry matter yield, nodule nitrogenase activity and total nitrogen content of *Acacia mellifera* were in higher order in plants inoculated with *Golmus fasciculatum* or *Rhizobium* or both as compared to the uninoculated control plants (Table 1). Also, arbuscular mycorrhizal fungi in association with nitrogen fixing bacteria increased the plant biomass, nodulation and nitrogen fixation (Chaturvedi and Kumar, 1991; Zaidey *et al*., 2010). Mobilization of phosphorus by arbuscular mycoorhizal fungi to the host plant could be reason for better nodulation and nitrogen fixation in dual inoculated plants. Total chlorophyll content in leaves of inoculated plants was higher than the uninoculated control plants (Table 1). This increase in chlorophyll content in inoculated plants is to meet the carbon requirements of the microsymbionts, since both *Rhizobium* and arbuscular mycorrhizal fungi depend of the host for their carbon requirements (Tester *et al*., 1986; Sivaprasad and Rai, 1987).

Impact of tripartite association on soil characteristics: Microbial inoculation induced significant changes in soil characteristics (Table 2). Dual inoculation significantly enhanced the N (60.0 mg kg^{-1} soil) and P (24.3 mg kg^{-1} soil) content of the soil but to a lesser extent in the case of K (155 mg kg^{-1} soil). The results of present study are in conformity with the findings of other workers (Dwivedi *et al*., 2003). Native barren soil had low microbial population. However, the total bacterial (4.7×10^7 cfu g^{-1} soil) and fungal (8×10^5 cfu/g soil) population and AM spores (256 spores/g soil) in soil increased substantially with dual inoculation. The activities of amylase, protease, chitinase and phosphatase in the soil also increased significantly following microbial inoculation (Table 2). For amylase and protease, the increase was much pronounced with dual inoculation, followed by single inoculation with *Rhizobium* or *Glomus fasiculatum*. For phosphatase and chitinase, the increase was less pronounced with *Rhizobium* inoculation as compared to *Glomus fasciculatum* inoculation. The increased phophatase activity in soil inoculated with *G. fasciculatum* could be the result of hydrolytic cleavage of organic P by the fungus, since a positive correlation has been established between soil enzymes and soil microbial biomass (Tabatabai, 1994).

In conclusion, inoculation of tree legumes species with *Rhizobium* and AM fungus enhanced plant growth by providing a balanced nutrient supply due to their beneficial association with root system of the host plant.

Table 1: Dual inoculation on percent colonization, nitrogenase activity, dry matter yield, total nitrogen content and total chlorophyll content in leaves of *Acacia mellifera*

	Treatments				
Parameters	Control	*Rhizobium*	*Glomus fasciculatum*	*R+Glomus fasciculatum*	LSD p<0.05
% Colonization	-	-	65b±11^b	80.3±9^c	2.717***
Dry weight (g/plant)	0.139±0.005^a	0.449±0.13^b(223)	0.356±0.056^b(156)	0.527±0.089^b(279)	0.159**
Total nitrogen content (mg N g^{-1} dry wt)	20.06±2.25^a	40.3±4.20^b(101)	22.4±3.27^a(12)	51.8±3.95^c(159)	6.593***
Nitrogenase Activity (n moles C_2H_4 formed/h/g fresh nodules)	3.70±0.71^a	9.55±2.82^c(157)	5.07±1.64^b(36)	11.62±3.35^d(213)	0.0266***
Total Chlorophyll content mg Chl g^{-1} fresh leaves	1.86±0.58^a	5.6 1.5 ^{b}(201)	4.2±0.79^b(125)	9.2±1.1^c(394)	1.982***

±: Standard deviation; values in parenthesis indicate percent increase over control. Values suffixed with different letter on same row indicate significant differences. *, **, *** Extent of significance p<0.05

Table 2: Impact of dual inoculation on NPK content and enzymes in the rhizosphere soil of *Acacia mellifera* at 45 DAI

	Treatments			
Parameters	Control	*Rhizobium*	*Glomus fasciculatum*	*R+Glomus fasciculatum*
Soil N (mg kg^{-1} soil)	14±2.51	56±2	52±1.73	60±3.05
Soil P (mg kg^{-1} soil)	1.1±0.25	11.3±0.87	8.8±0.47	24.3±1.17
Soil K (mg kg^{-1} soil)	115±1.82	145±2.64	140±2.52	155±1.71
Heterotrophic Bacteria (cfu/g soil)	1.1×10^7	2.6×10^7	1.8×10^7	4.7×10^7
Fungi (cfu/g soil)	2×10^5	5×10^5	3×10^5	8×10^5
AM spores Number/g soil	18	28	228	256
Soil amylase (μg starch degraded/h/g soil)	2187.0±123.3	7173.0±219.5(227)	5260.0±428.3(140)	11062.5±929.0(405)
Soil phosphatase (μg PNP formed/h/ g soil)	3348.1±103.4	5649.5±163.3(68)	6250.9±111.1(86)	8507.8±212.4(154)
Soil chitinase (μg glucose liberated/ h/g soil)	391.7±29.3	1315.6±217.5(235)	1372.1±35.03(250)	1555.2±236.6(297)
Soil protease (μg amino acid released/h/g soil)	104.06±6.93	384.3±9.43(269)	296.12±10.73(185)	479.3±16.9(360)

±: Standard deviation. Values in parenthesis indicate percent increase over control

APPENDIX: ANALYSIS OF VARIANCE

Supplemental nitrogen and dual inoculation on biomass content in *Acacia mellifera* (Fig. 1)

Source	SS	df	MS	F	p
Main effects					
St	0.2538715833	3	0.08462386111	11.7249516	0.0027**
Error	0.05773933334	8	7.2174167E-03		
Total	0.3116109167	11			

Supplemental nitrogen and dual inoculation on total nitrogen content (mg N/g dry wt) in *Acacia mellifera* (Fig. 2)

Source	SS	df	MS	F	p
Main effects					
St	2053.286667	3	684.4288889	55.81479216	0.0000***
Error	98.10000001	8	12.2625		
Total	2151.386667	11			

Supplemental nitrogen and dual inoculation on total phosphorus content (mg P/g dry wt) in *Acacia mellifera* (Fig. 3)

Source	SS	df	MS	F	p
Main effects					
St	124.6523667	3	41.55078889	20.77536927	0.0000***
Error	1.6000019E-03	8	2.0000024E-04		
Total	124.6539667	11			

*Significant, **, ***Highly Significant p<0.05

Supplemental phosphorus and dual inoculation on biomass content in *Acacia mellifera* (Fig. 4)

Source	SS	df	MS	F	p
Main effects					
St	0.5547265464	3	0.0158493299	8.686283089	0.0027**
Error	0.1313739998	8	1.8246389E-03		
Total	0.6861005462	11			

Supplemental phosphorus and dual inoculation on total nitrogen content (mg N g^{-1} dry wt) in *Acacia mellifera* (Fig. 5)

Source	SS	df	MS	F	p
Main effects					
St	0.2077171852	3	5.9347767E-03	13.87080196	0.0000***
Error	0.03080599991	8	4.2786111E-04		
Total	0.2385231851	11			

Supplemental phosphorus and dual inoculation on total phosphorus content (mg P g^{-1} dry wt) in *Acacia mellifera* (Fig. 6)

Source	SS	df	MS	F	p
Main effects					
St	0.09013121304	3	2.5751775E-03	2.247918107	0.0019***
Error	0.08248199991	8	1.1455833E-03		
Total	0.172613213	11			

*Significant, **, ***Highly Significant p<0.05

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