

Soil beneficial microbes and their role in *Sesbania grandiflora* growth in non-fertile soil

S. Lalitha

Department of Botany, School of life science, Periyar University, Salem

lara9k@gmail.com Mobile No: 09842985127

Abstract: Soil bacteria are very important in bio geochemical cycles and have been used for crop production. Plant microbial interactions in the rhizosphere are the important to the plant health and also soil fertility. 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 mg N 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 *Sesbania grandiflora* (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* Sg01, *Glomus fasciculatum* and *Sesbania grandiflora* on soil nutrients, soil enzymes and microbial dynamics.

Keywords: *Rhizobium*, *Glomus fasciculatum*, nitrogen, plant growth.

Introduction

Free-living soil bacteria beneficial to plant growth, usually referred to as plant growth promoting *rhizobacteria* (PGPR), are capable of promoting plant growth by colonizing the plant root (Zhou, 2014). These are associated with the rhizosphere and important soil ecological environment for plant microbe interactions. PGPR have the potential to contribute to sustainable plant growth promotion. Generally, PGPR function in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and lessening or preventing the plants from diseases. Plant growth promotion and development can be facilitated both directly and indirectly.

Sesbania grandiflora L. (Agast) is well known small, loosely branching, legume plant native to the Tropical Asia including, India, Indonesia, Malaysia, Myanmar and Philippines. It belongs to leguminosae; papilionoideae family. *Sesbania grandiflora* is mostly preferred for planning at field boundaries, hedge row intercropping or alley cropping and in home gardens (Sankaralingam, 2014 et al.,). It does not become a weed in managed agro-ecosystems; however it has moderate weed potential. *Sesbania grandiflora* grows fast enough to be used as an annual green manure crop. On the fields, it also acts as windbreak. It is excellent nitrogen fixing tree species for providing soil improvement through nutrient cycling and nutrient addition (Evans, et al., 2001). The sparse canopy of *Sesbania grandiflora* casts relatively little shade, hence its suitability close to sun-loving crops and gardens. It may grow along with grass species for improving eroded, waterlogged wastelands and acid soils. Soil microbes play a vital role in reclamation of waste lands and increasing biomass production. Soil enzymes are important for catalyzing innumerable reactions necessary for life processes of microorganisms in soils, decomposition of organic residues, cycling of nutrients and formation of organic matter and soil structure (Buono and Ladha, 2009). Although enzymes are

primarily of microbial origin, it can also originate from plants and animals. Soil enzymes are of great importance for agriculture because of their role in the recycling of the nutrients (Tabatabai, 1994; Dick, et al., 1997). The measurement of soil enzymes can be used as an indication of the biological activity or biochemical process. In general, increase in soil enzymes activities may be the result of soil physical and chemical changes, so there is a direct expression of microbial biomass and soil enzyme activities. Salam et al., (1999) have shown that there is a significant relationship between the changes in activities of the soil enzymes and soil organic carbon and total nitrogen. The activity of soil enzymes can be used to predict the fertility of soil. Various enzymes release specific plant nutrients from soil organic matter and their activity correlates with the fertility status of the soil. For example, increase in available phosphorus and nitrogen was the result of decomposition of carbohydrates (Spalding and Duxbury, 1977)

Materials and Methods:

Microbial inoculants:

One gram of soil based inoculum containing 180-200 spores and sporocarps of *Glomus fasciculatum* was spread over the lower layer of sterile soil (1.5kg). Then 1kg of sterile soil was layered over the inoculum. Seeds of *Sesbania grandiflora* obtained from the Oddukkam Seed Centre, Nallampatti, Tamil Nadu were surface sterilized with 0.1% HgCl₂ and sown in earthen pots containing garden soil and sand (2:1 ratio w/w). Plant growth conditions and *Rhizobium* (Cowpea miscellany isolated from *Sesbania grandiflora*) inoculation were as described by Rajagopalan and Raju (1972). The plants were watered with sterile tap water and harvested at 45 DAI.

Supplementation of inorganic fertilizers

An inorganic fertilizer like ammonium chloride and super phosphate was given as supplementary nutrients to *Sesbania grandiflora*. Calculated quantities of these fertilizers at 0, 50,

150 and 200 mg/Kg of soil of the respective plant nutrients were mixed with pot soil before sowing the seeds.

Dry matter yield (plant materials dried to constant weight), total nitrogen content by *microkjeldahl* method (Umbriet et al., 1972) and total phosphorus content by *Subba-Rao* as modified by Barlett (1959) 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) was performed. The data was subjected to statistical analysis by using Costat package for one-way ANOVA and Student Newman Kauls test.

Results and Discussion

Supplement nitrogen in combination with dual inoculation significantly 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; Byra Reddy et al., 1990). 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 NH₄⁺ or No₃⁻ from the soil and transfer it to the plant (Ames et al., 1983; Johnsson, et al., 1992). Thus it is clear that dual inoculation, improved the nitrogen nutrition of *Sesbania grandiflora*, in addition to nitrogen fixation.

Phosphorus is an important element required for rapid growth of plants (Hayman, 1986; Koide, 1991). This study demonstrated that a progressive increase in plant biomass, total nitrogen and phosphorus content of *Sesbania grandiflora* was possible due to 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 Uniyal, 1999). In an earlier report 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.

The results of this study also showed variations in effectiveness of different tree legume *rhizobia-arbuscular mycorrhizal* fungi associations. However, *Sesbania grandiflora* – cowpea *Rhizobium* (AcM05) *Glomus fasciculatum* association appears to be the best one in terms of accumulation of plant biomass, total nitrogen and phosphorus. Further, the aforesaid combination in nutrient poor unsterile soil is found to improve the soil activity such as soil NPK content (Table 1), soil enzymes and soil microbial populations (Table 2).

Table 1. Soil characteristics

Parameters	Control
E C (dsm ⁻¹)	0.3
pH	8.5
Soil N (mg/kg soil)	0.7 ± 0.14
Soil P (mg/kg soil)	0.7 ± 0.10
Soil K (mg/kg soil)	110 ± 1.35
Total bacterial population in soil (cfu/g soil)	1.1 X 10 ⁷
Total fungal population in soil (cfu / g soil)	2 X 10 ⁵
Total AM spores / g soil	18±2

± - Standard deviation

Table 2. Impact of tripartite association on soil characteristics at 45 DAI

Parameters	Control	cowpea <i>Rhizobium</i>	<i>Glomus fasciculatum</i>	cowpea <i>Rhizobium Glomus fasciculatum</i>
Soil N (mg/kg soil)	14 ± 2.51	56 ± 2.0	52 ± 1.73	60 ± 3.05
Soil P (mg/kg soil)	1.1 ± 0.25	11.3 ± 0.87	8.8 ± 0.47	24.3 ± 1.17
Soil K (mg/kg soil)	115 ± 1.82	145 ± 2.64	140 ± 2.52	155 ± 1.71
Total bacterial population in soil (cfu/g soil)	1.4 X 10 ⁷	2.6 X 10 ⁷	1.8 X 10 ⁷	4.7 X 10 ⁷
Total fungal population in soil (cfu / g soil)	2.8 X 10 ⁵	5 X 10 ⁵	3 X 10 ⁵	8 X 10 ⁵

± - Standard deviation

Figure – 1 Supplemental nitrogen and dual inoculation on biomass accumulation in *Sesbania grandiflora*

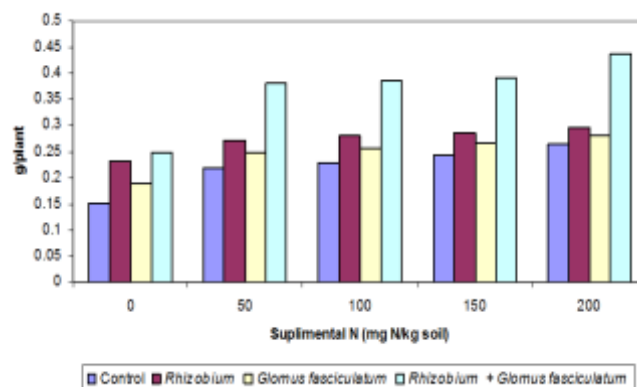


Figure – 2 Supplemental nitrogen and dual inoculation on total nitrogen content in *Sesbania grandiflora*

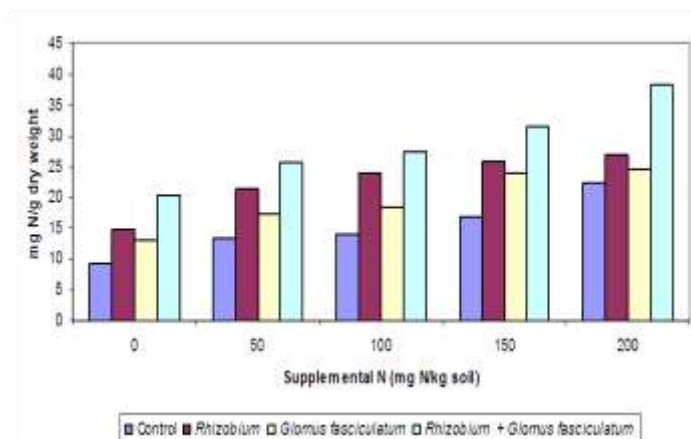


Figure – 3 Supplemental nitrogen and dual inoculation on total phosphorus content in *Sesbania grandiflora*

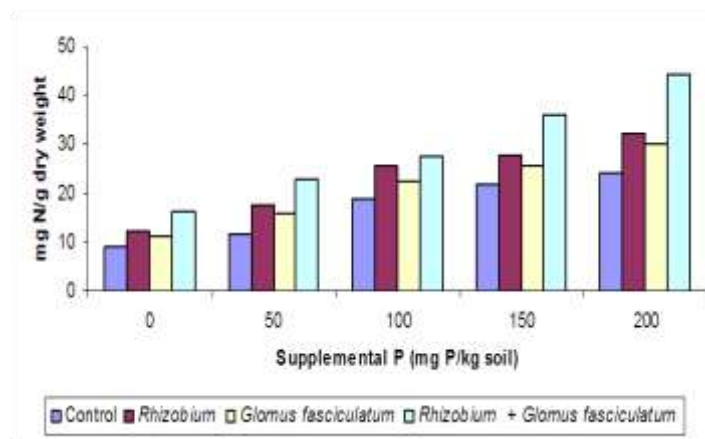


Figure – 6 Supplemental phosphorus and dual inoculation on total phosphorus in *Sesbania grandiflora*

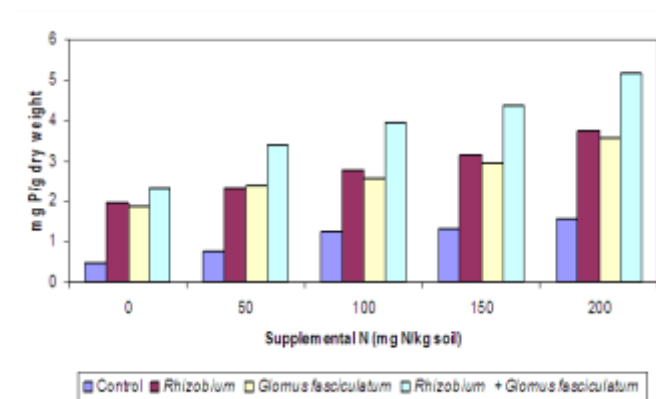


Figure – 4. Supplemental phosphorus and dual inoculation on biomass accumulation in *Sesbania grandiflora*

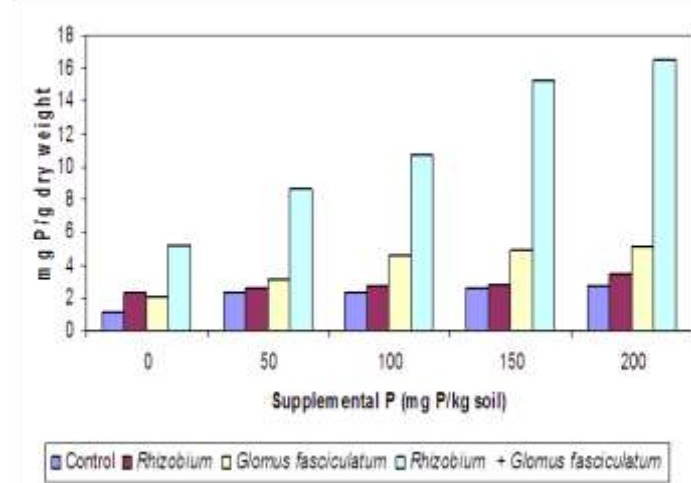


Figure – 5 Supplemental phosphorus and dual inoculation on total nitrogen in *Sesbania grandiflora*

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Author Profile:

Dr. S. LALITHA, Assistant Professor in Periyar University having 12+ years of experience in teaching and 15 years in research. She has 25 publications, 43 conference papers. She is guiding PhD/M.Phil.Scholars. She has visited KMITL, Thailand for special lecture. She has been well trained in AM Fungi, Bio fertilizer & Vermi technology, Mushroom cultivation.