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# Role of bioinoculants and organic fertilizers in fodder production and quality of leguminous tree species

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**Abstract**

The comparative effect of dual inoculation of native N fixer (*Rhizobium*) and AM fungi consortia with different organic fertilizers (vermicompost and farm yard manure) on fodder production and quality of two leguminous tree species (*Leucaena leucocephala* (Lam) de. Wit. and *Sesbania sesban* (L.) Merr.) in silvopastoral system and their impact on the fodder production of un-inoculated *Panicum maximum* Jacq. under cut and carry system. After three years of plantation maximum tree survival was in *L. leucocephala* in all the treatments in comparison to S. sesban while fodder production was more in *S. sesban* for initial two years and in third year it accelerated in *L. leucocephala.* Dual inoculation with vermicompost significantly improved fodder production, fodder quality and rhizosphere microflora in *L. leucocephala* but in *S. sesban* dual inoculation was at par with single inoculation of N fixer, AM fungi and control (without inoculation). The grass production was higher with L. leucocephala for two years while in third year it was more with *S. sesban.* The association of *Rhizobium* with AM fungi in *L. leucocephala* was better than in S. sesban.

**Key words**

Bio-inoculants, Biofertilizers, Silvopastoral system, Fodder production, Tree species

## Introduction

Fodder is the major component of economic animal production in semi- arid India. Their availability and potential of utilization in specific agro-ecological zones and in smallholder production systems indicate performance of livestock. Crop residues, concentrates and agro- industrial by- products constitute low quality basal feed that affects the productivity of livestock (Pathak *et al.*, 2005). Therefore, supplementation of green fodder becomes essential for sustaining livestock productivity because they are rich in macro and micro nutrients and economical than other fodder sources. A mixture of fodder grass and tree legume forage provide an appropriate mixed diet for ruminants, especially during the dry season when green forages from crops are in short supply (Seresinhe and Pathirana, 2000). Multipurpose tree species like *Sesbania* and *Leucaena* are potential top feed species with their ability to grow under a wide range of climatic conditions (Brewbaker *et al.*, 1990). *Panicum maximum* (Jacq.) is generally recognized as one of the best forage grass of the tropics with good yield potential even under shade (Javier, 1970).

By integrating high yielding varieties of green fodder in intensive production systems like silvopastoral system, the year round supply of green fodder can be ensured. But in intensive cutting systems there is a large demand of soil nutrients by plants (Hayishi, 1996). Hence, fertilizer inputs are required to sustain high level of forage production. Organic manures and biofertilizers owing to their high macro and micro nutrients and environment friendly properties (Wu *et al.*, 2005) should be used in fodder production system. Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert complex nutritionally important chemicals into simple form through chemical processes and make them available to plants (Vessey, 2003). It is well documented that arbuscular mycorhhizal fungi (AMF) colonization and arbuscular mycorrhizal (AM) fungal activity is enhanced by co inoculation with *Rhizobium*, resulting in

better plant performance in terms of production and quality (Biro *et al.*, 1993). Similarly, AM inoculation also improves nodulation in leguminous plants (Mishra *et al.*, 2008). Different studies have been done on the effect of single inoculation of AM fungi (Liasu *et al.*, 2005) and N fixers (Chalk *et al.*, 2006) in different agroforestry systems. But literature on the potential of dual inoculation of biofertilizers with different organic fertilizers especially in silvopastoral systems is lacking.

This paper attempts to study the fodder production and quality of leguminous tree species (*Leucaena leucocephala* (Lam) de. Wit. and *Sesbania sesban* (L.) Merr.) in silvopastoral system with native pure strain of N fixer (*Rhizobium*) and AM fungi consortia in different organic fertilizers [vermicompost (VC) and farmyard manure (FYM)]. The impact of tree species was also evaluated on the fodder production of associated grass *Panicum maximum* Jacq. under cut and carry system.

## Materials and Methods

**The study site:** The experiments were conducted during July 2004 - 2007 in field conditions at Micromodel experimental site of Indian Institute of Technology, New Delhi, India in two silvopastoral systems *i.e. L. leucocephala*+ *P. maximum* and *S. sesban*+ *P. maximum*. The experimental site is situated between 77.09´E longitude, 28.45´N latitude, and 228 m above sea level. The maximum and minimum temperature during study period varied between 18- 43°C and 3-15°C, respectively. The annual rainfall was in the range of 640 to 800 mm during the study period. The soil of the experimental field at 10- 15 cm depth was black, sandy loam having pH- 7.4, organic carbon- 0.35%, available N- 142 kg $ha^{-1}$, available P- 5.5 kg $ha^{-1}$ and available K–136 kg $ha^{-1}$. The density of indigenous rhizobia (CFU) was $0.6x10^2$ $100g^{-1}$ of soil and was found to be slow growing on YEMA media. The population of AMF spores was 12 spores 100 $g^{-1}$ of soil, which was very low.

**Preparation of materials:** Seed of selected plant species were obtained from Indian Grassland and Fodder Research Institute, Jhansi, India. Vermicompost (VC) was prepared from horticultural waste by raising *Eisenia fetida* earthworms for 40 days (pH-7.4, OC-27.3%, total N-0.6%, total P-1.34% total, K-1.8% and *Rhizobium* cells-0) while farm yard manure (FYM) was procured from a dairy farm (pH- 7.2, OC-12.2%, total N- 0.55%, total P-0.75% and total K-2.3% ).

The seed pretreatment of tree species was done by soaking seeds in hot water for 30 min while grass seeds were not given any treatment. The nursery raising of tree species [*Leucaena leucocephala* (cultivar S24) and *Sesbania sesban*] and grass sp. (*Panicum maximum*) was done as per treatments in poly bags and nursery beds, respectively in March 2004. Polythene bags were filled with soil, sand and vermicompost/ FYM in the ratio of 2:1:2. For the nursery raising of *P. maximum*, vermicompost/ FYM was mixed in soil of the nursery beds at the rate of 5t/ha and 10 t $ha^{-1}$, respectively with 2 kg of sand/plot to maintain the porosity of soil.

**Isolation and preparation of microbial inoculants:** The native rhizobia was isolated from the healthy nodules of respective tree species (*L. leucocephala* and *S. sesban*) grown in the experimental area without any inoculation. The rhizobia were isolated and multiplied on yeast extract mannitol agar media (YEMA) with congo red dye. Fast growing colonies were picked up and further multiplied on YEM broth at 27± 1° C on rotary shaker at 150 rpm. After incubation period pure cultures were formulated using sterilized charcoal (charcoal: broth culture 2:1 w/v) as a carrier base having ~ $10^7$ colony forming units (CFU) 100 $mg^{-1}$ carrier base (Vincent, 1970). Local AM consortia were obtained from the rhizosphere soil and rootlets of Castor (*Ricinus communis*) containing spores of *Glomus* sp. (*G. mossae, G. microcarpum, G. macrocarpum* and *G. fasciculatum*) and *Gigaspora* (*G. margarita and G. heterogama*).

**Site preparation and transplantation of seedlings:** The experiment was laid out in randomized block design with seven treatments in three replications. The vermicompost and FYM were mixed in the soil at the rate of 5 t $ha^{-1}$ and 10 t $ha^{-1}$, respectively as per treatment. The AM consortia was inoculated at the rate of 500 g consortia (containing ~200 spores 100 $g^{-1}$ soil) in every pit while rhizobia was inoculated by dipping roots of seedlings in slurry (carrier base and 10% jaggery solution in distilled water in 1:2 ratio) as per treatment;

T1 - N fixers with vermicompost (*Rhizobium* +VC)
T2 - AM consortia with vermicompost (AMF +VC)
T3 - N fixers + AM consortia with vermicompost (*Rhizobium* +AMF +VC)
T4 - N fixers with FYM (*Rhizobium*+ FYM)
T5 - AM consortia with FYM (AMF + FYM)
T6 - N fixers + AM consortia with FYM (*Rhizobium* + AMF + FYM)
T7 - Un inoculated Control (only soil)

The tree species of same size (80 -95 cm) were transplanted at the distance of 2 x1m (density of 5000 trees $ha^{-1}$) and root slips of *P. maximum* were planted at the distance of 50x30 cm. The treatments were randomly arranged in subplots having 1.5 m border in between to restrict any contamination. Standard management practices *i.e.*, watering and weeding were done for the maintenance of the system. The plots were irrigated weekly in summer (March-June) and at monthly interval in winter (October- February), while no irrigation was provided in rainy season (July-September).

**Data recording:** Trees were maintained at the height of 1.3 m and first cutting was done after 90 days of transplantation followed by subsequent cuttings after every 45 days. In *P. maximum* first cutting was done after 60 days of transplanting and subsequent cuttings were done after 45 days. From November to mid March of each year growth of trees as well as grass species was very slow. So, total four cuttings were obtained in both the tree species in first year while in second and third year five- five cuttings were obtained. In *P. maximum* total five cuttings were obtained in first year and six-six cuttings in second and third year in both the systems. At each harvesting after recording fresh weight of

samples dry weight was recorded after keeping samples in oven at 60°C until constant weight. The samples were mill ground for fodder quality analysis.

**Microbial and chemical analysis of plants and soil:** The density of colony forming unit of rhizobia was estimated annually in the rhizosphere soil of tree species by plate dilution method (Vincent, 1970). The AM spore number in the rhizosphere soil of each treatment were taken from three randomly selected points annually and estimated by the method of Gerdemann and Nicholson (1963) while to estimate the root infection percentage, 1 g fresh lateral roots were randomly taken from each subplot and cut to approximately 1cm segments. They were cleared and stained with acid glycerol trypan blue (Philips and Hayman, 1970). The gridline intersect method of Giovanetti and Mosse (1980) was used to determine the percentage root infection by AMF.

To study the effect of treatments on fodder quality in the tree species, macronutrients (N, P, K, Ca, Mg), micronutrients (Fe, Cu, Zn, Mn), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated annually in all treatments of the tree species. The organic C, N, P and K was estimated by the methods of Walkley and Black, Micro-Kjeldhal, Olsen and Flame Photometer methods, respectively in plant samples, soil, vermicompost and FYM as described by Rowell *et al.* (1994). Nutrients *viz.* iron (Fe), copper (Cu), calcium (Ca), manganese (Mn) and zinc (Zn) were estimated by the method of AOAC (1980), using different columns of atomic absorption spectrophotometer (model- AAnalyst 200, Perkin Elmer, USA)) by dry ashing of dried samples and further digestion in 3N hydrochloric acid. CP content in plant biomass was determined by multiplying 6.25 in N content of the plant. NDF and ADF content of plant material was estimated by the method given by Van Soest *et al.* (1991).

**Statistical analysis:** The experiment was conducted as a factorial randomized block design with each treatment replicated thrice. Statistical analysis of the data was done following the methods of analysis of variance (ANOVA) and the means were separated using LSD at $p<0.05$ level of significance.

## Results and Discussion

*L. leucocephala* had significantly more survival percentage than *S. sesban* after three years of growth in all the treatments (Table 1). There was a subsequent reduction in the survival of *S. sesban* (40-75%) in all the treatments over three years in comparison to *L. leucocephala*. This shows the potential of *L. leucocephala* over *S. sesban* to tolerate frequent cuttings for longer duration which is prerequisite for a fodder tree species in silvopastoral system (Mullen *et al.*, 2003). Both species survived best in mixed inoculation (100 and 90%, respectively) followed by single inoculation of *Rhizobium* and AMF with vermicompost in comparison to FYM and control treatments.

The total fodder production was more in *S. sesban* in initial two years but from third year onwards there was a reduction of 16% in all the treatments over *L. leucocephala* (Table 1). The fodder production was significantly best in mixed inoculation with vermicompost, 4.3-6.3 $kg^{-1}$ tree in *L. leucocephala* and in *S. sesban* it ranged from 2.3-3.8 kg $tree^{-1}$ during three years over other treatments. Although, in *S. sesban* all the biofertilizer

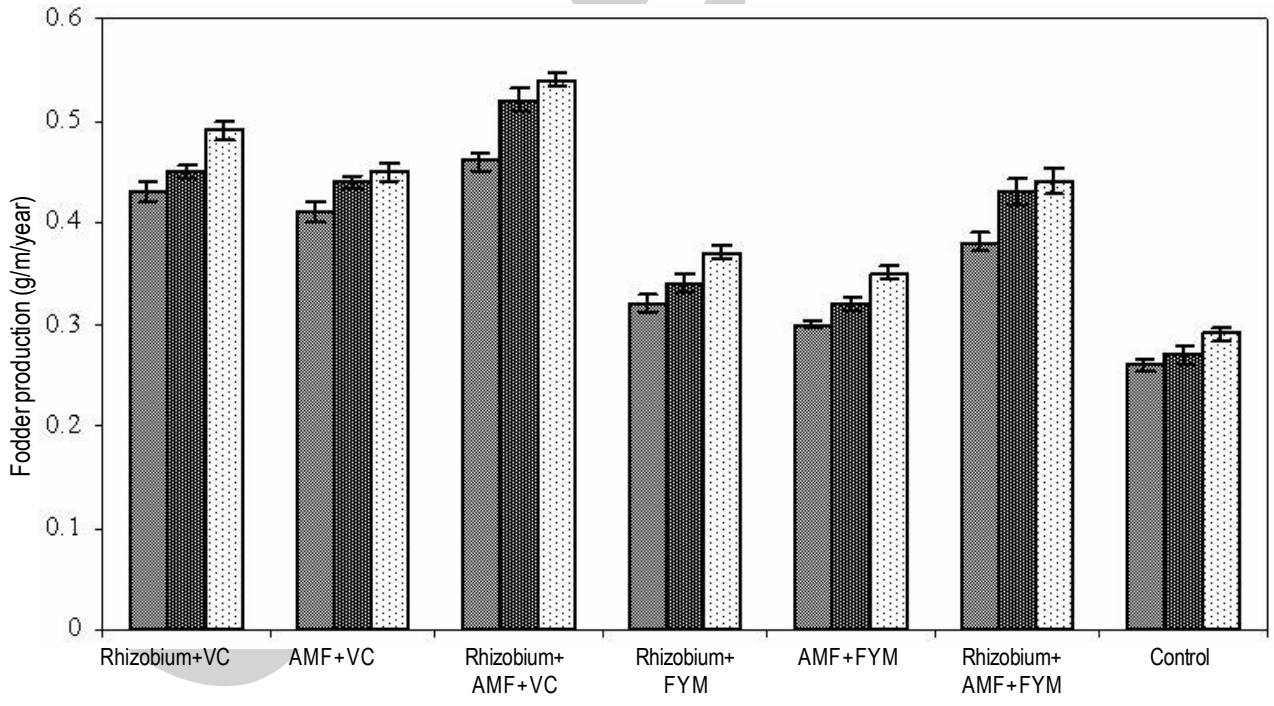


**Fig. 1:** Fodder production in *P. maximum* with *L. leucocephala* in response to biofertilizers in silvopastoral system. Bar represent ± standard error

**Table - 1:** Survival (%) and fodder production in tree species in response to biofertilizers in silvopastoral system*

| Tree species and treatments | Survival (%) | | | Fodder production (kg tree$^{-1}$ yr$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year |
| ***L. leucocephala*** | | | | | | |
| *Rhizobium* +VC | 100 $^{ab}$ | 100 $^{ab}$ | 100 $^{ab}$ | 3.60 $^{b}$ | 4.20 $^{b}$ | 5.60 $^{b}$ |
| AMF +VC | 90 $^{b}$ | 90 $^{b}$ | 90 $^{b}$ | 3.20 $^{c}$ | 3.90 $^{bc}$ | 4.60 $^{cd}$ |
| *Rhizobium*+AMF +VC | 100 $^{a}$ | 100 $^{a}$ | 100 $^{a}$ | 4.30 $^{a}$ | 5.30 $^{a}$ | 6.30 $^{a}$ |
| *Rhizobium* +FYM | 90 $^{b}$ | 90 $^{b}$ | 90 $^{b}$ | 3.0 $^{cd}$ | 4.10 $^{c}$ | 4.54 $^{cd}$ |
| AMF+ FYM | 80 $^{c}$ | 80 $^{bc}$ | 80 $^{bc}$ | 2.80 $^{d}$ | 3.60 $^{cd}$ | 4.30 $^{d}$ |
| *Rhizobium*+AMF+ FYM | 90 $^{b}$ | 90 $^{ab}$ | 90 $^{ab}$ | 3.20 $^{c}$ | 4.10 $^{bc}$ | 4.90 $^{c}$ |
| Control | 70 $^{d}$ | 50 $^{c}$ | 40 $^{c}$ | 2.50 $^{e}$ | 3.20 $^{d}$ | 3.80 $^{e}$ |
| *P* value at 0.05 level | 8.96 | 9.4 | 8.2 | 0.3 | 0.4 | 0.48 |
| ***S. sesban*** | | | | | | |
| *Rhizobium* +VC | 70 $^{ab}$ | 60 $^{ab}$ | 30 $^{b}$ | 4.20 $^{a}$ | 5.20 $^{a}$ | 5.36 $^{a}$ |
| AMF +VC | 60 $^{b}$ | 50 $^{b}$ | 20 $^{bc}$ | 3.90 $^{a}$ | 5.16 $^{a}$ | 5.28 $^{a}$ |
| *Rhizobium*+AMF +VC | 70 $^{a}$ | 70 $^{a}$ | 50 $^{a}$ | 4.34 $^{a}$ | 5.34 $^{a}$ | 5.45 $^{a}$ |
| *Rhizobium* +FYM | 60 $^{c}$ | 50 $^{bc}$ | 20 $^{c}$ | 3.45 $^{bc}$ | 4.49 $^{d}$ | 4.69 $^{b}$ |
| AMF+ FYM | 50 $^{cd}$ | 40 $^{cd}$ | 20 $^{c}$ | 3.22 $^{c}$ | 4.68 $^{c}$ | 4.70 $^{b}$ |
| *Rhizobium*+AMF+ FYM | 60b $^{c}$ | 50 $^{b}$ | 30 $^{bc}$ | 3.63 $^{b}$ | 4.85 $^{b}$ | 4.90 $^{b}$ |
| Control | 40 $^{d}$ | 30 $^{d}$ | 10 $^{d}$ | 3.12 $^{c}$ | 3.60 $^{e}$ | 3.69 $^{c}$ |
| *P* value at 0.05 level | 12.6 | 9.51 | 11.86 | 0.36 | 0.22 | 0.18 |

*Comparisons were made among different treatments of same plant species, Means in the same column followed by same letter are not significantly different at p<0.05

**Table - 2:** Micronutrient content (mg kg$^{-1}$ dry matter) of fodder tree species in response to biofertilizers in silvopastoral system*

| Tree species and treatments | Fe | | | Cu | | | Mn | | | Zn | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year |
| ***L. leucocephala*** | | | | | | | | | | | | |
| *Rhizobium* +VC | 336 $^{c}$ | 338 $^{c}$ | 339 $^{bc}$ | 28 $^{bc}$ | 29 $^{bc}$ | 30 $^{b}$ | 324 $^{b}$ | 324 $^{bc}$ | 325 $^{c}$ | 30 $^{c}$ | 34 $^{b}$ | 36 $^{c}$ |
| AMF +VC | 354 $^{b}$ | 354 $^{b}$ | 359 $^{b}$ | 31 $^{ab}$ | 32 $^{ab}$ | 33 $^{ab}$ | 311 $^{c}$ | 312 $^{c}$ | 315 $^{d}$ | 39 $^{b}$ | 42 $^{ab}$ | 44 $^{b}$ |
| *Rhizobium*+AMF+VC | 365 $^{a}$ | 366$^{a}$ | 369$^{a}$ | 32$^{a}$ | 34$^{a}$ | 35$^{a}$ | 339$^{a}$ | 341$^{a}$ | 343$^{a}$ | 43$^{a}$ | 45.3$^{a}$ | 47$^{a}$ |
| *Rhizobium* +FYM | 325$^{cd}$ | 329$^{cd}$ | 330$^{cd}$ | 21$^{c}$ | 22$^{d}$ | 23$^{c}$ | 310$^{cd}$ | 310$^{c}$ | 311$^{d}$ | 22$^{d}$ | 25.2$^{d}$ | 28$^{e}$ |
| AMF+FYM | 331$^{c}$ | 332$^{c}$ | 335$^{c}$ | 27$^{bc}$ | 28$^{c}$ | 29$^{bc}$ | 306$^{d}$ | 308$^{d}$ | 309$^{de}$ | 25$^{cd}$ | 28$^{c}$ | 29$^{d}$ |
| *Rhizobium*+AMF+ FYM | 342$^{b}$ | 345$^{bc}$ | 348$^{ab}$ | 29$^{b}$ | 30$^{b}$ | 31$^{ab}$ | 329$^{b}$ | 331$^{b}$ | 333$^{b}$ | 30$^{c}$ | 33.6$^{b}$ | 35$^{cd}$ |
| Control | 302$^{d}$ | 302$^{d}$ | 303$^{d}$ | 18$^{d}$ | 19$^{e}$ | 19$^{d}$ | 298$^{e}$ | 298$^{e}$ | 299$^{e}$ | 20$^{e}$ | 22$^{d}$ | 23$^{f}$ |
| *P* value at 0.05 level | 4.02 | 4.1 | 4.9 | 0.25 | 0.27 | 0.32 | 1.23 | 1.67 | 1.99 | 0.29 | 0.32 | 0.39 |
| ***S. sesban*** | | | | | | | | | | | | |
| *Rhizobium* +VC | 292$^{c}$ | 293$^{c}$ | 294$^{c}$ | 18$^{d}$ | 19$^{c}$ | 21$^{c}$ | 300$^{b}$ | 302$^{b}$ | 300$^{b}$ | 32$^{c}$ | 36$^{bc}$ | 37$^{c}$ |
| AMF +VC | 299$^{b}$ | 299$^{b}$ | 299$^{b}$ | 21$^{b}$ | 22$^{b}$ | 24$^{b}$ | 295$^{bc}$ | 298$^{bc}$ | 299$^{bc}$ | 40$^{b}$ | 41$^{b}$ | 41$^{bc}$ |
| *Rhizobium*+AMF +VC | 318$^{a}$ | 320$^{a}$ | 320$^{a}$ | 24$^{a}$ | 25$^{a}$ | 26$^{a}$ | 310$^{a}$ | 312$^{a}$ | 311$^{a}$ | 44$^{a}$ | 48$^{a}$ | 48$^{a}$ |
| *Rhizobium* +FYM | 285$^{cd}$ | 286$^{d}$ | 286$^{d}$ | 16.3$^{e}$ | 17$^{d}$ | 18$^{d}$ | 288$^{d}$ | 280$^{d}$ | 295$^{c}$ | 26$^{d}$ | 30$^{d}$ | 31$^{e}$ |
| AMF+ FYM | 284$^{d}$ | 289$^{d}$ | 289$^{cd}$ | 19.5$^{c}$ | 21$^{bc}$ | 21.9$^{c}$ | 279$^{e}$ | 283$^{d}$ | 280$^{d}$ | 31$^{cd}$ | 35$^{c}$ | 36$^{d}$ |
| *Rhizobium*+AMF+ FYM | 295$^{b}$ | 296$^{b}$ | 297$^{b}$ | 21.3$^{b}$ | 23$^{b}$ | 24$^{b}$ | 290$^{c}$ | 295$^{c}$ | 292$^{c}$ | 38$^{bc}$ | 41$^{b}$ | 43$^{b}$ |
| Control | 275$^{e}$ | 276$^{e}$ | 276$^{e}$ | 16.3$^{e}$ | 16.6$^{d}$ | 17$^{d}$ | 269$^{f}$ | 271$^{e}$ | 269$^{e}$ | 24$^{e}$ | 25$^{e}$ | 25$^{f}$ |
| *P* value at 0.05 level | 3.36 | 4.01 | 4.3 | 0.20 | 0.21 | 0.32 | 1.09 | 1.11 | 1.21 | 0.24 | 0.29 | 0.34 |

*Comparisons were made among different treatments of same plant species; Means in the same column followed by same letter are not significantly different at p<0.05

treatments performed better in vermicompost than FYM but the comparative effect of dual and single inoculation were at par with both organic fertilizers. The variation in the fodder production of these tree species interprets their growth pattern which varies from species to species; due to their different growth pattern (Larbi *et al.*, 2005).

In both the tree species (Table 2,3) dual inoculation with vermicompost had resulted significantly higher macronutrients (N, P, K, Ca and Mg) and micronutrients (Fe, Cu, Mn and Zn). After dual inoculation uptake of N, K and Mn was more in single inoculation of *Rhizobium*, whereas P, Ca, Mg, Fe, Cu and Zn the uptake was more in single AMF treatment with both organic fertilizers. Significantly

**Table - 3:** Macronutrient content (g 100 $g^{-1}$ dry matter) of fodder tree species in response to biofertilizers in silvopastoral system*

| Tree species and treatments | N | | | P | | | K | | | Ca | | | Mg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year |
| ***L. leucocephala*** | | | | | | | | | | | | | | | |
| *Rhizobium* +VC | 4.3$^{ab}$ | 4.4$^{b}$ | 4.6$^{ab}$ | 0.21$^{d}$ | 0.21$^{d}$ | 0.23$^{d}$ | 2.1$^{b}$ | 2.1$^{b}$ | 2.16$^{b}$ | 1.3$^{d}$ | 1.3$^{c}$ | 1.36$^{cd}$ | 0.3$^{b}$ | 0.3$^{b}$ | 0.31$^{b}$ |
| AMF +VC | 3.7$^{b}$ | 3.9$^{bc}$ | 4.0$^{bc}$ | 0.29$^{b}$ | 0.31$^{b}$ | 0.33$^{b}$ | 2.0$^{b}$ | 2.05$^{b}$ | 2.1$^{b}$ | 1.42$^{b}$ | 1.45$^{b}$ | 1.53$^{b}$ | 0.32$^{ab}$ | 0.32$^{ab}$ | 0.33$^{ab}$ |
| *Rhizobium*+AMF +VC | 4.7$^{a}$ | 4.8$^{a}$ | 4.9$^{a}$ | 0.36$^{a}$ | 0.37$^{a}$ | 0.40$^{a}$ | 2.4$^{a}$ | 2.46$^{a}$ | 2.51$^{a}$ | 1.72$^{a}$ | 1.76$^{a}$ | 1.8$^{a}$ | 0.33$^{a}$ | 0.33$^{a}$ | 0.34$^{a}$ |
| *Rhizobium* +FYM | 3.55$^{c}$ | 3.6$^{c}$ | 3.7$^{c}$ | 0.20$^{d}$ | 0.20$^{d}$ | 0.21$^{d}$ | 1.02$^{c}$ | 1.09$^{c}$ | 1.1$^{d}$ | 1.2$^{d}$ | 1.21$^{d}$ | 1.29$^{d}$ | 0.26$^{c}$ | 0.26$^{c}$ | 0.27$^{c}$ |
| AMF+ FYM | 3.2$^{cd}$ | 3.4$^{cd}$ | 3.5$^{c}$ | 0.22$^{cd}$ | 0.24$^{cd}$ | 0.25$^{cd}$ | 0.9$^{d}$ | 1.0$^{c}$ | 1.06$^{d}$ | 1.36$^{bc}$ | 1.39$^{c}$ | 1.40$^{c}$ | 0.27$^{bc}$ | 0.28$^{c}$ | 0.28$^{bc}$ |
| *Rhizobium*+AMF+FYM | 3.8$^{b}$ | 4.0$^{b}$ | 4.1$^{b}$ | 0.28$^{c}$ | 0.29$^{c}$ | 0.31$^{c}$ | 1.3$^{c}$ | 1.31$^{bc}$ | 1.36$^{c}$ | 1.49$^{b}$ | 1.49$^{b}$ | 1.51$^{b}$ | 0.29$^{b}$ | 0.29$^{bc}$ | 0.30$^{b}$ |
| Control | 3.0$^{d}$ | 3.04$^{d}$ | 3.2$^{d}$ | 0.19$^{e}$ | 0.19$^{e}$ | 0.2d$^{e}$ | 0.85$^{d}$ | 0.88$^{d}$ | 0.91$^{e}$ | 1.0$^{e}$ | 1.1$^{d}$ | 1.23$^{e}$ | 0.21$^{d}$ | 0.21$^{d}$ | 0.22$^{d}$ |
| *P* value at 0.05 level | 0.42 | 0.39 | 0.49 | 0.043 | 0.049 | 0.043 | 0.15 | 0.18 | 0.21 | 0.041 | 0.036 | 0.05 | 0.12 | 0.14 | 0.15 |
| ***S. sesban*** | | | | | | | | | | | | | | | |
| *Rhizobium* +VC | 3.9$^{ab}$ | 4.1$^{ab}$ | 3.9$^{b}$ | 0.12$^{c}$ | 0.13$^{c}$ | 0.13$^{c}$ | 2.2$^{b}$ | 2.26$^{b}$ | 2.3$^{b}$ | 0.93$^{c}$ | 0.93$^{c}$ | 0.95$^{c}$ | 0.28$^{b}$ | 0.28$^{b}$ | 0.29$^{b}$ |
| AMF +VC | 3.7$^{bc}$ | 3.9$^{b}$ | 3.5$^{c}$ | 0.17$^{b}$ | 0.18$^{b}$ | 0.20$^{b}$ | 2.13$^{b}$ | 2.19$^{b}$ | 2.2$^{bc}$ | 1.0$^{b}$ | 1.0$^{b}$ | 1.0$^{b}$ | 0.30$^{ab}$ | 0.30$^{ab}$ | 0.31$^{ab}$ |
| *Rhizobium*+AMF+VC | 4.03$^{a}$ | 4.14$^{a}$ | 4.0$^{a}$ | 0.21$^{a}$ | 0.23$^{a}$ | 0.23$^{a}$ | 2.36$^{a}$ | 2.39$^{a}$ | 2.42$^{a}$ | 1.23$^{a}$ | 1.23$^{a}$ | 1.21$^{a}$ | 0.32$^{a}$ | 0.32$^{a}$ | 0.33$^{a}$ |
| *Rhizobium* +FYM | 3.6$^{c}$ | 3.7$^{c}$ | 3.6$^{c}$ | 0.11$^{d}$ | 0.11$^{d}$ | 0.11$^{d}$ | 1.9$^{c}$ | 1.96$^{c}$ | 1.99$^{c}$ | 0.89$^{d}$ | 0.89$^{d}$ | 0.9$^{cd}$ | 0.25$^{c}$ | 0.25$^{d}$ | 0.26$^{d}$ |
| AMF+ FYM | 3.5$^{cd}$ | 3.6$^{cd}$ | 3.4$^{d}$ | 0.13$^{bc}$ | 0.14$^{c}$ | 0.15$^{c}$ | 2.0$^{bc}$ | 2.13$^{bc}$ | 2.19$^{bc}$ | 0.96$^{bc}$ | 0.97$^{bc}$ | 0.97$^{c}$ | 0.28$^{bc}$ | 0.28$^{c}$ | 0.29$^{bc}$ |
| *Rhizobium*+AMF+FYM | 3.8$^{b}$ | 3.9$^{b}$ | 3.8$^{b}$ | 0.18$^{b}$ | 0.19$^{b}$ | 0.21$^{ab}$ | 2.29$^{b}$ | 2.3$^{b}$ | 2.32$^{b}$ | 1.03$^{b}$ | 1.05$^{b}$ | 1.05$^{b}$ | 0.29$^{b}$ | 0.30$^{b}$ | 0.30$^{b}$ |
| Control | 3.18$^{d}$ | 3.3$^{d}$ | 3.09$^{e}$ | 0.10$^{d}$ | 0.10$^{d}$ | 0.11$^{d}$ | 1.83$^{d}$ | 1.89$^{d}$ | 1.9$^{d}$ | 0.85$^{d}$ | 0.85$^{d}$ | 0.81$^{d}$ | 0.25$^{d}$ | 0.25$^{d}$ | 0.26$^{d}$ |
| *P* value at 0.05 level | 0.36 | 0.42 | 0.45 | 0.03 | 0.032 | 0.04 | 0.09 | 0.01 | 0.012 | 0.039 | 0.041 | 0.044 | 0.1 | 0.12 | 0.15 |

*Comparisons were made among different treatments of same plant species, Means in the same column followed by same letter are not significantly different at p<0.05

**Table - 4:** Crude protein and fiber content of tree species in response to biofertilizers in silvopastoral system*

| Tree species and treatments | CP (g 100 $g^{-1}$ DM) | | | NDF (g 100 $g^{-1}$ DM) | | | ADF (g 100 $g^{-1}$ DM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year |
| ***L. leucocephala*** | | | | | | | | | |
| *Rhizobium* +VC | 26.42$^{b}$ | 27.7$^{b}$ | 28.57$^{b}$ | 53.4$^{bcd}$ | 52.47$^{cd}$ | 52.27$^{c}$ | 30.6$^{cd}$ | 29.63$^{bc}$ | 29.3$^{c}$ |
| AMF +VC | 23.25$^{c}$ | 24.53$^{c}$ | 25.1$^{c}$ | 56.87$^{a}$ | 53.47$^{bc}$ | 52.2$^{c}$ | 32.47$^{ab}$ | 30.8$^{b}$ | 29.87$^{bc}$ |
| *Rhizobium*+AMF +VC | 29.5$^{a}$ | 29.9$^{a}$ | 30.7$^{a}$ | 52.0$^{d}$ | 51.17$^{d}$ | 50.37$^{d}$ | 30.2$^{d}$ | 28.97$^{c}$ | 27.9$^{d}$ |
| *Rhizobium* +FYM | 22.17$^{c}$ | 22.7$^{d}$ | 23.2$^{d}$ | 55.4$^{a}$ | 52.5$^{cd}$ | 52.33$^{c}$ | 3108$^{b}$ | 31.2$^{ab}$ | 30.73$^{ab}$ |
| AMF+ FYM | 20.18$^{d}$ | 21.17$^{e}$ | 22.3$^{e}$ | 56.33$^{a}$ | 55.73$^{a}$ | 54.77$^{ab}$ | 32.0$^{b}$ | 31.0$^{b}$ | 30.97$^{ab}$ |
| *Rhizobium*+AMF+ FYM | 23.79$^{c}$ | 24.7$^{c}$ | 25.03$^{c}$ | 54.4$^{bc}$ | 54.27$^{ab}$ | 53.47$^{bc}$ | 31.67$^{bc}$ | 31.17$^{ab}$ | 30.37$^{abc}$ |
| Control | 18.58$^{d}$ | 19.0$^{f}$ | 19.87$^{f}$ | 56.6$^{a}$ | 55.1$^{a}$ | 55.07$^{a}$ | 33.4$^{a}$ | 32.43$^{a}$ | 31.13$^{a}$ |
| *P* value at 0.05 level | 1.65 | 0.8 | 0.76 | 1.63 | 1.52 | 1.4 | 1.2 | 1.6 | 1.17 |
| ***S. sesban*** | | | | | | | | | |
| *Rhizobium* +VC | 24.25$^{ab}$ | 25.6$^{a}$ | 24.07$^{ab}$ | 48.27$^{bc}$ | 48.3$^{bc}$ | 48.9$^{bc}$ | 24.43$^{bc}$ | 24.53$^{ab}$ | 24.9$^{ab}$ |
| AMF+VC | 23.4$^{b}$ | 24.8$^{bc}$ | 22.1$^{bc}$ | 48.89$^{b}$ | 49.0$^{ab}$ | 49.0$^{abc}$ | 25.37$^{b}$ | 25.7$^{a}$ | 25.63$^{a}$ |
| *Rhizobium*+AMF +VC | 25.21$^{a}$ | 25.9$^{a}$ | 24.83$^{a}$ | 48.0$^{bc}$ | 47.17$^{c}$ | 48.2$^{c}$ | 23.4$^{a}$ | 23.73$^{b}$ | 23.77$^{b}$ |
| *Rhizobium*+FYM | 22.63$^{cd}$ | 23.3$^{c}$ | 22.6$^{c}$ | 49.0$^{ab}$ | 48.47$^{ab}$ | 48.57$^{ab}$ | 24.5$^{bc}$ | 24.43$^{ab}$ | 25.17$^{a}$ |
| AMF+ FYM | 21.78$^{d}$ | 22.2$^{d}$ | 21.23$^{d}$ | 49.3$^{ab}$ | 48.8$^{ab}$ | 49.5$^{ab}$ | 24.3$^{b}$ | 24.1$^{b}$ | 24.8$^{ab}$ |
| *Rhizobium*+AMF+FYM | 23.55$^{bc}$ | 24.3$^{b}$ | 23.87$^{abc}$ | 46.87$^{c}$ | 47.7$^{bc}$ | 48.17$^{ab}$ | 23.07$^{cd}$ | 23.4$^{b}$ | 23.8$^{b}$ |
| Control | 19.89$^{e}$ | 20.78$^{e}$ | 19.36$^{e}$ | 50.77$^{a}$ | 49.8$^{a}$ | 50.57$^{a}$ | 26.73$^{a}$ | 25.8$^{a}$ | 25.57$^{a}$ |
| *P* value at 0.05 level | 1.03 | 0.93 | 1.28 | 1.8 | 1.49 | 1.6 | 1.03 | 1.44 | 1.25 |

*Comparisons were made among different treatments of same plant species, Means in the same column followed by same letter are not significantly different at p<0.05

higher amount of macro and micronutrients in dual inoculated treatment in tree species obtained due to their enhanced uptake from soil because of better association of AMF with host plant. The AMF increase the surface area of root and improves the uptake of nutrients (Tawaraya *et al.*, 2001). The results pertaining to fodder quality of tree species demonstrates that there was a significant effect ($p<0.05$) of treatments on CP, NDF and ADF content of both the tree species (Table 4). Inoculation enhanced CP content (9-35%) and decreased NDF (9%) and ADF (11%) in both the tree species in comparison to control. In both tree species CP% increased up to three years in inoculated treatments over control while NDF and ADF content reduced. This was also found with *Panicum maximum* and

**Table - 5:** Effect of treatments on the density of inoculated bioinoculants in silvopastoral system*

| Tree species and treatments | *Rhizobium* (CFU 100g$^{-1}$ soil) | | | No. of AM spores 100g$^{-1}$ soil | | | AM root infection (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year | I$^{st}$ year | II$^{nd}$ year | III$^{rd}$ year |
| ***L. leucocephala*** | | | | | | | | | |
| *Rhizobium* +VC | $5.3x10^{4b}$ | $6.2x10^{4}$ | $7.6x10^{4ab}$ | $35.1^{d}$ | $42.5^{d}$ | $49.0^{b}$ | $22^{c}$ | $30^{e}$ | $47^{ab}$ |
| AMF+VC | $6.6x10^{3c}$ | $5.7x10^{3}$ | $0.6x10^{3b}$ | $57.0^{b}$ | $59.3^{b}$ | $68.3^{a}$ | $36^{b}$ | $52^{c}$ | $62^{a}$ |
| *Rhizobium*+AMF+VC | $5.7x10^{4a}$ | $7.8x10^{4}$ | $8.0x10^{4a}$ | $66.0^{a}$ | $65.0^{a}$ | $71.0^{a}$ | $65^{a}$ | $70^{a}$ | $75^{c}$ |
| *Rhizobium*+FYM | $8.6x10^{3bc}$ | $3.7x10^{4}$ | $3.6x10^{4ab}$ | $27.4^{e}$ | $31.0^{e}$ | $36.3^{cd}$ | $15^{c}$ | $22^{e}$ | $40^{c}$ |
| AMF+FYM | $3.7x10^{3c}$ | $3.0x10^{3}$ | $4.7x10^{2bc}$ | $36.0^{d}$ | $42.3^{d}$ | $45.0^{bc}$ | $25^{c}$ | $40^{d}$ | $42^{c}$ |
| *Rhizobium*+AMF+FYM | $1.7x10^{4bc}$ | $4.0x10^{4}$ | $4.2x10^{4ab}$ | $45.0^{c}$ | $55.0^{c}$ | $63.0^{a}$ | $42^{b}$ | $58^{b}$ | $56^{b}$ |
| Control | $1.1x10^{2d}$ | $2.0x10^{2}$ | $2.2x10^{2c}$ | $18.0^{f}$ | $28.5^{f}$ | $36.5^{d}$ | $5^{d}$ | $10^{f}$ | $15^{d}$ |
| *P* value at 0.05 level | 1.3 | 5.08 | 3.62 | 5.73 | 2.9 | 10.63 | 7.33 | 5.08 | 10.63 |
| ***S. sesban*** | | | | | | | | | |
| *Rhizobium*+VC | $6.6x10^{3ab}$ | $5.7x10^{4ab}$ | $6.3x10^{4ab}$ | $36.0^{c}$ | $28^{b}$ | $32^{bc}$ | $30^{c}$ | $32^{cd}$ | $43^{b}$ |
| AMF+VC | $5.7x10^{2bc}$ | $3.2x10^{3cd}$ | $5.0x10^{3bc}$ | $45.7^{b}$ | $38^{a}$ | $40^{ab}$ | $40^{c}$ | $44^{b}$ | $50^{b}$ |
| *Rhizobium*+AMF+VC | $7.9x10^{3a}$ | $7.2x10^{4a}$ | $7.5x10^{4a}$ | $55.0^{a}$ | $40^{a}$ | $55^{a}$ | $65^{a}$ | $62^{a}$ | $70^{a}$ |
| *Rhizobium* +FYM | $2.8x10^{3b}$ | $8.8x10^{3b}$ | $9.2x10^{3bc}$ | $26.0^{d}$ | $29^{b}$ | $36^{c}$ | $25^{d}$ | $22^{d}$ | $27^{c}$ |
| AMF+FYM | $4.2x10^{2c}$ | $1.2x10^{3c}$ | $1.8x10^{3c}$ | $37.1^{c}$ | $32^{b}$ | $40^{bc}$ | $35^{c}$ | $28^{cd}$ | $32^{c}$ |
| *Rhizobium*+AMF+FYM | $5.0x10^{3ab}$ | $0.6x10^{4bc}$ | $1.3x10^{4b}$ | $46.3^{b}$ | $40^{a}$ | $46^{ab}$ | $50^{b}$ | $35^{bc}$ | $45^{b}$ |
| Control | $0.8x10^{2d}$ | $1.2x10^{2d}$ | $1.4x10^{2d}$ | $10.3^{e}$ | $9.0^{c}$ | $12.3^{d}$ | $10^{e}$ | $10^{e}$ | $12^{d}$ |
| *P* value at 0.05 level | 1.82 | 2.52 | 1.9 | 3.9 | 4.44 | 11.09 | 8.3 | 10.06 | 13.37 |

*Comparisons were made among different treatments of same plant species, Means in the same column followed by same letter are not significantly different at p<0.05

**Table - 6:** Effect of treatments on the nutrient quality of rhizosphere soil in silvopastoral system*

| Treatments | Organic C (%) | Available N (kg ha$^{-1}$) | Available P (kg ha$^{-1}$) | Available K (kg ha$^{-1}$) |
|---|---|---|---|---|
| ***L. leucocephala*** | | | | |
| *Rhizobium* +VC | $0.40^{ab}$ | $177^{b}$ | $6.3^{c}$ | $139^{b}$ |
| AMF+VC | $0.38^{ab}$ | $164^{de}$ | $7.2^{b}$ | $134^{b}$ |
| *Rhizobium*+AMF+VC | $0.43^{a}$ | $185^{a}$ | $7.9^{a}$ | $142^{a}$ |
| *Rhizobium*+FYM | $0.37^{ab}$ | $169^{cd}$ | $5.6^{e}$ | $137^{ab}$ |
| AMF+FYM | $0.36^{bc}$ | $161^{e}$ | $6.1^{d}$ | $136^{b}$ |
| *Rhizobium*+AMF+ FYM | $0.39^{ab}$ | $173^{c}$ | $6.9^{b}$ | $138^{ab}$ |
| Control | $0.36^{c}$ | $159^{f}$ | $5.7^{e}$ | $136^{b}$ |
| *P* value at 0.05 level | 0.05 | 3.5 | 0.3 | 4.03 |
| ***S. sesban*** | | | | |
| *Rhizobium*+VC | $0.41^{a}$ | $168^{b}$ | $5.8b^{cd}$ | $138^{c}$ |
| AMF+VC | $0.37^{b}$ | $159^{d}$ | $6.1b^{c}$ | $140^{ab}$ |
| *Rhizobium*+AMF+VC | $0.42^{a}$ | $172^{a}$ | $6.8^{ab}$ | $141^{a}$ |
| *Rhizobium*+FYM | $0.36^{b}$ | $165^{bc}$ | $5.7^{ab}$ | $136^{bc}$ |
| AMF+FYM | $0.35^{b}$ | $161^{cd}$ | $5.8^{d}$ | $136^{c}$ |
| *Rhizobium*+AMF+ FYM | $0.37^{b}$ | $169^{b}$ | $6.2^{cd}$ | $138^{abc}$ |
| Control | $0.35^{b}$ | $158^{d}$ | $5.5^{d}$ | $135^{c}$ |
| *P* value at 0.05 level | 0.05 | 2.9 | 0.43 | 4.0 |

*Comparisons were made among different treatments of same plant species, Means in the same column followed by same letter are not significantly different at p<0.05

*Stylosanthes seabrana* in our earlier pot studies (Mishra *et al.*, 2008; 2009), where single and mixed inoculation of *Azospirillum* and *Rhizobium* with AM fungi enhanced CP content and reduced NDF, ADF. In *S. sesban* percentage increase in CP in third year was reduced that could be due to the reduction of fodder production.

Fodder production from *P. maximum* was more (10%) under *L. leucocephlala* than in *S. sesban* during the initial two years (Fig. 1,2). But after second year, grass production was more (26%) under *S. sesban* than in *L. leucocephala* (4%). It might be due more competition between *S. sesban* and *P. maximum* during initial years for resources that lead to less survival of *S. sesban* (Durr and Rangel, 2000). Grasses with their dense fibrous root networks may affect woody plant performance by extracting near surface resources and preventing their percolation to deeper soil depths. In this competition, performance of trees reduces and needs further fertilization (Simmons *et al.*, 2007). The overall production of grass

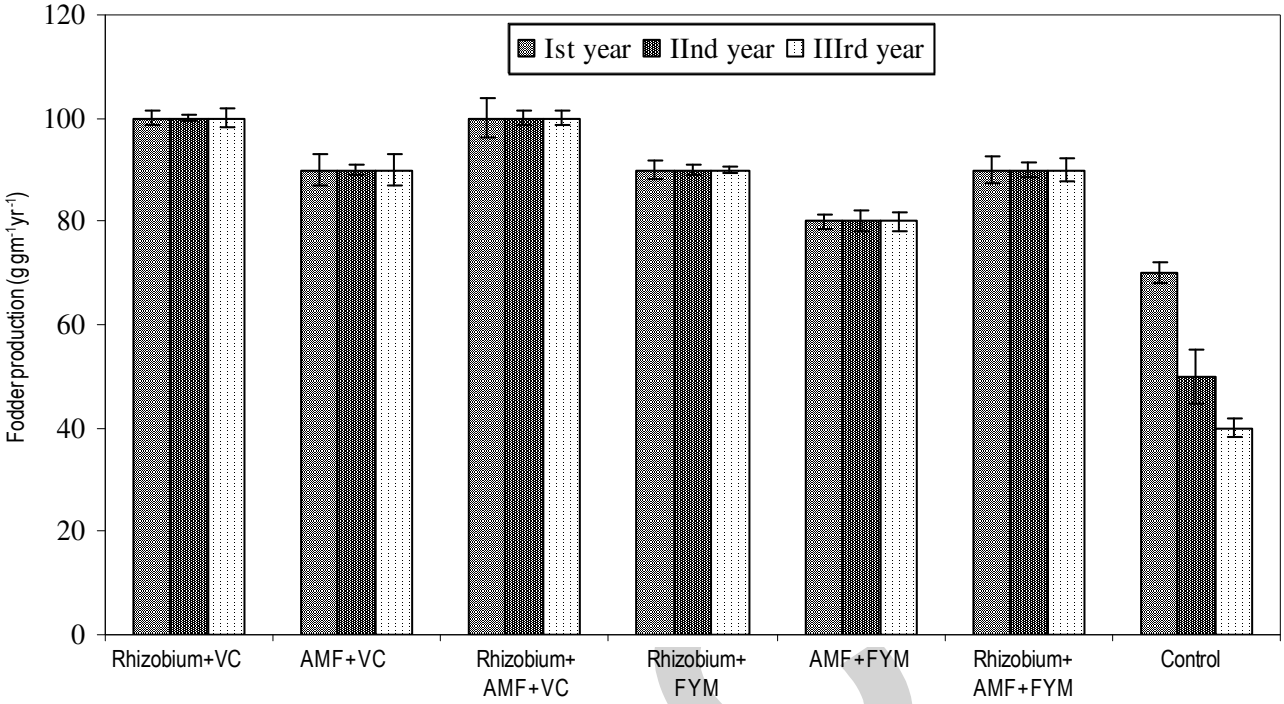


**Fig. 2:** Fodder production in *P. maximum* with *S. sesban* in response to Biofertilizer in silvopastoral system. Bar represent ± standard error

was more in biofertilizers treatments with vermicompost in both tree species exhibits the comparative potential of vermicompost over FYM. Organic fertilizers and biofertilizers are the important components of integrated nutrient supply system which improve the productivity of crop as well as soil (Wu *et al.*, 2005; Zaller, 2007). Better association of *P. maximum* with *L. leucocephala* indicates their suitability in pasture development programme.

The density of *Rhizobium* cells and of AM spores in the soil and AM root infection significantly increased ($p<0.05$) over the years in dual inoculation in vermicompost over FYM and control in *L. leucocephala* while in *S. sesban* it was at par in all the biofertilizer treatments (Table 4). However, in third year there was an improvement in microbial population. It could be due to reduced survival of *S. sesban* in third year that improved access to soil organic matter for microbes by mineralization of the decaying roots (Liasu *et al.*, 2005). After three year of plantation dual inoculation in vermicompost had maximum soil nutrients (organic C, N, P and K) followed by single inoculation of native *Rhizobium* and AM fungi than FYM and control in *L. leucocephala* (Table 5). In *S. sesban* the soil nutrient increase was at par in biofertilizer treatment. However, it was higher with vermicompost.

In our study the overall effect of dual inoculation was found to be better than single inoculation in *L. leucocephala.* It may be due to positive interactions among inoculated microbes with native microflora of the soil that resulted in their synergistic association and overall performance of tree species (Giller and Caddish, 1995). In *S. sesban* the overall performance of dual and single inoculation of native *Rhizobium* were at par followed by single inoculation of AM fungi and control. It could be due to more competition of native *Rhizobium* and inoculated AM fungi for colonizing sites on legume roots and also different plant species stimulate the amount and occurrence of different species of AM fungi which may results in poor effectiveness of symbiosis (de Varnnes and Goss, 2007; Chalk *et al.*, 2006). However, increase in AM spores density in rhizosphere soil and root infection in *S. sesban* suggested that the effect of inoculated native *Rhizobium* was more stimulating than AM fungi which may be slow growing to act positively. Moreover, the magnitude and potential of microbial inoculants on plant- rhizosphere functioning depends upon the interactions among microbial (indigenous and exotic) and environmental (temperature, drought, organic matter, acidity etc.) components involved (Biro *et al.*, 2000; Bayoumi *et al.*, 1995).

The results in this study suggest that improved yield and fodder quality from silvopastoral system are possible through inoculation with proper AM fungi species with native *Rhizobium* strain.

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