Afforestation in barren laterite lands with *Swietenia macrophylla* G. King and plant growth promoting microbes

Arumugam Karthikeyan

Institute of Forest Genetics and Tree Breeding, Coimbatore, India

✉ karthikarumugam13@gmail.com

ARTICLE INFO

Citation:
Karthikeyan A (2020) Afforestation in barren laterite lands with *Swietenia macrophylla* G. King and plant growth promoting microbes. Reforesta 9:54-65.
DOI: https://dx.doi.org/10.21750/REFOR.9.07.81

Editor: **Vladan Ivetić, Serbia**
Received: **2020-06-08**
Accepted: **2020-06-26**
Published: **2020-06-30**

Abstract

Barren laterite lands are available plenty in Kasargode District of Kerala, India and being used mainly for extracting laterite bricks for building construction. These lands are found barren due to lack of vegetation and rocky nature. Afforestation in these lands is very essential to avoid extraction of laterite bricks so as to prevent degradation of lands. To develop vegetation in these laterite lands the soil properties of laterite was examined as a first step of afforestation process. It was found that the soils have lack of plant growth promoting microbes (PGPM) and poor in major nutrients (N, P, K). Therefore, the PGPM specifically arbuscular mycorrhizal fungi (*Glomus fasciculatum* and *Glomus geosporum*) and bacteria *(Azospirillum brasilense* and *Bacillus megaterium*) were used for afforestation in laterite lands along with *Swietenia macrophylla* G. King a commercially important tree of Kerala, India. The laterite soils were collected and used as potting media for growing seedlings of *S. macrophylla* in nursery and the cultured PGPM were inoculated in to the seedlings of *S. macrophylla* and maintained for 3 months. The PGPM inoculated seedlings showed improved growth, biomass and nutrient uptake. Thereafter the seedlings were transplanted at laterite lands at Karmanthodi, Bovikanam, Kasargode, Kerala, India and monitored their growth for 12 months. The seedlings inoculated with PGPM showed up to 98% survival rate with improved growth. From this study it was understood that PGPM have the potential to increase the efficiency of plant growth system in *S. macrophylla* seedlings through supply of essential levels of N, P and K that helped for successful afforestation in hardy laterite lands.

Keywords

Swietenia macrophylla; PGPM; Laterite lands; afforestation

Contents

1 Introduction

Laterite is a residual ferruginous rock commonly found in tropical regions and has close genetic association with bauxite. Laterite rocks are predominantly available in Kerala, Karnataka, Maharashtra, Orissa and Assam states of India. Lateritic soils are rich in iron and aluminum usually formed in hot tropical areas and showed rusty-red colour due to iron oxides. They develop by intensive and long lasting weathering of the underlying parent rock. Laterite soil are widely used for arable cultivation, grazing and constructional purposes in the Northern part of Kerala (India). They are fine to medium texture, acidic, low in organic matter, poor in exchangeable bases, deficient in available phosphorus and total nitrogen. The hard nature of laterite rocks is unfit to cultivate any crop or tree due to their unfavorable physical and chemical factors to the plants. For a successful afforestation in the laterite rocks the suitable biological properties need to be amended along with tree species. Plant growth promoting microbes (PGPM) are the only potential tool for afforestation in laterite lands as biological amendments. PGPM is a group of root associated microorganisms intimately interact with the plant roots and consequently influence plant health and soil fertility.

Different PGPM including associative bacteria such as *Azospirillum, Bacillus, Pseudomonas, Enterobacter* groups have been used for plant growth promotion (Kloepper and Beauchamp 1992). The mechanisms of PGPM are mobilization of nutrients (Lifshitz et al. 1987) stimulation of root growth by production of phytohormones (Bothe et al. 1992; Kloepper et al. 1980) and antagonism against soil borne plant pathogens (Kloepper et al. 1988). All of these mechanisms happened due to direct contact of PGPM and plant roots by inoculation. The inoculation of PGPM in to the plant rhizosphere or roots is a prerequisite for early establishment in the harsh lands (Karthikeyan et al. 2009). Several studies clearly showed the effect of PGPM on plant growth of different trees in problematic sites like mine out lands (Karthikeyan and Krishnakumar 2012; Diagne et al. 2013).

In this study the PGPM, arbuscular mycorrhizal (AM) fungi (*Glomus fasciculatum* and *Glomus geosporum*), N fixing bacteria (*Azospirillum brasilense*) and phosphate solubilizing bacteria (*Bacillus megaterium*) were used for afforestation in laterite lands at Kasargode District, Kerala, India along with *Swietenia macrophylla* G. King a commercially important tree species of Kerala, India. The main focus of the study is to

develop a tree cover with *S. macrophylla* and PGPM as an eco friendly approach so as to convert the barren laterite lands into productive lands.

S. macrophylla is an evergreen tree native to Mexico and South America and well known for its fast growth. This tree was introduced to India in 1872 for ornamental and timber purpose. The seeds of *S. macrophylla* are being used for ethno botanical medicine to cure hypertension, diabetes and Malaria in Indonesia (Dewanjee and Maiti 2011). *S. macrophylla* is commonly found in northern parts of Kerala, India hence this tree was selected for afforestation in barren laterite lands as it has natural adaptation.

2 Materials and methods

2.1 Study site

One hectare laterite land was selected at Karmanthodi, Bovikanam (taluk) Kasargode, Kerala India (12°31'48.93'N; 75°09'28.93'E) for this study (Figure 1).

Figure 1. Map of the study site (Source: Google earth).

2.2 Soil analysis

The physico chemical properties of laterite soils (pH, electric conductivity, organic carbon, available N, P and K) were analyzed according to Jackson (1973).

2.3 Isolation and culture of AM fungi

AM fungal spores were isolated from the collected rhizosphere soils samples of *Acacia auriculiforms* found grown naturally adjacent to the barren laterite lands at Kasargode, Kerala, India by the method of Gerdemann and Nicolson (1963). The soil samples contain 4.6 (±0.22) infective propagules (spores, hypha) as examined by most probable number method (Porter 1979). The isolated AM fungal spores were identified with the manual of Schenck and Perez (1990). The identified AM fungal species found in the rhizosphere soils are *Glomus fasciculatum* Gerd. & Trappe emend., Walker & Koske and *Glomus geosporum* Nicol & Gerd., Gerd & Trappe. These AM fungi were multiplied in sterile media (alfisoil and sand) with *Zea mays* L as host species under

green house conditions (mean relative humidity of 60 % and mean temperature of 24°C) in pot cultures for three months.

2.4 Isolation of *Bacillus megaterium* (Phosphate solubilizing bacteria)

0.1 ml of laterite soil was diluted in sterile water (10^{-6}) were spread on the selective medium for Phosphobacterium (Pikovaskya medium: 10 g sucrose, 5.0 g $Ca_3(PO_4)$, 0.27 NH_4NO_3, 0.2 g KCl, 0.1 g $MgSO_4 X 7H_2O$, 0.1 g yeast extract, 1.0 mg $MnSO_4 X 6H_2O$, 1.00 mg $FeSO_4 X 7 H_2O$, 0.1 g Yeast extract and 15 g agar) culture. The plates were incubated at 32°C for 6 days. The colony in medium was identified as *Bacillus megaterium* that formed halo zone around the bacterial colonies.

2.5 Isolation of *Azospirillum brasilense* (Nitrogen fixing bacteria)

Congo red medium (gL^{-1} of distilled water: KH_2PO_4 – 5 g; $MgSO_4 X 7H_2O$ - 0.2 g; NaCl - 0.1 g; Yeast extract - 0.5 g; $FeCl_3 X 6H_2O$ - 0.2 g; NaCl - 0.1 g, DL. Malic Acid - 5.0 g; KOH - 4.8 g, Agar - 12 g; and 15 ml of 1:4000 solution of congo red) was used for isolation of *A. brasilense.* 0.1 ml laterite soil diluted in sterile water (10^{-5}) was poured in the medium and incubated at 37°C for 6 days. Pink and scarlet colonies of bacteria *A. brasilense* were found after 6 days.

2.6 Nursery experiments

The seeds of *S. macrophylla* were sown with following the standard pretreatment (soaked in cold water for 24 hours) in the nursery beds containing pure sand. The seeds germinated 7 to 10 days after sowing. Germinants were transplanted to polybags with size of 4.15 L volume (14 x 27 cm) containing sieved laterite soils collected from the latertie extracted areas of Kasargode, Kerala India.

2.7 Inoculation of PGPM

AM fungi obtained from the pot cultures of *Z. mays* containing chlamydospores were placed at the rate of 20 g $polybag^{-1}$ at 5 cm below the soil surface in each polythene bag containing laterite soils as potting media. Inoculation of cultured *A. brasilense* and *B. megaterium* individually and in combinations was executed by applying at the rate of 10 ml of each PGPM. Uninoculated control treatment was also maintained along with PGPM inoculated treatments. Total of 10 replicates of each of 7 treatments were used in the experiment, specifically : (1) Control, (2) AM fungi (20 g), (3) *A. brasilense* (10 ml), (4) *B. megaterium* (10 ml), (5) AM fungi (20g) + *A. brasilense* (10 ml), (6) AM fungi (20 g) + *B. megaterium* (10 ml) and (7) AM fungi (20 g) + *A. brasilense* (10 ml) + *B. megaterium*(10 ml). Each replicate consists of 5 seedlings hence totally 350 seedlings were used in this experiment. The PGPM inoculated and uninoculated control treatments were arranged in a Randomized block design for 90 days under shade house conditions (Temperature; 24.2°C (±1.1); Relative humidity; 66.7 (±1.3). All the seedlings were watered regularly to maintain the plants growth and survival.

2.8 Measurement of growth parameters

Ninety days after inoculation, the seedlings of *S. macrophylla* (only 5 replicates) were harvested with shoot and roots intact. The root length, seedling height, root collar

diameter, root biomass and number of leaves of each seedling were measured. The shoot and root biomass were measured after drying in oven at 50°C for 48 hours.

2.9 Nutrients assimilation

5 g dried root and shoot samples were digested with potassium sulphate and copper sulphate (5:1) + triple acid (nitric + sulphuric + perchloric (9:3:1) in a Kjeltec digestion system at 420°C for one hour. After that the digested samples were analyzed for the major nutrients N, P and K assimilation according to Jackson (1973).

2.10 Transplantation of seedlings

The rest of *S. macrophylla* seedlings with PGPM (remaining 5 replicates) of each treatment were transplanted in barren laterite lands in 120 cm deep planting spots following the square pattern (3 x 3 m) at Karmanthodi, Bovikanam (taluk) Kasargode, Kerala India. The initial height, root collar diameter and number of leaves were measured after planting. The same growth parameters were measured 12 months after transplanting in barren laterite lands.

2.11 Assessment of survival (%)

Survival (%) was calculated as follows for each treatment:

$$Survival(\%) = \frac{\text{Number of survival seedlings of } S.macrophylla \text{ in barren laterite land}}{\text{Total number of } S.macrophylla \text{ seedlings transplanted}} \times 100$$

2.12 Statistical analysis

All the growth parameter drawn from nursery and field experiments were analyzed for ANOVA by Duncan's Multiple Range Test using SPSS ver. 16.

3 Results

3.1 Soil analysis

The physico-chemical properties of the laterite soils showed that the soil is acidic and low in major nutrients (Table 1).

Table 1. Physico chemical properties of laterite soils (+ SE of mean).

	pH	E.C (ds m^{-1})	Bulk density (g cm^{-3})	Organic C (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
Laterite soils	6.3 (±0.02)	0.12 (±0.012)	1.4 (±0.22)	0.87 (±0.04)	135.9 (±2.3)	19.7 (±1.8)	0.0

3.2 Nursery experiments

Overall the results of this study showed the compared seedlings of *S. macrophylla* inoculated with PGPM, improved the growth and biomass than the uninoculated control seedlings (Table. 2).

Table 2. Growth and biomass of *S. macrophylla* inoculated with PGPM (mean of 5 replicates).

Treatments	Seedling height (cm)	Root length (cm)	No.of. leaves plant^{-1}	Root collar diameter (cm)	Seedling Biomass (g plant^{-1})		R/S ratio
					Root	Shoot	
Control	15.3^{a}	12.7^{a}	6.4^{a}	0.74^{a}	1.8^{a}	2.2^{a}	0.81^{d}
AM fungi	18.4^{b}	13.9^{b}	8.7^{bc}	0.96^{c}	2.8^{b}	4.3^{b}	0.65^{c}
A. brasilense	18.8^{b}	14.1^{b}	7.3^{b}	0.89^{b}	2.4^{b}	4.2^{b}	0.57^{b}
B. megaterium	18.5^{b}	13.0^{a}	7.2^{b}	0.85^{b}	2.2^{b}	4.1^{b}	0.53^{b}
AM fungi + *A. brasilense*	32.6^{c}	14.4^{b}	10.1^{c}	1.2^{c}	3.0^{c}	5.5^{c}	0.54^{b}
AM fungi + *B. megaterium*	31.6c^{c}	14.2^{b}	10.5^{c}	1.6^{c}	3.3^{c}	5.2^{c}	0.57^{b}
AM fungi + *A. brasilense* + *B. megaterium*	45.2^{d}	25.8^{c}	16.8^{d}	2.8^{d}	4.6^{d}	10.2^{d}	0.45^{a}

Means followed by same letter(s) are not significantly different according to Duncan's multiple range test (P<0.05).

The dual PGPM (AM fungi + *A. brasilense,* AM fungi + *B. megaterium*) inoculations were significantly (p<0.05) improved the growth and biomass than individual PGPM inoculations. The combinations of all PGPM (AM fungi + *A. brasilense* + *B. megaterium*) significantly (p<0.05) improved shoot length (45.2 cm), root length (25.8 cm), stem girth (2.8 cm), number of leaves (16.8 plant^{-1}) and seedling biomass (shoot: 10.2 g plant^{-1}; root 4.6 g plant^{-1}). Significant lower root to shoot ratio (0.45) was found in the combined PGPM inoculated seedlings than other treatments.

3.3 Nutrients assimilation

The PGPM (AM fungi + *A. brasilense* + *B. megaterium*) inoculated *S. macrophylla* seedlings had significantly increased (p<0.05) N, P and K assimilation than compared to control and other treatments (Figure 2).

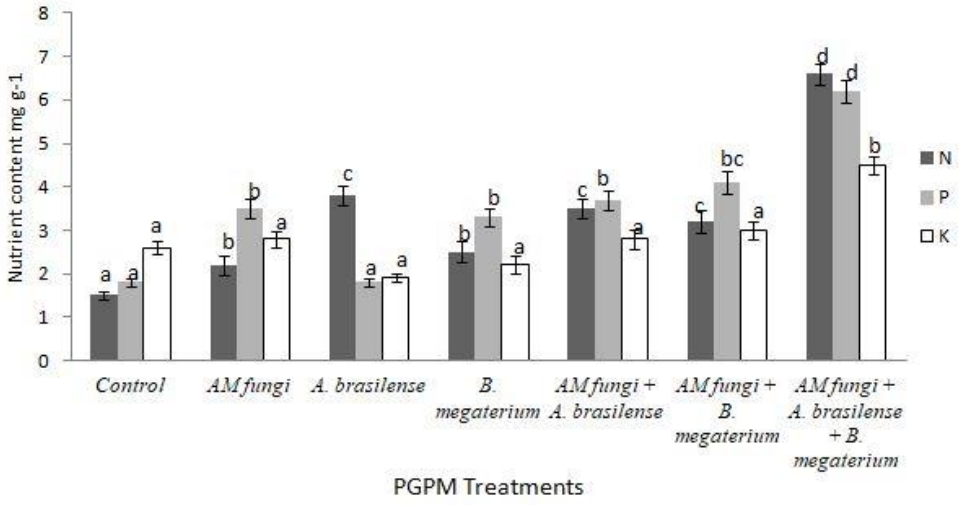

Figure 2. Nutrient content of *S. macrophylla* seedlings inoculated with PGPM (mean of 5 replicates).

3.4 Field experiments

The PGPM inoculated seedlings of *S. macrophylla* showed significant ($p<0.05$) growth improvement at barren laterite lands 3 months after planting (Table 3). Twelve months after planting the combination of PGPM (AM fungi + *A. brasilense*+ *B. megaterium*) significantly improved the height (190.4 cm), stem girth (5.22 cm) and number of leaves (36.8 plant^{-1}) (Table 3).

Table 3. Growth performance of *S. macrophylla* seedlings inoculated with PGPM at barren laterite lands (mean of 5 replicates).

Treatments	Height (cm)	Root collar diameter(cm)	No. of leaves plant^{-1}
3 months after planting			
Control	15.5^{a}	0.76^{a}	7.5^{a}
AM fungi	19.0^{b}	0.98^{c}	9.0^{bc}
A. brasilense	18.5^{b}	0.90^{b}	7.8^{b}
B. megaterium	18.4^{b}	0.88^{b}	7.6
AM fungi + *A. brasilense*	32.1^{c}	1.30^{c}	12.4^{c}
AM fungi + *B. megaterium*	32.4^{c}	1.70^{c}	12.7^{c}
AM fungi + *A. brasilense* + *B. megaterium*	47.2^{d}	3.10^{d}	17.2^{d}
12 months after planting			
Control	55.2^{a}	1.82^{a}	9.2^{a}
AM fungi	82.7^{b}	2.028^{c}	12.4^{bc}
A. brasilense	74.5^{b}	2.05^{b}	10.6^{b}
B. megaterium	73.8^{b}	2.01^{b}	10.8^{b}
AM fungi + *A. brasilense*	128.4^{c}	2.84^{c}	20.5^{c}
AM fungi + *B. megaterium*	127.6^{c}	2.78^{c}	21.6^{c}
AM fungi + *A. brasilense* + *B. megaterium*	190.4^{d}	5.22^{d}	36.8^{d}

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P<0.05$)

3.5 Survival performance

The individual and dual PGPM inoculated seedlings of *S. macrophylla* showed 75 to 86 % survival rate whereas the combined PGPM (AM fungi + *A. brasilense* + *B. megaterium*) showed 98 % survival (Figure 3).

Results of this study shows afforestation success with *S. macrophylla* be improved in barren laterite lands with use of PGPM (Figures 3, 4, 5 and 6).

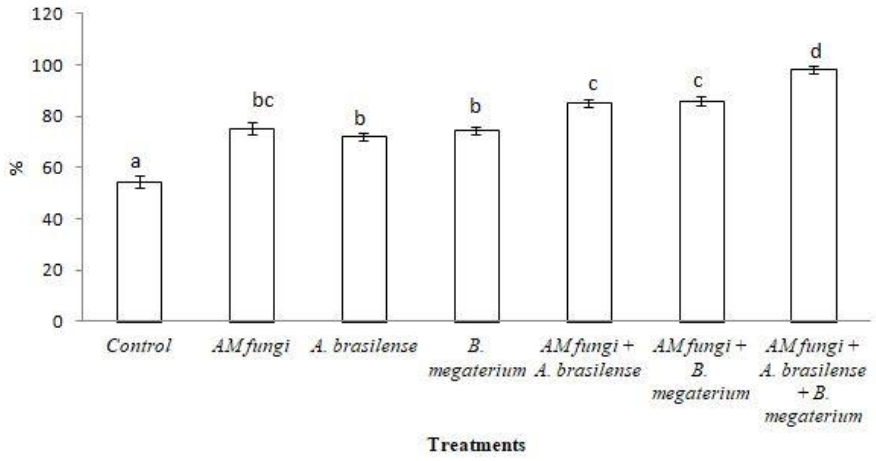

Figure 3. Survival (%) of *S. macrophylla* transplanted in barren laterite lands.

Figure 4. Barren laterite lands before planting.

Figure 5. Barren laterite lands after planting with *S. macrophylla* seedlings and PGPM.

Figure 6. A single *S. macrophylla* seedlings inoculated with PGPM at laterite land.

4 Discussion

Though the laterite soils are poor in nutrients due to absence of organic matter they were used as potting media for growing *S. macrophylla* seedlings in nursery. This method helped for conditioning of seedlings and their easier establishment at barren and rocky laterite lands. The same technique was used earlier by Karthikeyan et al. (2009) for reclamation of bauxite mined out areas. The nursery experiment showed that the PGPM inoculated seedlings improved the growth and biomass of *S. macrophylla* seedlings. These PGPM mobilized the phosphorus from soil and fix the atmospheric nitrogen in the seedlings that helped to improve the growth of seedlings compared to control. Similar results were found in many tree crops such as *Azadirachta indica* (Muthukumar et al. 2001), *Acacia auriculiformis* and *A. mangium (*Diouf et al. 2005), *A. holosericia* (Dupponnois et al. 2007), *Casuarina equisetifolia* (Muthukumar and Udaiyan 2010), *Bruguiera sexangula* (Karthikeyan and Sivapriya 2018) and *Pterocarpus santalinus* (Karthikeyan and Arunprasad 2019). Combined inoculation of PGPM gives better results than single and dual inoculations of PGPM, as shown in this study, because combined inoculation always promotes the plant growth in multiple ways particularly P and N uptake (Muthukumar and Udaiyan 2018). Number of leaves in *S. macrophylla* was increased in the PGPM inoculated seedlings possibly due to the effect of AM fungi, as it is reported the earlier studies of *Tectona grandis* (Rajan et al. 2000) and *Macadamia tetraphylla* (Yeoyoungwech et al. 2013). *S. macrophylla* seedlings inoculated with combined PGPM showed a decreased root to shoot ratio. It may be due to improvement of above ground production and decrement of below ground production through nutrient transfer of PGPM (Smith and Smith 2012). Improved tissue nutrients assimilation is recorded in PGPM inoculated seedlings of *S. macrophylla* compared to control seedlings. The increased accumulation of nutrients showed as results of nutrient transfer attributed by the combined effect of PGPM. AM fungi and *B. megaterium* significantly improved the P content where as *A. brasilense* improved the N content (Khan et al. 2014; Muthukumar and Udaiyan 2018; Karthikeyan and Arunprasad 2019).

The increased K status in *S. macrophylla s*eedlings inoculated with PGPM showed that K was absorbed by the seedling from decomposed soil K by PGPM (Meena et al. 2014).

In the field experiment at laterite lands the growth and survival performance of PGPM inoculated *S. macrophylla* seedlings were higher than uninoculated control seedlings and the seedlings were well established. The seedlings of *S. macrophylla* were planted with improved height and biomass due to inoculated PGPM that is very much required to early and successful establishment in the field. This is the reason that *S. macrophylla* survives in low nutrient laterite lands along with PGPM. Similar successful studies were carried out in *Casuarina equisetifolia* and *Eucalyptus tereticornis* planted at bauxite mine spoils (Karthikeyan et al. 2009; Karthikeyan and Krishnakumar 2012). The improved survival rate at laterite land is due to nutrient uptake of N and P through PGPM. Further the significant growth increase of *S. macrophylla* seedlings with PGPM may be due to increased microbial population and activity in the soil. The infection and colonization of PGPM increased the growth and survival also due to decreasing the nutrient deficiency and water stress (Joner and Layval 2001; Sanchaz-DinzHonrubia 1994) particularly phosphate solubilizing bacteria playing a major role in supply of P to *S. macrophylla* seedlings at field conditions (Chen et al. 2000). These effects of PGPM have improved the growth and survival of *S. macrophylla* in the barren and hardy laterite lands.

5 Conclusion

The results of this study draw a conclusion that establishing plants like *S. macrophylla* in barren laterite lands inoculated with PGPM is essential for successful afforestation. The PGPM have the potential to increase the efficiency of plant growth system through supply of essential levels of N, P and K. This study showed that barren waste lands like laterite lands can be converted to productive lands with useful trees/plants with inoculation of PGPM.

6 Acknowledgment

The author thanks Department of Forests & Wildlife, Government of Kerala, India for financial support in form of research project (No IFGTB- KFD2) for this study. Thanks also given to Indian Council of Forestry Research and Education, Dehra Dun, India for necessary support and facilities to conduct experiments under this study.

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