Establishment of *Gmelina arborea* plantation in an uncultivated farmland inoculated with arbuscular mycorrhizal fungi and plant growth promoting bacteria

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Abstract

Beneficial microbes are very essential for establishing tree plantations, particularly in wastelands or abandoned lands that make them suitable for cultivation. To establish a plantation in uncultivated farmland *Gmelina arborea* Roxb. tree seedlings were previously inoculated with the beneficial microbes (Arbuscular mycorrhizal (AM) fungi - *Gigaspora albida, Glomus aggregatum*), plant growth promoting rhizo bacteria (PGPRs) - *Azospirllum brasilense*, *Bacillus megaterium*) in single and combinations. The effect of these beneficial microbes were analysed and it was found that the seedlings inoculated with AM fungi + $PGPRs_1$+ $PGPRs_2$ have improved in growth and biomass (shoot length: 85.3 cm $plant^{-1}$; root length: 40.2 cm $plant^{-1}$; number of leaves: 42.3 $plant^{-1}$; stem girth: 9.5 mm $plant^{-1}$; shoot dry weight: 8.89 g $plant^{-1}$; root dry weight: 1.81 g $plant^{-1}$) than the control and individual microbe inocuations. The tissue nutrients (N: 9.1 mg g^{-1}; P: 9.5 mg g^{-1}; K: 6.1 mg g^{-1}) found higher particularly in AM fungi + PGPRs inoculated seedlings. Thus, improved seedlings were transplanted in an uncultivated farmland and monitored for growth and survival. The seedlings planted in the uncultivated farmland showed significant growth improvement. The single (AM fungi, $PGPRs_1$, $PGPRs_2$) and dual (AM fungi + $PGPRs_1$/$PGPRs_2$) inoculated seedlings of *G. arborea* showed 75 to 87% survival whereas the combined treatment (AM fungi + $PGPRs_1$ + $PGPRs_2$) showed 96% survival in farmland. These results confirmed that these beneficial microbes significantly contributed to the establishment of *G. arborea* seedlings in the uncultivated farmland through the transfer of nutrients.

Keywords

Mycorrhiza; *Gigaspora albida*; *Glomus aggregatum*; *Azospirillum*; *Bacillus*; White teak

Contents

1 Introduction

The development of forest tree plantations for industrial purpose is considerably increasing over the last period (Mc Ewan et al. 2020). The plantations are mainly developed for paper and pulp, plywood, furniture wood and wooden handicrafts. Normally, the plantations are created with short rotation trees like Casuarina and Eucalyptus, however, the fast-growing long rotation forest trees like *Tectona grandis* L.f (Teak) also receive much attention due to their high economic value. *Gmelina arborea* Roxb. is as equal as *T. grandis* in terms of wood value. *Gmelina arborea* is a medium sized deciduous tree and also called as white teak. This tree is naturally distributed in tropical and sub tropical regions of Asia particularly in Indonesia, Myanmar, Thailand, Indonesia, Philippines and Sri Lanka (Jensen 2005). In India, this tree is widely distributed in Kerala and Andaman Nicobar Islands. Also, *G. arborea* is cultivated in many parts of Southern India for wood. Further, *G. arborea* leaves are used for fodder and the roots are used in Indian medicine for promoting digestion (Kala 2005), whereas the stem and bark are used as antidiarrheal (Warrier et al. 2021). It is very good for making plywood, pulp and paper (Wang 2004). This species can survive well at altitudes up to 1300 m a.s.l with a range of 750-5000 mm of rainfall (Lauridsen 2004). *Gmelina arborea* is usually propagated by seeds in nurseries, however, the application and effect of beneficial microbes on seedlings are not known. Arbuscular mycorrhizal (AM) fungi are one of the symbiotic beneficial microbes (Giovannini et al. 2020) associated with angiosperms that was used in this study for their effect in *G. arborea* seedlings. Barua et al. (2010), reported the effects of AM fungi on growth improvement of *G. arborea* in arsenic soils. However, the other beneficial microbes such as plant growth promoting rhizobacteria (PGPRs) (Karthikeyan 2020) on the growth improvement in *G. arborea* also are not known yet. Hence, the aims of this study were to test the effect of individual and combined application of these beneficial microbes (AM fungi, PGPRs) to *G. arborea* seedlings at nursery and field conditions in order to improve the growth and biomass of this valuable species. The results of this study may help in selection of the suitable AM fungi and PGPRs in *G. arborea* nursery to produce quality seedlings with better performance.

2 Material and methods

2.1 Study site and physical and chemical properties of soil

An uncultivated farm land was selected at Dharmapuri, Tamilnadu, India (12.0898°N, 78.4061°E) for establishment of *G. arborea* plantation with inoculations of AM fungi + PGPR. Priorly the physical and chemical properties of the soil of land determined according to Jackson (1973). The soil of the land is acidic (pH. 6.6±0.1) and red on color. The electrical conductivity is 0.12 (±0.01), nitrogen (N) 15.3 (±0.6) mg kg^{-1}, phosphorous 13.5 (±0.5) mg kg^{-1} and potassium 27.2 ± 1.2) mg kg^{-1}. The average temperature of the study site is 28.81°C and receives rain fall about 118 (mm) in a year.

2.1.1 Isolation and multiplication of AM fungi

The AM fungi *Gigaspora albida* N.C Schenck & G.S. Sm (Figure 1A) and *Glomus aggregatum* N.C. Schenck & G.S Sm (Figure 1B) were isolated by the method of Gerdemann and Nicolson (1963) from the rhizosphere soils of *G. arborea* and identified according to the Schenck and Perez (1990) identification manual. These AM fungi were multiplied in sterile media pot cultures (alfisoil and sand; 1;1) with *Zea mays* L as host species under green house conditions (mean relative humidity of 60 %; mean temperature 28°C) for four months. After four months the multiplied AM fungal spores were stored in vermiculite media along with *Z. mays* root for inoculation.

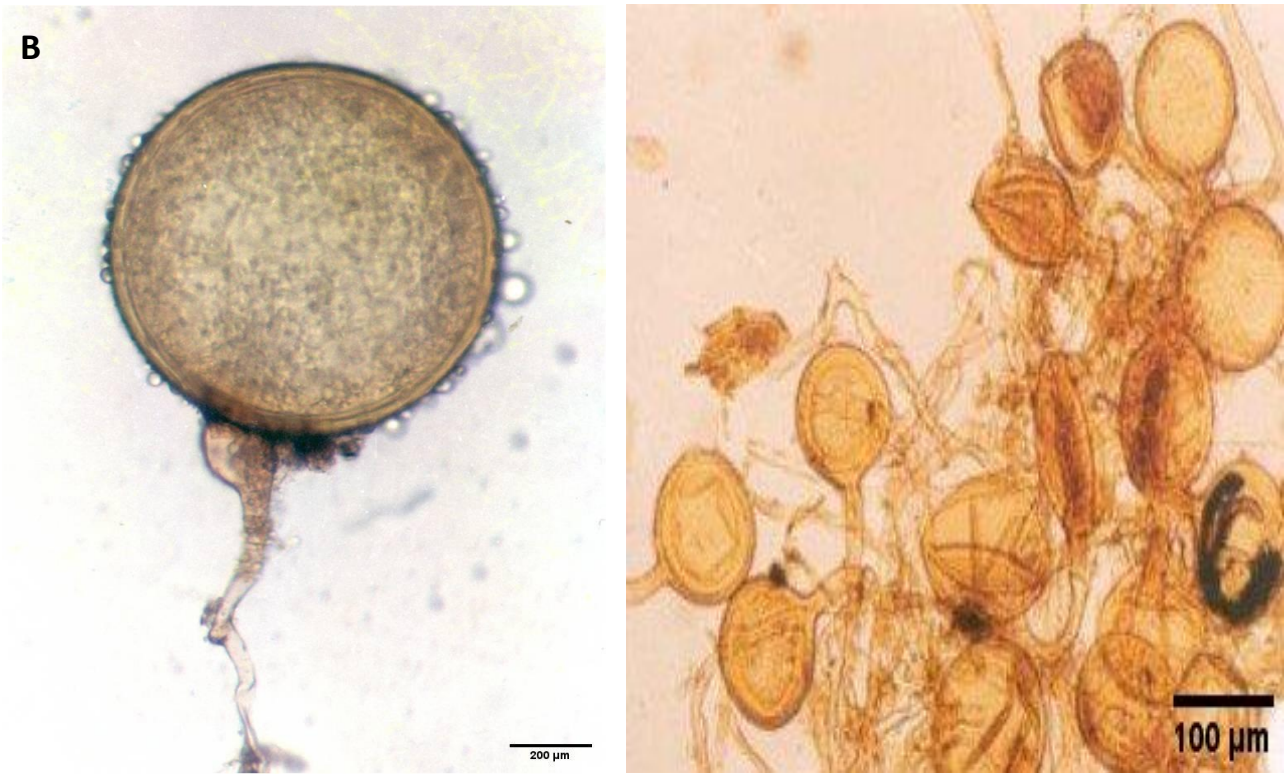


Figure 1. A (left) - Chlamydospore of *Gigaspora albida* showing three wall layers and bulbous suspensor and B (right) - Aggregated Chlamydopsores of *Glomus aggregatum*.

2.2 Culture of PGPRs

Phosphobacteria one of the PGPRs was isolated from the 0.1 ml of rhizosphere soil of *G. arborea* in sterile water (10^{-6}) on the Pikovaskya medium (Wollum 1982) petriplates contains 10 g of $C_{12}H_{22}O_{11}$, 5.0 g of $Ca_3(PO_4)$, 0.27 g of NH_4NO_3, 0.2 g of KCl, 0.1 g of $MgSO_4$ x $7H_2O$, 1.0 mg of $MnSO_4$ x $6H_2O$, 1.0 mg of $FeSO_4$ x 7 H_2O, 0.1 g of Yeast extract and 15 g of agar per liter of distilled water. These petriplates were incubated at 34°C for 6 days. The colony in Pikovaskya medium was identified as *Bacillus megaterium* phosphate solubilizing bacteria which formed halo zone around the white bacterial colonies (Figure 2A). The bacteria was again stained by gram staining method (Coico 2005) to find the positive or negative. The endospores of *B. megaterium* were stained according to Schaeffer Fultron method (Volk and Wheeler 1988) by using malachite green. The techniques of these staining methods confirmed that *B. megaterium* is gram positive as showed violet crystal color and malachite green stained endospores showed two membranes. Because of these characters confirmed that the PGPRs is *B. megaterium* (Andriani et al. 2017).

The another PGPR, nitrogen fixing bacteria *Azospirillum brasilense* was also form the same rhizosphere soils of *G. arborea* through Congo red medium contains gL^{-1} of distilled water KH_2PO_4 - 5g; $MgSO_4$ x $7H_2O$ - 0.2 g; NaCl - 0.1 g; incentive excerpt - 0.5 g; $FeCl_3$ x $6H_20$ - 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. This medium was used for multiplying the *A. brasilense* in petriplates by placing 0.1 ml of soil adulterated in sterile water (10^{-5}) and incubated at 36°C for seven days. After seven days of incubation, pink and scarlet colonies (Figure 2B) of the bacteria was observed and confirmed as *A. brasilense* (Rodrígues-Cáceres 1982). These cultures were also tested for gram staining that showed helical shaped with single flagellum and gram negative (Sung and New 1998). The endospores were showed double membrane due to staining of malachite green.

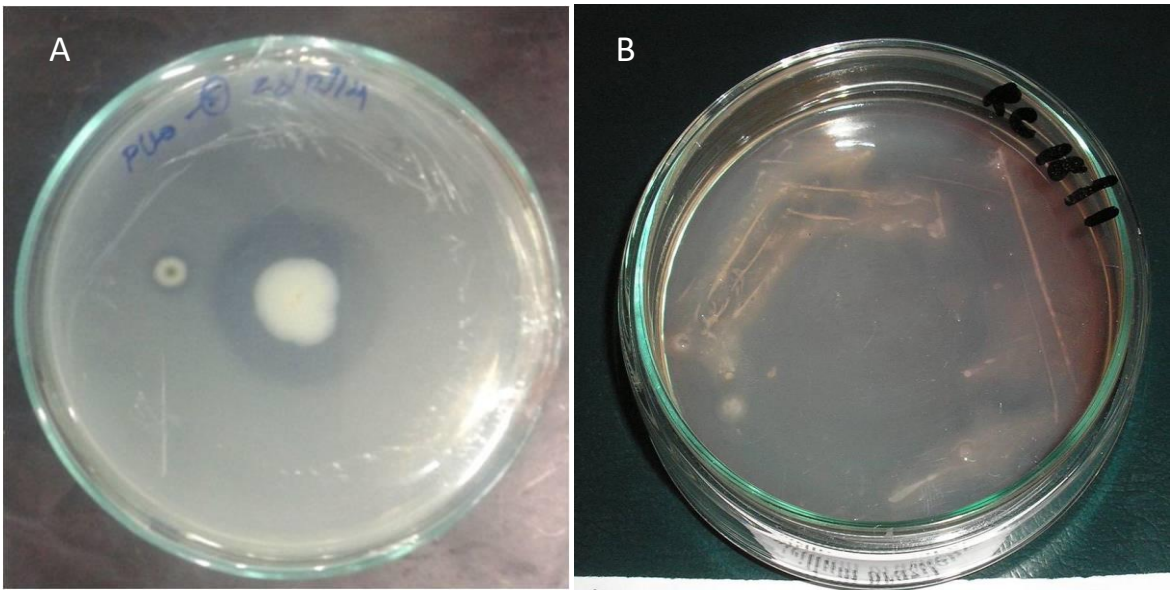


Figure 2. A (left) - Chlamydospore *Bacillus megaterium* : Bacterial colonies showing halozone in Pikovaskya medium after 6 days of incubation at 34°C and B (right) - *Azospirllum brasilense* showing scarlet colonies in Congo red medium after 7 days of incubation at 36°C.

2.3 Seed propagation of *G. arborea*

Dried *G. arborea* seeds were soaked in cold water for 24 hours for speedy germination. Thus pre treated seeds were sown in the germination beds containing pure sand. The seeds were found germinated after 25 to 30 days after sowing. Later the seedlings with size of 7 cm length were transferred to polythene bags containing alfi soils (red soil) having the size of 14 x 27 cm (v. 4.15 L).

2.4 Inoculation of AM fungi and PGPRs

The inoculation of AM fungi and PGPRs in to the seedlings of *G. arborea* was carried out as follows. The combined AM fungi *G. albida* and *G. aggregatum* multiplied as pot cultures with *Z. mays* plants were applied at the rate of 20 g per polythene bag at 5 cm below the soil surface of each polythene bag. The PGPRs of *A*. *brasilense* ($PGPRs_1$: 5 x 10^7cfu g^{-1}) and *B. megaterium* ($PGPR_2$: 2 x10^5cfu g^{-1}) were inoculated individually and in combinations by applying at the rate of 5 ml of each per seedling. A control treatment without AM fungi and PGPRs inoculations was also maintained. Thus a total of 7 treatments with 10 replicates of contain 5 seedlings were designed for the nursery experiment as mentioned in the table below:

1. Control (0)
2. AM fungi (20g)
3. PGPRs1 (5 ml)
4. PGPRs2 (5 ml)
5. AM fungi (20g) + PGPRs1 (5 ml)
6. AM fungi (20g) + PGPRs2 (5 ml)
7. AM fungi (20g) + $PGPRs_1$ (5 ml) + $PGPRs_2$ (5 ml)

Totally 350 seedlings were placed in a randomized block design for six months under green house conditions maintained in the temperature of 26.2°C (±1.2) and relative humidity 65.4 (±1.1). Proper irrigation was provided to the seedlings for their necessary survival.

2.5 Growth measurement of *G. arborea* seedlings from nursery

Six months after inoculation, the *G. arborea* seedlings from five replicates were harvested without disturbing shoot and roots and measured for root length, shoot length, root collar diameter, root biomass and number of leaves. After the growth measurement the seedlings were dried in oven at 50°C for 48 hours to measure the biomass. The tissue nutrient contents of the seedlings were also carried out by digestion of 5g dried root and shoot with potassium sulphate and copper sulphate (5:1) + triple acid (nitric + sulphuric + perchloric) at the ratio of 9:3:1 in a Kjeltec equipment at 420°C for one hour. These digested *G. arborea* samples were analysed the tissue nutrient contents of Nitrogen (N), Phosphorus (P) and Potassium (K) (Jackson 1973).

2.6 Seedling quality index

Seedling quality index (SQI) of AM fungi and PGPRs inoculated *G. arborea* seedlings was calculated using the formula of Dickson et al. (1960):

$$SQI = \frac{\text{total dry weight (g)}}{\frac{\text{height (cm)}}{\text{root collar diameter (mm)}} + \frac{\text{shoot dry weight (g)}}{\text{root dry weight (g)}}}$$

2.7 Microbial inoculation effect

Microbial inoculation effect (MIE) was assessed in *G. arborea* seedlings inoculated with AM fungi + PGPRs using the formula of Bagyaraj (1992):

$$MIE = \frac{\text{Dry weight of inoculated}*\text{seedlings} - \text{Mean dry weight of inoculated}**\text{seedlings}}{\text{Dry weight of inoculated}*\text{seedlings}} \times 100$$

*AM fungi and PGPRs; **Control

2.8 Field evaluation

Rest of other five replicates of each treatment was planted in a uncultivated farmland located at Dharmapuri, Tamilnadu, India at 60 cm deep pits with an escapement of 3 x 3 m. This land was uncultivated for the past 5 years however it was naturally vegetated with grasses and small herbs. The height, root collar diameter and number of leaves were measured after six months of planting. The same growth parameters were measured twelve months after planting grown in this land. The survival (%) was assessed according to the following formula:

$$\text{Survival (\%)} = \frac{\text{Number of seedlings survived}}{\text{Total number of seedlings planted}} \times 100$$

2.9 Statistical analyses of data

Duncan's Multiple Range Test (DMRT) was executed for ANOVA analysis of all the growth parameters of nursery and field experiments using SPSS ver. 17.

3 Results

3.1 Growth response of *G. arborea* seedlings to AM fungi and PGPRs

The inoculation of AM fungi and PGPRs in *G. arborea* seedlings showed that the seedlings improved in terms of growth and biomass (Table 1). Single inoculations (AM fungi, $PGPRs_1$, $PGPRs_2$) showed significant higher growth improvement that the control seedlings. Dual inoculations of AM fungi + $PGPRs_1$/$PGPRs_2$ showed significant ($p<0.05$) improvement in stem girth than the single (AM fungi, $PGPRs_1$, $PGPRs_2$) and control seedlings. The combined inoculations of AM fungi + $PGPRs_1$ + $PGPRs_2$ result significant improvement in shoot length (85.3 cm plant^{-1}), root length (40.2 cm plant^{-1}), number of leaves (42.3 plant^{-1}), stem girth (9.5 mm plant^{-1}) and biomass (shoot 8.89 g plant^{-1}, root 1.81 g plant^{-1}). Significant lower root to shoot ratio (0.20) also found in this treatment (Table 1). Significant higher accumulation of nutrients (N: 9.1 mg g^{-1}; P: 9.5 mg g^{-1}; K: 6.1 mg g^{-1}) in AM fungi + $PGPR_1$ + $PGPR_2$ inoculated seedlings (Figure 3).

Table 1. Growth and biomass of *G. arborea* inoculated with AM fungi + PGPRs in nursery (mean of 5 replicates).

Treatments	Shoot length (cm)	Root length (cm)	No.of leaves plant^{-1}	Stem girth (mm)	Seedling Biomass (g plant^{-1})		R/S ratio
					Root	Shoot	
Control	38.1^{a}(±1.2)	18.2^{a}(±0.9)	16.7^{a}(±1.2)	5.8^{a}(±0.1)	1.22^{a}(±0.01)	4.23^{a}(±0.1)	0.49^{c}(±0.01)
AM fungi	58.2^{b}(±1.1)	25.1^{b}(±1.0)	22.3^{b}(±1.1)	6.5^{b}(±0.1)	1.32^{b}(±0.02)	5.46^{b}(±0.1)	0.24^{b}(±0.01)
PGPR$_1$	60.8^{b}(±1.1)	28.7^{b}(±11)	24.3^{bc}(±1.1)	6.8^{b}(±0.1)	1.35^{b}(±0.02)	5.32^{b}(±0.1)	0.25^{b}(±0.01)
PGPRs$_2$	61.3^{b}(±1.1)	29.6^{c}(±1.1)	24.8^{bc}(±1.2)	7.2^{c}(±0.1)	1.46^{b}(±0.02)	6.44^{c}(±0.2)	0.22^{a}(±0.01)
AM fungi + PGPRs$_1$	72.6^{c}(±1.2)	34.2^{d}(±1.1)	31.2^{c}(±1.1)	7.9^{c}(±0.1)	1.65^{bc}(±0.02)	6.58^{c}(±0.1)	0.25^{b} (±0.01)
AM fungi + PGPRs$_2$	73.8^{c}(±1.1)	35.3^{d}(±1.1)	32.3^{c}(±1.1)	8.1^{cd}(±0.1)	1.63^{bc}(±0.02)	6.88^{c}(±0.1)	0.23^{ab}(±0.01)
AM fungi + PGPRs$_1$ +PGPRs$_2$	85.3^{d}(±1.1)	40.2^{e}(±1.1)	42.3^{d}(±1.1)	9.5^{d}(±0.1)	1.81^{b}(±0.02)	8.89^{d}(±0.2)	0.20^{a}(±0.01)

± SE of the mean
Mean superscripted by the same letter(s) are not significantly different according to Duncan's multiple range test (p<0.05)

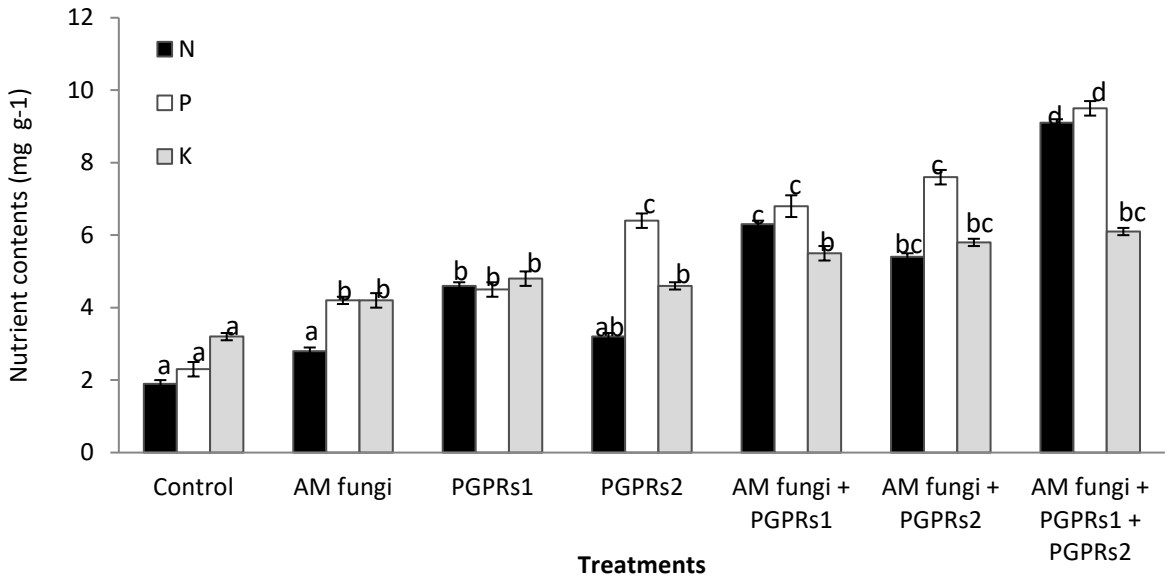


Figure 3. Tissue nutrient contents of *G. arborea* seedlings inoculated with AM fungi and PGPR (mean of 5 replicates).

3.2 Seedling quality index

Inoculation of AM fungi + PGPRs have improved the seedling quality by 24% than the control seedlings. The dual inoculated seedlings got improved by 12% than the control seedlings. However there is no significant quality improvement in individual inoculations (AM fungi, PGPRs$_1$, PGPRs$_2$) (Figure 4).

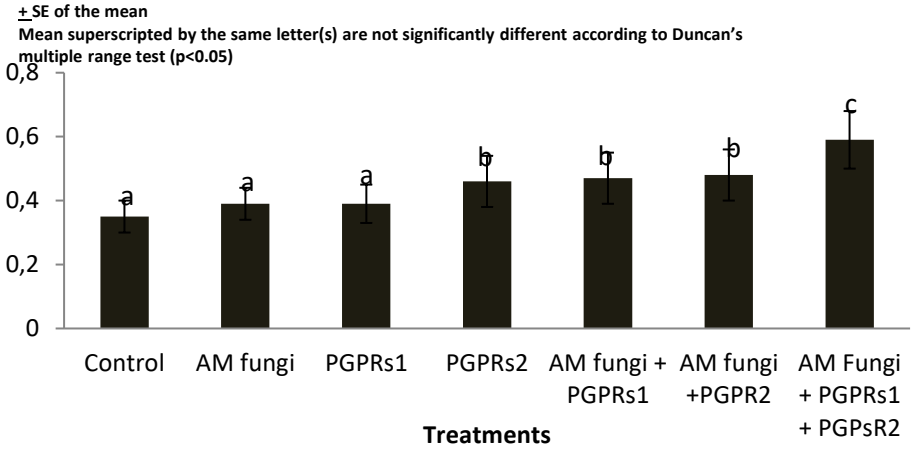


Figure 4. Seedling quality index in *G. arborea* seedlings.

3.3 Microbial inoculation effect (MIE)

More than 96 % MIE was found in AM fungi + $PGPRs_1$ + $PGPRs_2$ treated *G. arborea* seedlings. In the dual inoculated seedlings the MIE more than 50% was found and in single inoculations more than 20% MIE was recorded than the control seedlings (Figure 5).

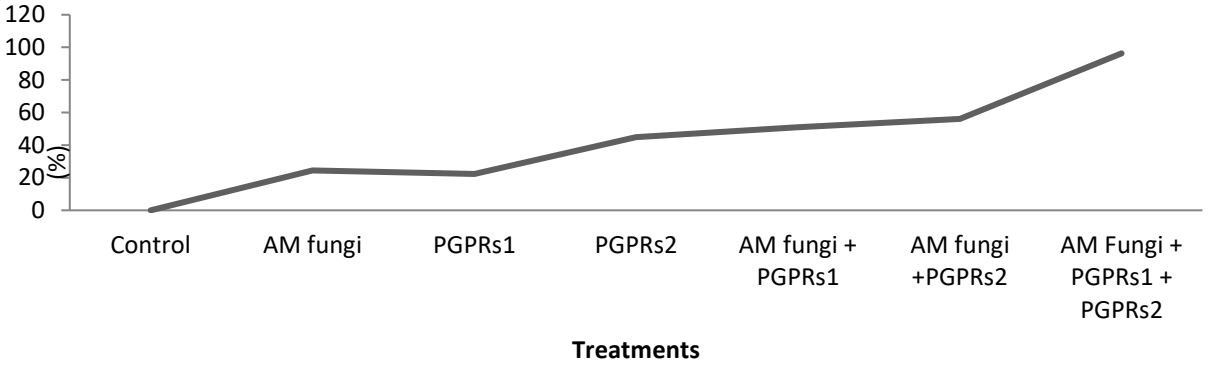


Figure 5. Microbial inoculation effect in *G.arborea* seedlings.

3.4 Growth response of *G. arborea* in uncultivated farmland

The transplanted seedlings of *G. arborea* in farmland with inoculations of AM fungi and PGPRs showed significant ($p<0.05$) growth improvement in terms of height, stem girth and number of leaves in 6 months as well as 12 months after planting than the control seedlings (Table 2). The seedlings showed 12 months after planting with combined inoculations (AM fungi + $PGPRs_1$ + $PGPRs_2$) received significant improvement in height (114.3 plant^{-1}), stem girth (16.6 cm plant^{-1}) and number of leaves (152.6 plant^{-1}). The number of leaves was increased four times higher in this combined treatment than the control seedlings (Figure 6). The single (AM fungi, $PGPR_1$, $PGPR_2$) and dual (AM fungi + $PGPR_1$/$PGPR_2$) inoculated seedlings of *G. arborea* showed 75 to 87% survival whereas the combined treatment (AM fungi + $PGPRs_1$ + $PGPRs_2$) showed 96% survival

in farmland (Figure 7). In overall, the uncultivated land has been afforestated with *G. arborea* and AM fungi + PGPRs (Figure 8).

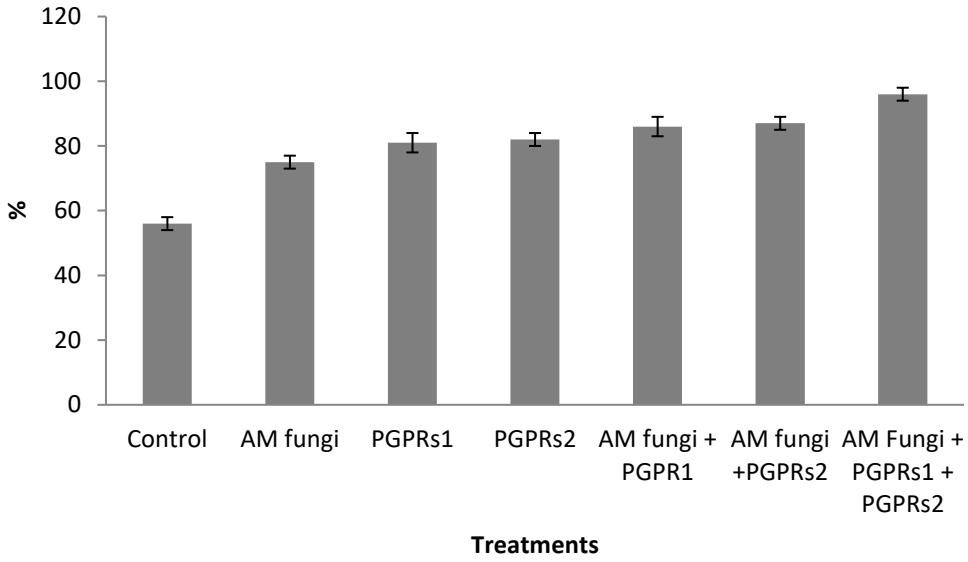
Table 2. Growth performance of *G. arborea* seedlings inoculated with AM fungi + PGPRs in farmland (mean of 5 replicates).

Treatments	Months after planting	Height (cm)	Stem girth (cm)	No. of leaves plant^{-1}
Control	6	42.7^{a} (±1.1)	7.1^{a} (±1.1)	19.8^{a} (±1.0)
	12	62.5 ^{a}(±1.1)	9.8^{a} (±1.1)	40.3^{a}(±1.2)
AM fungi	6	54.4^{ab} (±1.0)	8.5^{ab} (±1.1)	26.3^{b} (±1.2)
	12	73.5^{b} (±1.1)	12.3^{b}(±1.1)	53.2^{b}(±1.2)
PGPRs$_1$	6	60.4^{b} (±1.1)	8.8^{ab} (±1.1)	35.2^{c} (±1.2)
	12	75.5^{b} (±1.1)	13.5^{b} (±1.1)	72.3^{c}(±1.1)
PGPRs$_2$	6	62.4^{b} (±1.1)	9.1^{b} (±1.2)	38.4^{d} (±1.1)
	12	77.6^{c} (±1.1)	13.7^{b}(±1.1)	74.2^{c}(±1.1)
AM fungi + PGPRs$_1$	6	65.6^{c} (±1.2)	9.4^{b} (±1.2)	40.3^{de} (±1.1)
	12	81.8^{d} (±1.1)	14.3^{bc}(±1.1)	95.4^{d}(±1.1)
AM fungi + PGPRs$_2$	6	66.6^{c} (±1.2)	9.7^{b} (±1.2)	41.2^{de} (±1.1)
	12	83.7^{d} (±1.0)	14.6^{bc}(±1.0)	98.6^{d}(±1.0)
AM fungi + PGPRs$_1$ + PGPRs$_2$	6	75.8^{d} (±1.2)	10.2^{bc} (±1.2)	54.6^{e} (±1.1)
	12	114.3^{e}(±1.0)	16.6^{c} (±1.0)	152.6^{e}(±1.0)

± SE of the mean

Mean superscripted by the same letter(s) are not significantly different according to Duncan's multiple range test ($p<0.05$)

Figure 6. Un inoculated *G. arborea* plants showing 60 cm in height and less number of leaves (left) and *G. arborea* inoculated with AM fungi + PGPRs showing >114 cm height and more number of leaves (right).



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Mean superscripted by the same letter(s) are not significantly different according to Duncan's multiple range test ($p<0.05$)

Figure 7. Survival of *G. arborea* in farmland.

Figure 8. Established *G. arborea* plantation in an uncultivated farmland inoculated with AM fungi + PGPRs.

4 Discussions

This study explicates the importance of AM fungi and PGPRs in plant growth promotion at uncultivated farmland. Making use of uncultivated or abandoned lands for growing trees helps for productivity and the establishment of green cover (Wardle et al. 2004). Generally the soil organic carbon and essential nutrients for plants are usually lower or absent in waste lands/uncultivated lands (Yang et al. 2018) so it is necessary to introduce some of the beneficial microbes like AM fungi and PGPRs along with selected plants in those lands. Thus introduced these kind of beneficial microbes have a capability to promote the plant growth and enrich the soil nutrients (Vessey 2003) that improve the fertility of the lands.

The nursery experiment from this study showed that the beneficial microbes of AM fungi and PGPRs inoculated *G. arborea* seedlings gained improvement in growth and biomass. The reason behind is these microbes have mobilized the soil nutrient phosphorus from the soil and fixed the atmospheric nitrogen in the seedlings (Karthikeyan 2021). In earlier studies also was proved in *Acacia auriculiformis* (Diouf et al. 2005), *A. holosericia* (Dupponnois et al. 2007), *Casuarina equisetifolia* (Karthikeyan 2016), *Pterocarpus santalinus* (Karthikeyan and Arunprasad 2021) and recently in *Santalum album* (Muthu Kumar et al. 2023). Combined inoculations of AM fungi + $PGPRs_1$ +$PGPRs_2$ showed significantly results on growth and biomass better than single and dual inoculations. In this treatment the increased microbial biomass has promoted the plant growth through phosphorus and nitrogen assimilation (Gunina et al. 2017). The number of leaves in *G. arborea* was increased in AM fungi + PGPRs inoculated seedlings because of AM fungal effect. It was proved in the previous studies on *Tectona grandis* (Rajan et al. 2016), *Macadamia tetraphylla* (Yooyongwech et al. 2013) *Elaeis guineensis* (Ajeng et al. 2020) *Swietenia macrophylla* (Karthikeyan 2020) and *Ailanthus tryphysa* (Karthikeyan 2021). Seedlings of *G. arborea* inoculated with AM fungi + $PGPRs_1$ + $PGPRs_2$ showed a lower root to shoot ratio that confirmed the increment of above ground production and the decrement of below ground production through nutrient transfer (Hetrick 1991; Smith and Smith 2012). Increased microbial inoculation effects in *G.arborea* with AM fungi + PGPRs treatments showed that the plant fully utilised the nutrients through microbes for their growth without any additional fertilizer. Improved seedling quality index also showed improved dry mass that indicates sturdier stem and a equal stem dry weight (Muthukumar and Udaiyan 2008). Tissue nutrient contents were found higher in AM fungi + $PGPRs_1$ + $PGPRs_2$ inoculated *G. arborea* seedlings compared to control seedlings. This is due to the accumulation of nutrients attributed to the combined effect of these microbes (Khan et al. 2014).

Established *G. arborea* seedlings in uncultivated farmland showed improved growth and survival performance due to AM fungi + PGPRs inoculation. Because *G. arborea* seedlings were planted with improved height and biomass that made a successful establishment in those uncultivated lands. Bessadok et al. (2021) reported similar results on establishment of *Anthyilis heroniana* with *Rhizobium* sp. in vegetation poor sites. The improved survival (96%) in the uncultivated land and growth is likely a consequence of nutrients uptake through beneficial AM fungi + PGPRs as they produced organic acids for nutrient solubilization (Joner and Leyval 2001; Burtan et al. 2010; Sharma et al. 2022).

5 Conclusions

From this study, it was understood that AM fungi and PGPRs (*Azsosprillum brasilense and Bacillus megaterium*) are the suitable beneficial microbes for production of quality planting seedlings of *G. arborea* and successful establishment in uncultivated farmlands thereafter. This study concluded that AM fungi and PGPRs have the potential to increase the efficiency of plant growth system through supply of essential levels of major nutrients that made a productive land from uncultivated waste lands.

6 Acknowledgements

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