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## Establishment of *Ailanthus tryphysa* (Dennst.) Alston inoculated with beneficial microbes in barren laterite rocks

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### ABSTRACT

Developing countries including India are using laterite bricks extracted from laterite rocks for building construction. Laterite rocks are found barren usually as they are not fit for cultivation. Extraction of laterite bricks from these barren laterite rocks causing land degradation and posing environmental threats in the laterite excavated lands. Hence, planting or establishing of trees on laterite rocks can prevent land degradation and environmental problems. In this study, it was decided to establish *Ailanthus tryphysa* (Dennst.) Alston a multi-purpose native tree species of India on laterite rocks with suitable beneficial microbes as growth promoters. The laterite soils dug out from laterite rocks were tested and found that the soils have lack of beneficial microbes and poor in major nutrients (N, P, K). Therefore the beneficial microbes, arbuscular mycorrhizal fungi and growth promoting bacteria were used for *A. tryphysa* as they are one of the important soil properties. The laterite soils were also used as potting media for the seedlings of *A. tryphysa* in nursery and thereafter the cultured beneficial microbes were inoculated in to the seedlings and maintained for three months. After three months the seedlings were planted at laterite rocks and monitored for their growth and survival. The results of the experiment showed that beneficial microbes inoculated seedlings improved the growth, biomass, tissue nutrient content and 95% of survival rate after twelve months in laterite rocks. These results confirmed that the beneficial microbes have successfully established the *A. tryphysa* seedlings in laterite rocks through transfer of essential nutrients.

### 1. Introduction

Laterite bricks extracting from laterite rocks are widely used for building construction in developing countries. Laterite rocks are rich in Iron, Aluminium and Manganese oxides. Laterite soils accumulated in laterite rocks are considered as very good alternative building material for river sand (Akinyemi et al., 2020) They are hardened soil that turns into solid laterite rocks hence it is being used for building construction. However, they are not suitable for developing any vegetation or planting tree species due to poor nutrients availability and hardy in nature (Karthikeyan, 2020). To establish vegetation in this kind of hardy and nutrient poor sites the beneficial microbes are the only eco friendly solution. The beneficial microbes are capable to interact with plant roots and improve their plant growth and soil fertility. Among beneficial microbes the bacteria such as *Azospirillum*, *Bacillus*, *Pseudomonas* and *Enterobacter* genera are generally used for plant growth improvement as they secrete growth promoting hormones such as gibberellin, Indole acetic acid and cytokinin (Saad et al. 2020). The another prime groups of beneficial microbes are arbuscular mycorrhizal fungi which are

symbiotically associated with mostly flowering plants and helps the plants to uptake phosphorous from soil. So, the main role of these beneficial microbes are enhancing the availability of minerals through solubilization, chelation and mineralization (Rouphael and Colla, 2020). They are also good protectors of plants against soil pathogens (Liu et al., 2019) and they have the ability to improve the plant tolerance against biotic and abiotic stress (Koskey et al., 2021). However, it is essential to inoculate the beneficial microbes along with plants in the barren sites like laterite rocks where there is no vegetation as well as micro flora. The inoculation of beneficial microbes in to the plant rhizosphere or roots is a prerequisite for successful establishment in the problematic or hardy lands (Karthikeyan et al., 2009). Several previous studies have showed the positive effect of beneficial microbes on plant growth through nutrient transfer of different trees in problematic sites like mined out overburdens (Diagne et al., 2013; Karthikeyan and Krishnakumar, 2012). As the nutrients are very essential to plants for establishment in any hard sites like laterite rocks it was decided in this study to use the beneficial microbes for successful development of vegetation. The beneficial microbes such as arbuscular mycorrhizal

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(AM) fungi (*Glomus fasciculatum* and *Glomus geosporum*), nitrogen fixing bacteria (*Azospirillum brasilense*) and phosphate solubilizing bacteria (*Bacillus megaterium*) were attempted in laterite rocks at Kasargode District of Kerala, India along with *Ailanthus tryphisa* (Dennst.) Alston a native tree species of India. This kind of a study is lacking in *A. tryphisa* and the application of beneficial microbes in *A. tryphisa* seedlings will improve the growth and biomass in nursery and field conditions.

*A. tryphisa* is a medium sized evergreen tree having multipurpose uses. It mostly occurs in Eastern countries and Australia however it is naturally distributed abundantly in the Western Ghats of India up to 1500 m a.s.l (Shujauddin and Kumar, 2003). It is also a plantation species in India due to it uses in plywood, matchwood and packingcase industries (Kumar et al., 2001). A resinous gum produced from this tree is largely used in perfume industry. Because of these qualities and nativity this tree was selected for establishment in barren laterite rocks. This study helps to produce quality seedlings of *A. tryphisa* and vegetate barren sites like laterite rocks that can be converted in to productive sites.

## 2. Materials and methods

### 2.1. Study site

This study was carried out at barren laterite rocks in the state of Kerala, India located at 12 °31'48.93"N; 75 °09'28.93"E.

#### 2.1.1. Soil analysis

The pH, electric conductivity, organic carbon, available nitrogen, phosphorus, and potassium of dug out laterite soils from laterite rocks were analyzed according to Jackson (1973).

### 2.2. Isolation and culture of arbuscular mycorrhizal fungi (AM)

The beneficial microbe AM fungi were isolated from the rhizosphere soils of *Crotalaria juncea* L. found grown naturally adjacent to the barren laterite rocks by the method of Gerdemann and Nicolson, (1963). The soils were analyzed for AM propagules by most probable number method (Porter, 1979) and found 5.5 ( $\pm 0.21$ ) infective propagules (spores, hypha). Later, the AM fungal spores were identified as *Glomus fasciculatum* (Gerd & Trappe) emend., Walker & Koske (Fig. 1 a) and *Glomus geosporum* (Nicol & Gerd), Gerd & Trappe.(Fig. 1b) with the help of Schenck and Perez (1990) identification manual. These AM fungi were grown for increasing the infective propagules in sterile media pot cultures (alfisoiil and sand; 1:1) with *Zea mays* L as host species under green house conditions (mean relative humidity of 65%; mean temperature 25°C) for three months.

#### 2.2.1. Culture of other beneficial microbes

The other beneficial microbes were also isolated from the laterite soils and multiplied for inoculation in *A. tryphisa* seedlings as follows. 0.1 ml of sieved laterite soil in sterile water ( $10^{-6}$ ) was spread on the Pikovaskya medium petriplates contains 10 g of  $C_{12}H_{22}O_{11}$ , 5.0 g of  $Ca_3(PO_4)_2$ , 0.27 g of  $NH_4NO_3$ , 0.2 g of KCl, 0.1 g of  $MgSO_4 \cdot 7H_2O$ , 1.0 mg of  $MnSO_4 \cdot 6H_2O$ , 1.0 mg of  $FeSO_4 \cdot 0.7H_2O$ , 0.1 g of Yeast extract and 15 g of agar. The petriplates were incubated at 34 °C for 6 days. The colony in Pikovaskya medium was identified as *Bacillus megaterium* a phosphate solubilizing bacteria as formed halo zone around the white bacterial colonies (Fig. 2a) (Wollum, 1982). The cultures were tested by gram staining (Coico, 2005) for positive and negative reaction. The endospores were stained by the method of Schaeffer Fultron (Volk and Wheeler, 1988) using malachite green. These micro techniques showed that the bacteria is gram positive as stained by violet crystal and stained endospores with malachite green showed the bacteria have two membranes. These characters were noticed in the research Microscope (1000x) and confirmed that the culture is *B. megaterium* (Andriani et al., 2017).

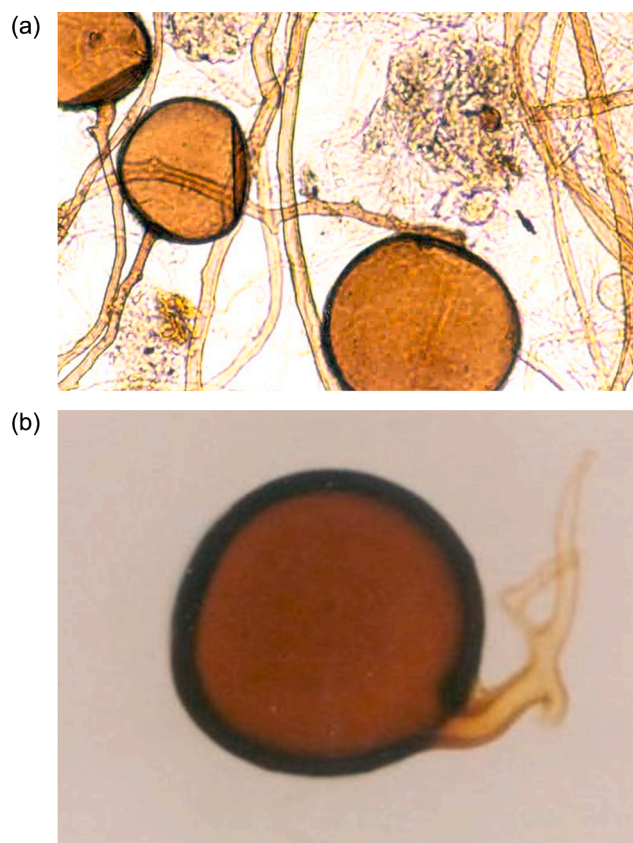


Fig. 1. a. *G. fasciculatum* b. *G. geosporum*.

The nitrogen fixing beneficial microbe *Azospirillum brasilense* was isolated in Congo red medium contains  $gL^{-1}$  of distilled water:  $KH_2PO_4$ ; 5 g;  $MgSO_4 \cdot 7H_2O$ ; 0.2 g; NaCl; 0.1 g; Yeast extract; 0.5 g;  $FeCl_3 \cdot X6H_2O$ ; 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. The petriplates containing this medium were used for isolation of *A. brasilense* as follows. 0.1 ml laterite soil diluted in sterile water ( $10^{-5}$ ) was poured in the medium and incubated at 36°C for seven days. After seven days of incubation, pink and scarlet colonies of the bacteria were identified as *A. brasilense* (Fig. 2b) (Rodríguez-Cáceres, 1982). These cultures were also tested for gram staining that showed spiral shaped with single flagellum and found further gram negative reaction (Sung and New, 1998). The endospores were also responded to malachite green and showed double membrane.

#### 2.2.2. Seed propagation of A. tryphisa

The seeds of *A. tryphisa* were collected from the matured trees and soaked in cold water for 24 h (Abijith et al., 2020). Later the seeds were sown in the nursery beds containing pure sand. The seeds were found germinated 10 to 15 days after sowing and the seedlings with uniform size (5 cm length; 25 days old) were transplanted to polythene bags (v 4.15 L Size: 14 × 27 cm) containing laterite soils collected from dug out laterite rocks (Figs 3,4). The laterite soils contain exchangeable cations of Ca ( $1.27 \text{ cmol } (p^+) \text{ kg}^{-1}$ ), Mg ( $0.32 \text{ cmol } (p^+) \text{ kg}^{-1}$ ) and Na ( $0.04 \text{ cmol } (p^+) \text{ kg}^{-1}$ ) according to Jackson (1973) while placing in poly bags.

#### 2.2.3. Inoculation of beneficial microbes

The inoculation of beneficial microbes in to the seedlings of *A. tryphisa* was executed as follows. AM fungi grown from the pot cultures of *Z. mays* were applied at the rate of 15 g per polythene bag at 5 cm below the soil surface in each polythene bag. Cultured other beneficial microbes of *A. brasilense* ( $5 \times 10^7 \text{ cfu } g^{-1}$ ) and *B. megaterium* ( $2 \times 10^5 \text{ cfu } g^{-1}$ ) individually and in combinations were executed by applying

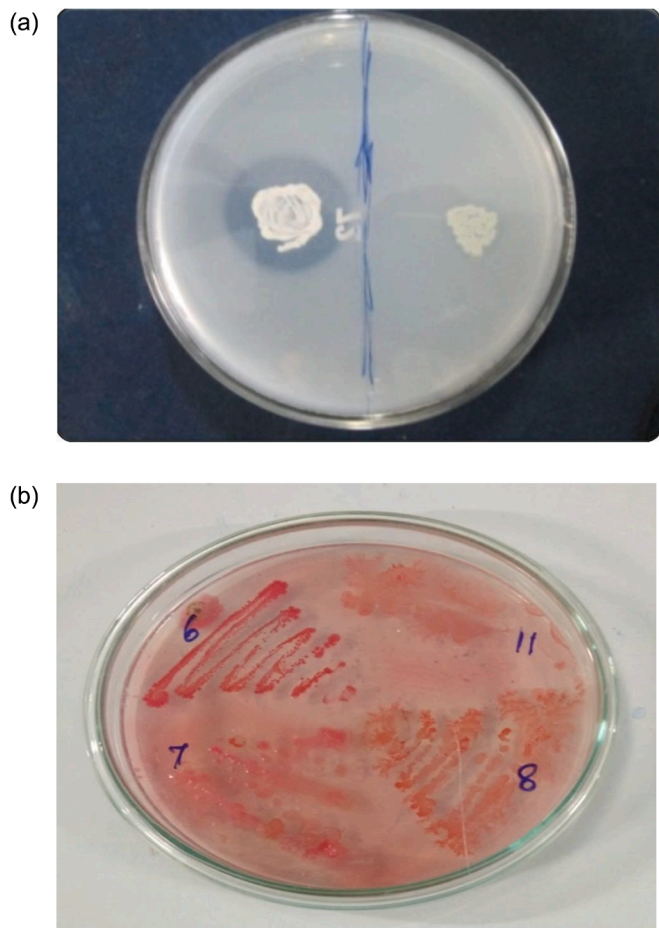


Fig. 2. a. *Bacillus megaterium* b. *Azospirillum brasilense* (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.).



Fig. 3. Germinated seedlings of *A. tryphysa*.

at the rate of 10 ml of each per seedling. Uninoculated control treatment was also maintained along with the inoculated treatments. A total of 10 replicates of each of 7 treatments were designed in this experiment viz., (1) Control, (2) AM fungi (15 g), (3) *A. brasilense* (10 ml), (4) *B. megaterium* (10 ml), (5) AM fungi (15 g) + *A. brasilense* (10 ml), (6) AM fungi (15 g) + *B. megaterium* (10 ml) and (7) AM fungi (15 g) + *A. brasilense* (10 ml) + *B. megaterium* (10 ml). These inoculation methods



Fig. 4. *A. tryphysa* seedlings grown in laterite soils along with beneficial microbes.

were followed with an earlier work of Karthikeyan and Sivapriya (2018). In this experiment it was maintained five seedlings in each replicate hence totally 350 seedlings were kept in a randomized block design for three months under shade house conditions with temperature  $25.4 \text{ }^\circ\text{C} (\pm 1.1)$  and relative humidity  $67.5 (\pm 1.2)$ . The seedlings were maintained with sufficient irrigation for their growth and survival.

#### 2.2.4. Harvest and evaluation of nursery experiment

Three months after inoculation, the seedlings from five replicates were harvested with entire shoot and roots. Then the seedlings were measured for root length, seedling height, root collar diameter, root biomass and number of leaves. The seedlings were also dried in oven at  $50 \text{ }^\circ\text{C}$  for 48 h. to measure the biomass. Later 5 g dried root and shoot samples were digested with potassium sulphate and copper sulphate (5:1) + triple acid (nitric + sulphuric + perchloric at the ratio of 9:3:1 in a Kjeltac equipment at  $420 \text{ }^\circ\text{C}$  for one hour to analyze the tissue nutrient contents of Nitrogen (N), Phosphorus (P) and Potassium (K) according to Jackson (1973).

#### 2.2.5. Field experiment

Rest of other five replicates of each treatment were planted in the barren laterite rocks at 120 cm deep pits with an espacement of  $3 \times 3 \text{ m}$ . The height, root collar diameter and number of leaves were measured after three months of planting. The same growth parameters were measured twelve months after planting grown in barren laterite rocks. The survival (%) was calculated according to the following formula.

$$\text{Survival} = \frac{\text{Number of seedlings survived}}{\text{total number of seedlings planted}} \times 100$$

This formula to be displayed in a single line. In the paginated proof it was showed two lines.

#### 2.2.6. Statistical analyses

Finally, the data of all the growth parameters derived from nursery and field experiments were analyzed for ANOVA by Duncan's Multiple Range Test using SPSS ver. 16.

### 3. Results

#### 3.1. Soil analyses

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

**Table 1**  
Physico chemical properties of dug out laterite soils from laterite rocks (mean of 5 replicates).

	pH	E.C (ds/m)	Bulk density (gm/cc)	Organic C (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
Dug out Laterite soils from laterite rocks	6.1 (± 0.01)	0.12 (± 0.010)	1.5 (± 0.12)	0.67 (± 0.02)	68.8 (± 1.2)	15.2 (± 1.6)	0.0

± SE of mean.

### 3.2. Nursery experiment

In nursery experiments the results showed that the seedlings of *A. tryphya* inoculated with AM fungi, *A. brasilense* and *B. megaterium* as single inoculations have got improvement in growth and biomass than the uninoculated control seedlings. However, no significant difference was found among single beneficial microbe inoculated seedlings in terms of growth and biomass (Table 2; Fig. 5). The double combinations of beneficial microbes (AM fungi + *A. brasilense*, AM fungi + *B. megaterium*) inoculations showed significant ( $p < 0.05$ ) improvement in growth and biomass than the individual inoculations. All combined beneficial microbes (AM fungi + *A. brasilense* + *B. megaterium*) significantly ( $p < 0.05$ ) improved the shoot length (40.7 cm plant<sup>-1</sup>), root length (20.8 cm plant<sup>-1</sup>), stem girth (2.1 cm plant<sup>-1</sup>), number of leaves (15.3 plant<sup>-1</sup>) and seedling biomass (shoot: 4.4 g plant<sup>-1</sup>; root 10.5 g plant<sup>-1</sup>). Significant lower root to shoot ratio (0.41) was found in all combined beneficial microbes inoculated seedlings (Table 2). The beneficial microbes (AM fungi + *A. brasilense* + *B. megaterium*) inoculated *A. tryphya* seedlings were significantly ( $p < 0.05$ ) increased nitrogen (6.5 mg g<sup>-1</sup>), phosphorus (5.8 mg g<sup>-1</sup>), and potassium (4.1 mg g<sup>-1</sup>) nutrients content when compared to control and other treatments (Fig. 6).

### 3.3. Field experiment

In field experiment the beneficial microbes inoculated seedlings of *A. tryphya* showed significant ( $p < 0.05$ ) growth improvement in barren laterite rocks at three months after planting (Table 3). The seedlings showed twelve months after planting the all combined beneficial microbes (AM fungi + *A. brasilense* + *B. megaterium*) got significant improvement in height (120.7 cm plant<sup>-1</sup>), stem girth (4.52 cm plant<sup>-1</sup>) and number of leaves (41.8 plant<sup>-1</sup>) (Table 3). The individual and dual beneficial microbes inoculated seedlings of *A. tryphya* showed 68 to 80% survival rate whereas the all combined beneficial microbes (AM fungi + *A. brasilense* + *B. megaterium*) showed 95% survival in barren laterite rocks (Fig. 7). Overall, the results of this study showed the successful establishment of *A. tryphya* in laterite rocks due to inoculation of beneficial microbes (Figs. 8,9).

## 4. Discussion

Establishment or afforestation of tree species in abandoned lands is getting much attention nowadays for improving productivity and carbon sequestration (Wardle et al., 2004). The soil organic carbon and other



**Fig. 5.** Harvested seedlings of *A. tryphya* inoculated with beneficial microbes Cont; Control, 1; AM fungi 2; *A. brasilense* 3; *B. megaterium* 4; AM fungi + *A. brasilense* 5; AM fungi + *B. Megaterium* 6; AM fungi + *A. brasilense* + *B. megaterium*.

nutrients were usually lower in abandoned site (Yang et al., 2018) so it is essential to introduce the beneficial microbes along with selected plants as the beneficial microbes are capable to multiply rapidly to promote the plant growth (Vessey, 2003). The soil analysis showed poor nutrients availability due to absence of organic matter including soil microbes. In this study the laterite soils were used as potting media for growing *A. tryphya* seedlings in nursery to enhance the adaptability for field plantation. Similar method was used for growing *Swietenia macrophylla* by Karthikeyan (2020). This technique has helped for adopting the seedlings and their earlier establishment in barren laterite rocks. As the beneficial microbes capable to improve the plant growth by enhancing the nutrient availability to *A. tryphya* seedlings through N fixation and P solubilization (Soumare et al., 2020) the seedlings were established successfully in the laterite rocks. In an earlier study also (Karthikeyan et al., 2009) the bauxite mined out soils were used as potting media to grow *Casuarina equisetifolia* with beneficial microbes and thereafter the seedlings were planted for reclamation in bauxite mined out overburdens.

Nursery experiment conducted in this study showed that the beneficial microbes inoculated seedlings have improved the growth and biomass of *A. tryphya* that grown in laterite soils as potting media.

**Table 2**  
Growth and biomass of *A. tryphya* inoculated with beneficial microbes (mean of 5 replicates).

Treatments	Shoot length (cm)	Root length (cm)	No.of. leaves plant <sup>-1</sup>	Stem girth (cm)	Seedling Biomass (g plant <sup>-1</sup> )	R/S ratio
					Root	Shoot
Control	08.8 <sup>a</sup>	06.4 <sup>a</sup>	4.2 <sup>a</sup>	0.22 <sup>a</sup>	0.8 <sup>a</sup>	0.9 <sup>a</sup>
AM fungi	14.8 <sup>b</sup>	09.9 <sup>b</sup>	7.7 <sup>bc</sup>	0.48 <sup>b</sup>	1.4 <sup>b</sup>	2.1 <sup>b</sup>
<i>A. brasilense</i>	14.4 <sup>b</sup>	10.5 <sup>b</sup>	7.5 <sup>b</sup>	0.85 <sup>b</sup>	1.4 <sup>b</sup>	2.2 <sup>b</sup>
<i>B. megaterium</i>	13.8 <sup>b</sup>	10.1 <sup>a</sup>	7.8 <sup>b</sup>	0.84 <sup>b</sup>	1.3 <sup>b</sup>	2.1 <sup>b</sup>
AM fungi + <i>A. brasilense</i>	22.2 <sup>c</sup>	12.3 <sup>b</sup>	10.6 <sup>c</sup>	1.5 <sup>c</sup>	2.5 <sup>c</sup>	3.6 <sup>c</sup>
AM fungi + <i>B. megaterium</i>	24.5 <sup>c</sup>	12.7 <sup>b</sup>	11.1 <sup>c</sup>	1.6 <sup>c</sup>	2.8 <sup>c</sup>	3.8 <sup>c</sup>
AM fungi + <i>A. brasilense</i> + <i>B. megaterium</i>	40.7 <sup>d</sup>	20.8 <sup>c</sup>	15.3 <sup>d</sup>	2.1 <sup>d</sup>	4.4 <sup>d</sup>	10.5 <sup>d</sup>

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

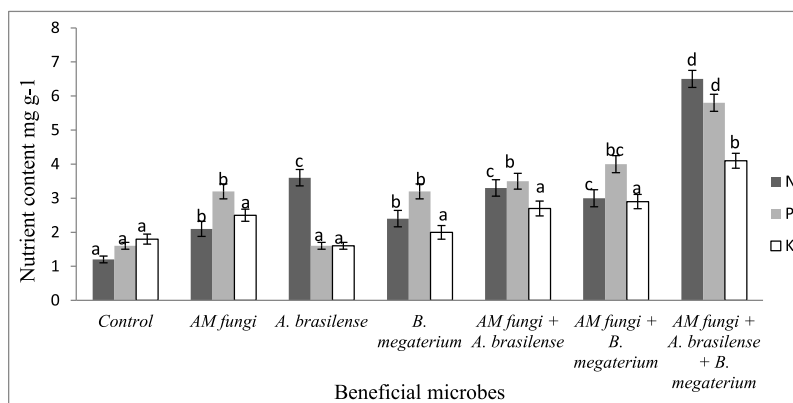


Fig. 6. Nutrient content of *A. tryphysa* seedlings inoculated with beneficial microbed (mean of 5 replicates).

Table. 3

Growth performance of *A. tryphysa* seedlings inoculated with beneficial microbes at laterite barren rocks (mean of 5 replicates).

Treatments	Height (cm)		Stem girth (cm)		No. of leaves plant <sup>-1</sup>	
	3 MAP*	12 MAP	3 MAP	12 MAP	3 MAP	12 MAP
Control	11.5 <sup>a</sup>	25.6 <sup>a</sup>	0.44 <sup>a</sup>	1.44 <sup>a</sup>	6.5 <sup>a</sup>	10.7 <sup>a</sup>
AM fungi	17.0 <sup>b</sup>	55.3 <sup>b</sup>	1.21 <sup>c</sup>	2. 2 <sup>c</sup>	9.5 <sup>b</sup>	17.2 <sup>bc</sup>
<i>A. brasilense</i>	18.2 <sup>b</sup>	58.1 <sup>b</sup>	1.24 <sup>b</sup>	2.1 <sup>b</sup>	10.8 <sup>b</sup>	16.9 <sup>b</sup>
<i>B. megaterium</i>	18.6 <sup>b</sup>	60.2 <sup>b</sup>	1.28 <sup>b</sup>	2.2 <sup>b</sup>	10.6 <sup>b</sup>	17.4 <sup>b</sup>
AM fungi + <i>A. brasilense</i>	29.5 <sup>c</sup>	85.2 <sup>c</sup>	1.40 <sup>c</sup>	2.85 <sup>c</sup>	14.3 <sup>c</sup>	23.8 <sup>c</sup>
AM fungi + <i>B. megaterium</i>	31.3 <sup>c</sup>	88.2 <sup>c</sup>	1.42 <sup>c</sup>	2.88 <sup>c</sup>	14.2 <sup>c</sup>	24.1 <sup>c</sup>
AM fungi + <i>A. brasilense</i> + <i>B. megaterium</i>	45.7 <sup>d</sup>	120.7 <sup>d</sup>	3.11 <sup>d</sup>	4.52 <sup>d</sup>	25.2 <sup>d</sup>	41.8 <sup>d</sup>

MAP\*: months after planting.

Means in a column followed by the same letter(s) are not significantly different according to Duncan’s multiple range test ( $P < 0.05$ ).

These beneficial microbes probably mobilized the phosphorus from soil and fix the atmospheric nitrogen in the seedlings to improve the growth of *A. tryphysa* than uninoculated seedlings. Similar kind of results were obtained in many tree seedlings 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), *Brugeira sexangula* (Karthikeyan and Sivapriya, 2018), *Swietenia macrophylla* (Karthikeyan, 2020) and *Pterocarpus santalinus* (Karthikeyan and Arunprasad, 2021). All combined inoculation of beneficial microbes showed significant results than single and dual inoculations because the increased microbial biomass has promotes the plant growth in various ways particularly phosphorus and nitrogen assimilation (Gunina et al., 2016). Number of leaves in *A. tryphysa* was increased in the beneficial microbes inoculated seedlings due to the effect of AM fungi. These results were matched with the earlier studies on *Tectona grandis* (Rajan et al., 2000) *Macadamia tetraphylla* (Yooyongwech et al., 2013) and *Elaeis guineensis* (Ajang et al., 2020). *A. tryphysa* seedlings inoculated with all combined beneficial microbes showed a lower root to shoot ratio that expressed the increment of above ground production and decrement of below ground production through nutrient transfer of beneficial microbes (Smith and Smith, 2012). Improved tissue nutrients content is recorded in beneficial microbes inoculated seedlings of *A. tryphysa* compared to control seedlings. This increased nutrients accumulation showed as results of nutrient transfer attributed by the combined effect of beneficial microbes. The beneficial microbes AM fungi and *B. megaterium* significantly improved the phosphorus content whereas *A. brasilense* improved the nitrogen content (Khan et al., 2014; Muthukumar and Udaiyan, 2018). The increased potassium content in *A. tryphysa* seedlings with beneficial microbes showed that potassium may be absorbed by the seedlings from decomposed soil by *B. megaterium* as reported by Meena

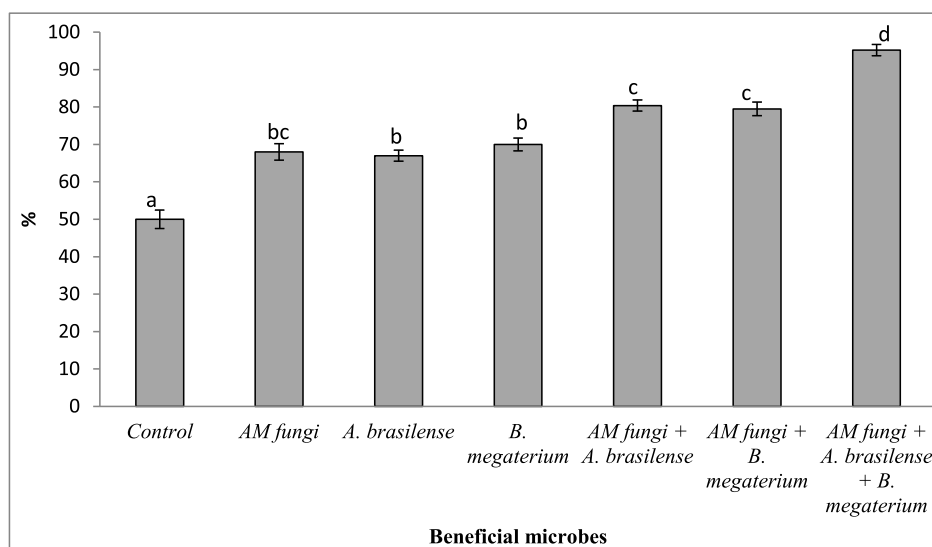


Fig. 7. Survival of *A. tryphysa* transplanted at barren laterite lands.



Fig. 8. Established *A. tryphysa* at barren laterite rocks with beneficial microbes.



Fig. 9. *A. tryphysa* seedling established at laterite rocks with AM fungi + *A. brasilense* + *B. megaterium*.

et al., (2014).

Field experiment at laterite rocks showed improved growth and survival performance of beneficial microbes inoculated *A. tryphysa* seedlings. *A. tryphysa* seedlings were planted with improved height and biomass due to inoculated beneficial microbes that aids successful establishment in the laterite rocks. This is the reason that *A. tryphysa* survives in low nutrient laterite rocks along with these beneficial microbes. Recently, Bessadok et al. (2021); reported the similar results that *Rhizobium* strains enhanced the growth and adaptability of *Anthyllis heroniana* in vegetation poor sites. Likewise, successful studies were carried out in *Casuarina equisetifolia* and *Eucalyptus tereticornis* at bauxite mined out lands (Karthikeyan and Krishnakumar, 2012; Karthikeyan et al., 2009). Further, Fofana et al. (2020) have also reported that AM fungi and N fixing bacteria are the potential agents for land restoration. The improved survival rate at laterite rocks is due to nutrient uptake of N and P through beneficial microbes as they produced organic acids for nutrient solubilization (Ajeng et al., 2020). Further the significant growth increase of *A. tryphysa* seedlings with beneficial microbes are due to nutrient solubilization by the beneficial microbes (Burton et al., 2010) that decreased the nutrient deficiency (Joner and Leyval, 2001; Sanchez-Diaz and Honrubia, 1994). These effects of beneficial microbes made the successful establishment of *A. tryphysa* in the barren laterite rocks.

## 5. Conclusion

The beneficial soil microbes are the main contributors of forest ecosystem in nutrient cycling and creators of organic materials. The beneficial microbes provide essential nutrient availability that supports soil fertility. The beneficial microbes used in this study have the potential to increase the efficiency of plant growth system through supply of essential levels of major nutrients of N and P. This study has proven that barren wastelands like laterite rocks can be successfully afforested with useful trees/plants along with beneficial microbes.

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## CRedit authorship contribution statement

**Arumugam Karthikeyan:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The author declare that no known competing financial interests or personal relationships that could appeared to influence the work reported in this paper.

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