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Effect of Biofertilizers and Organic Amendments on Germination and Seedling Growth of Common Dry Zone Forest Species in Sri Lanka: Sustainable Reforestation Practices in Sri Lanka[#]

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ABSTRACT

Most of the dry zone forests in Sri Lanka are arisen after unsustainable farming practices. Therefore, the natural regeneration of plant species in these forests reported to be very poor due to the reduced soil fertility. This study was conducted to find possible measures for the successful seed germination and seedling establishment of common dry zone forest tree species by sustainable soil fertility management. A pot trial was carried out in a greenhouse at the Faculty of Applied Sciences, Rajarata University, Mihintale, Sri Lanka. The experiment comprised of 128 pots and four replicates. Pots were assigned with different soil fertility managements: T0: control (Top soil only), T1: Arbuscular Mycorrhizal Fungi (AMF), T2: a traditional mixed microbial culture (*Jeewamurthum*), T3: Compost, T4: Biochar, T5: T1+T2, T6: T1+T3, T7: T1+T4; and different forest plant species: *Manilkara hexandra*, *Feronia limonia*, *Pterospermum conscens* and *Bauhinia racemosa*. Seed germination percentage was measured two weeks after sowing, and shoot biomass, relative plant growth rate, and AMF colonization percentage were measured after four months of germination. The results revealed that inoculation of AMF enhance the seed germination percentage of all the selected plant species. Both sole and combined application of AMF and compost gives more benefits to all the tested plant species by enhancing all the measured growth parameters. The findings of present study would be useful in reforestation programs of dry zone forests in Sri Lanka by practicing sustainable procedures.

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Introduction

Dry zone of Sri Lanka has nearly two-third of the total land area and contains 76% of the total forest ecosystem (Ranagalage, 2020). Natural forests types in dry zone comprise mainly tropical dry mixed evergreen forests, tropical thorn forests and tropical sub montane seasonal forests (Nissanka et al., 2010). These forest ecosystems rich in very important species of flora and fauna. Also, they provide a wide range of goods and services which are highly valuable for mankind, animals and environment for giving better life on the earth. However, the biodiversity of dry zone forests in Sri Lanka are adversely affected by various biotic and abiotic influences. During the past 27 years, the dry zone forest cover has been undergone a rapid deforestation and it is 8.0% of the total forest loss in the country (Ranagalage, 2020). Therefore, immediate actions should be taken to regenerate and protect the dry zone forest cover in Sri Lanka.

In Sri Lanka, the recovery rate of dry zone forests is slow due to the reduced soil fertility resulted by unsustainable farming practices (Perera et al., 1995). Because most of the dry zone forests in Sri Lanka are secondary in origin and arisen from the shifting cultivation (Perera, 2001). As a result, natural regeneration of dry zone forest species reported to be very poor (Perera, 2012). Therefore, finding possible measures for the successful seed germination and seedling establishment of forest tree species would be important in the dry zone forest regeneration programs in Sri Lanka. The present study was designed to investigate the potential of soil fertility management for the seedling establishment of some common dry zone forest tree species.

The application of chemical fertilizer is known to adversely effects on natural microbial communities in ecosystems and thereby affect soil fertility and plant productivity (Senevirathne, 2009). Therefore, the use of synthetic fertilizer for the seedling establishment by means of soil fertility management would not be sustainable. Biofertilizer are sustainable alternatives for synthetic fertilizer (Adesemoye et al., 2009; Kulasooriya et al., 2017). These are the inoculants of live microorganisms which are capable of promoting plant growth via various biological processes either by colonizing within the rhizosphere or inside of the plant (Vessey et al., 2003; Cong et al., 2011). Such beneficial microorganisms including AMF, nitrogen fixing bacteria and other plant growth promoting microbes are vital for nutrient availability and plant uptake in restoration programs (Jasper, 2007; Madhushan et al., 2021).

Use of soil amendments together with biofertilizer leads to increase plant survival and seedling quality (Karličić et al., 2016). Compost is an organic soil amendment capable of improving the soil quality, fertility, structure, texture, and moisture retention. Compost that fails to meet standards of agricultural crop production i.e. those produced by sewage sludge could be used in forest soil fertility management, as an environmentally friendly option (Wei et al., 2000). Biochar also known to a better soil amendment which capable of improving soil nutrient availability, moisture retention and microbial activity (Baldock and Smernik, 2002).

In this study we investigate the effectiveness of AMF inoculum and a mixed microbial culture (*jeewaamurthum*), organic soil amendments such as compost and biochar and their combinations for seed germination and seedling establishment of some important dominant dry zone forest species in Sri Lanka (*Manilkara hexandra*, *Feronia limonia*, *Pterospermum conscens* and *Bauhinia racemosa*) with the objective of evaluating the prospects for using them in sustainable forest regeneration.

Materials and Methods

Experiment site and design

A pot experiment was conducted in a greenhouse at the Faculty of Applied Sciences, Rajarata University, Mihinthale, Sri Lanka. The study was planned as a two-factor factorial experiment following randomized completely block design (RCBD). The two factors used were different soil fertility managements (Factor-1) and different forest plant species (Factor-2). Factor-1 comprised with eight levels, T0: Control (Top soil only), T1: AMF, T2: Mixed Microbial Culture (*Jeewamurthum*), T3: Compost, T4: Biochar, T5: T1+T2, T6: T1+T3, T7: T1+T4). Factor-2 comprised with four levels, *Manilkara hexandra*, *Feronia limonia*, *Pterospermum conscens* and *Bauhinia racemosa*.

Preparation of biofertilizer and soil amendments

Compost

Compost was produced on-farm by windrow composting using rice straw, *Gliricidia sepium* leaves and cow manure. Materials allowed to decompose for 90 days and maintained 60-65 ℃ temperature inside. Windrows were turned periodically and moistened.

Biochar

Biochar was prepared using paddy husks by double-barrel method (Agegnehu et al., 2016). Dried paddy husks heated to about 450 – 550 °C for 1 hour by burning with firewood. After about one hour when pyrolysis of the biomass had aborted, charcoal was collected, and air-dried, ground and sieved before use.

AMF inoculum

AMF inoculum was prepared by trap culture method. Soil samples containing fine root fragments of herbs were collected from an undisturbed site in dry zone environment at a depth of 0-10 cm. Then mixed with fine sand and cow manure, and trap cultures were established using maize (*Zea mays* L.). After six weeks, maize rhizospheric soil containing spores, fungal hyphae and colonized root fragments (65% of colonization) were used as the AMF inoculum (Chaurasia et al., 2005).

Mixed microbial culture

A traditional liquid organic fertilizer named "*Jeewaamurthum*" was used as the mixed microbial culture, which was prepared by mixing forest soil, cow urine, cow dung, sugar, *Gliricidia* leaves in water.

Treatment establishment

There were 32 (factor1- 8 × factor2- 4) treatment combinations and each was 4 replications, hence there were total of 128 pots. Pots of control were filled sieved top soil obtained from an area with previously no added synthetic fertilizer, at the depth of 0-15 cm. Required potting mixtures of other treatments were prepared by adding compost (500 g/pot), biochar (250 g/pot), AMF inoculum (250 g/pot), *jeewaamurthum* (500 ml/pot) and inorganic fertilizer (20 g/pot). The final weight of each potting mixture was adjusted into 3kg by changing the weight of top soil. Seeds of *Manilkara hexandra*, *Feronia limonia*, *Bauhinia racemosa* and *Pterospermum conscens* were soaked in distilled water for three days and five seeds were sown per pot.

Data collection

After two weeks of seeds sowing, seed germination percentage (GP) was determined by following eqation: GP = (Number of germinated seeds/Total number of sown seeds) × 100. After three weeks of seed germination, excess seedlings were thinned out leaving two healthy seedlings per pot. After four months of seed germination, relative growth rate (increase of plant height per month) and dry shoot biomass was measured as the growth parameters. Composite roots samples were prepared and percentage AMF colonization was determined by following the procedures described by Phillips and Hayman (1970) and McGonigle et al., (1990).

Data analysis

The measured parameters were analyzed with a Kruskal–Wallis nonparametric analysis of variance (ANOVA) test. The means were separated using the Least Significant Difference (LSD) method at the 5% probability level. Mean separation was sliced by the plant species in the factor-02. All data analyses were performed using Minitab version 17.1.

Results and Discussion

The influence on seed germination

In the plant growth cycle, seed germination and establishment of seedlings are critical stages that determine the species survival in natural habitats (Hadas, 2005). Results showed that seed germination of different plant species are differently responsive for soil treatments (Figure 1). Adding of biochar and AMF alone and together was shown significantly higher seed germination rates in *P. conscens*; it was significantly higher in *F. limonia* when added AMF, biochar, compost and compost added with AMF; and the germination of *M. hexandra* seeds were more responsive for AMF and compost treatments. However, among the treatments added to soil, incorporating of AMF has significantly enhanced the seed germination percentage of all the selected tree species. Strigolactones secreted by plant roots as a signaling compound to initiate AMF colonization are found to stimulate seed germination while inducing spore germination and hyphal activities of AMF (Parniske, 2008; Kretzschmar et al., 2012). Seeds of *M. hexandra* have very poor germination thereby need pre-treatments to enhance the germination ability (Shinde and Malshe, 2015). According tothe present study, AMF and compost could be used to enhance the seed germination in *M. hexandra*.

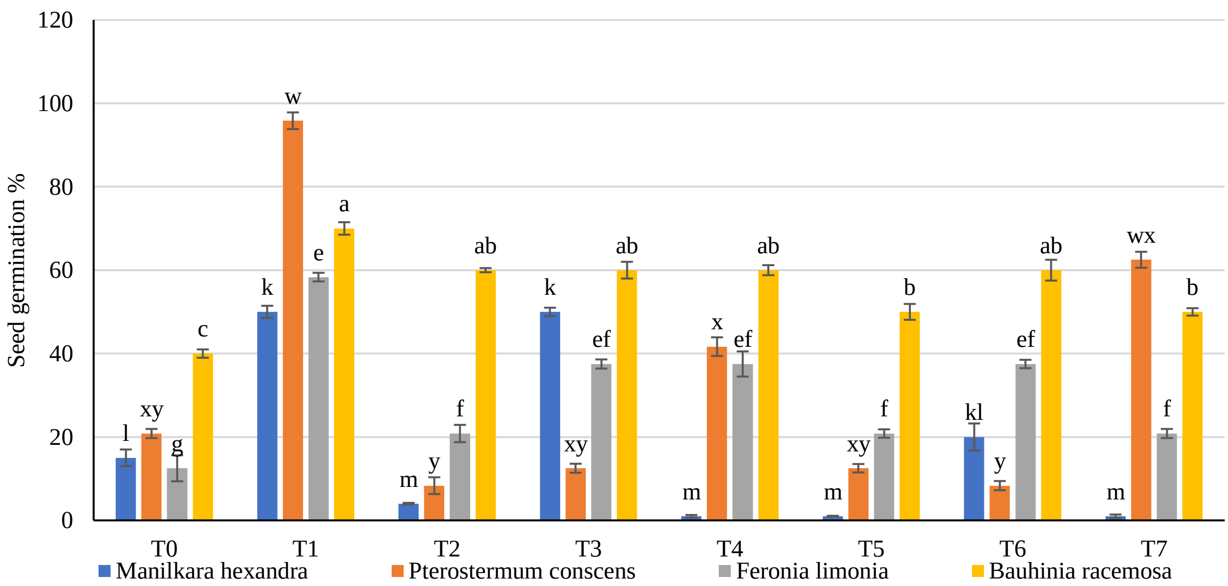

Figure 1. Effect of treatments on seed germination of selected forest plant species.
(T0: Control, T1: AMF, T2: Mixed Microbial Culture, T3: Compost, T4: Biochar, T5: T1+T2, T6: T1+T3, T7: T1+T4. The same letters within each plant species are not significantly different at P<0.05 (LSD), mean separation was sliced by plant species.)

The influence on shoot dry biomass and relative growth rate

Among the treatments, adding AMF, and compost + AMF have given significantly higher shoot biomass (Figure 2A) in all the selected plant species. Relative growth rate, which is an indirect measurement of the resource acquisition rate of plants (Lowry and Smith, 2018) was shown to be significantly higher among the treatments for some forest plant species particularly *F. limonia* and *B. racemosa* (Figure 2B). Compared to the control, adding of AMF alone and together with other soil amendments and biofertilizer has resulted a significantly higher relative growth rates in all the plant species.

These findings were suggested that AMF has the positive influence on the seedling development by elongating shoots and roots, increasing biomass accumulation, and increasing the growth rate. Sreeramulu et al. (1998), also reported that enhanced plant growth in *M. hexandra* (Roxb.) Dub. seedlings by the inoculation of AMF. However, the physiology behind such early plant development remains unknown (Gutowski, 2015). Plant growth regulators of auxin and cytokinin play a major role in the elongation of root and shoot meristematic tissues (Schaller et al., 2015). AMF found to be increased the production of auxins and cytokinin (Fusconi, 2014) while improving the plant uptake of soil added nutrients by forming a bridge between plant roots and the soil. Also, application of compost can improve soil physical, chemical and biological fertility and productivity of crop production by replenishing soil organic matter and supplying nutrients (Adugna, 2016). Biochar is capable of decreasing the soil bulk density (Mukherjee and Lal, 2013), hence facilitating the better root growth by improving the soil structure.

The influence on AMF colonization rate

Incorporation of AMF and compost alone and together have given significantly higher AMF colonization in all the selected plant species (Figure 3). There is a poor AMF colonization in selected plant species when the soil treated with mixed microbial culture. This would be due to the negative interactions of microorganisms present in the mixed microbial culture towards the added and naturally soil available AMF. In the rhizosphere there are deleterious microorganisms which are producing different unfavorable compounds including phytotoxins, competing for resources with other soil microbes as well as inhibiting the activity of AMF (Miransari, 2011).

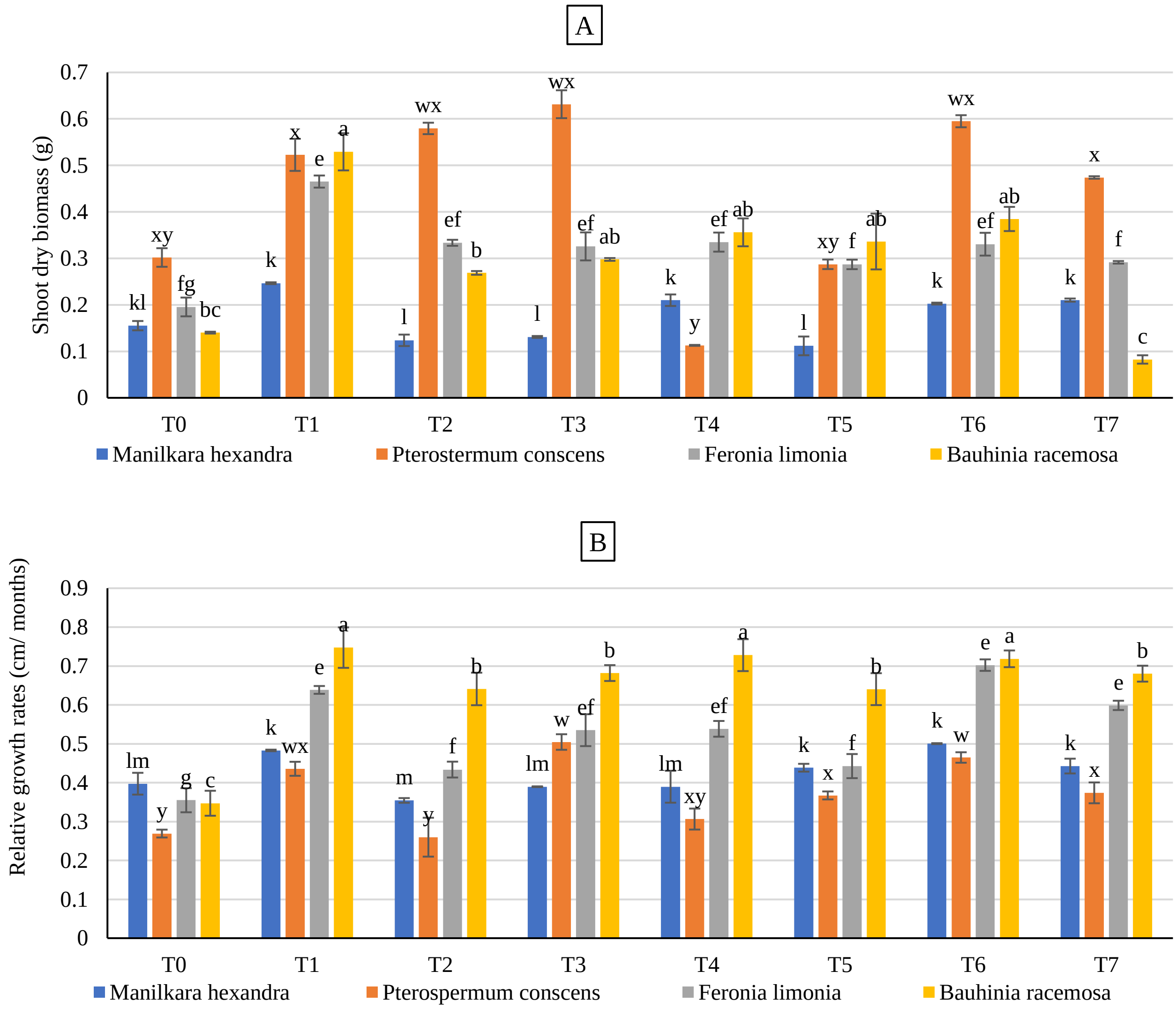

Figure 2. Effect of treatments on growth parameters
(A: Shoot dry biomass, B: Relative growth rate) of selected forest plant species. (T0: Control, T1: AMF, T2: Mixed Microbial Culture, T3: Compost, T4: Biochar, T5: T1+T2, T6: T1+T3, T7: T1+T4. The same letters within each plant species are not significantly different at P<0.05 (LSD), mean separation was sliced by plant species.)

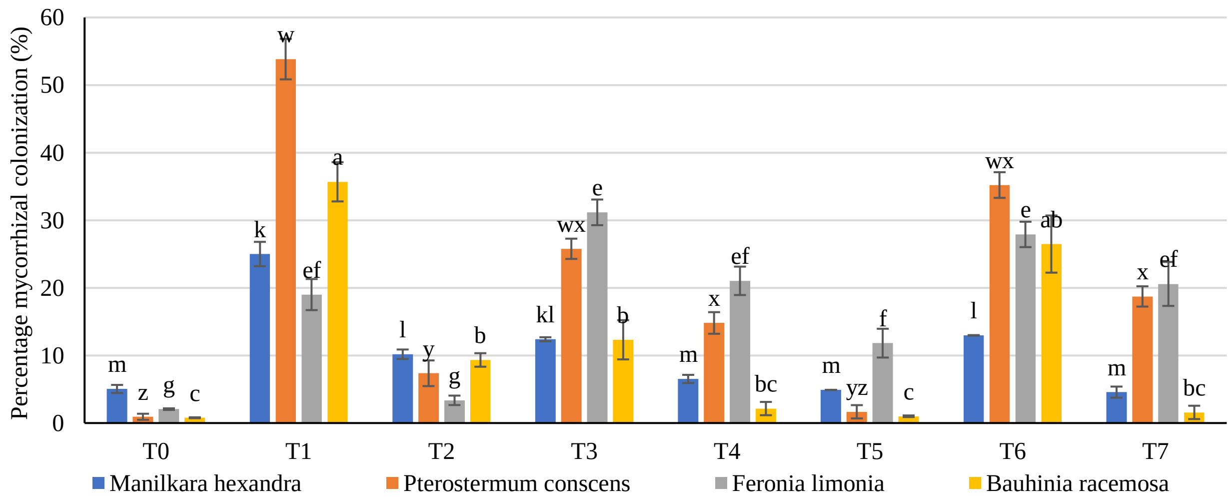

Figure 3. Effect of treatments AMF colonization of selected forest plant species.
(T0: Control, T1: AMF, T2: Mixed Microbial Culture, T3: Compost, T4: Biochar, T5: T1+T2, T6: T1+T3, T7: T1+T4. The same letters within each plant species are not significantly different at P<0.05 (LSD), mean separation was sliced by plant species.)

Conclusions

Inoculation of AMF enhances the seed germination percentage, and the application of AMF and compost alone and together increased the seed germination, relative growth rate and the shoot biomass of the tested plant species such as *M. hexandra*, *F. limonia*, *P. conscens* and *B. racemosa*. The findings of present study would be useful in sustainable reforestation programs of the dry zone forests in Sri Lanka.

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