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Growth Performance of *Pentaspadon motleyi* Seedlings Inoculated with Arbuscular Mycorrhiza Fungi for Waterway Rehabilitation

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ABSTRACT

Stream development causes changes to the ecosystems of an area. Planting of trees in cleared area along waterways can help in reviving and create new habitats. Planting of riverine species such as *Pentaspadon motleyi* is one way to rehabilitate disturbed riverine area. The ability of seedlings to survive in new and harsh open environment depends on the seedlings' early growth as well as species adaptability. Arbuscular mycorrhiza fungi (AMF) have the capability to promote superior and stronger seedlings with better growth performance when planted in the fields. Thus, the objectives of this study were: (i) to measure the growth performance of *P. motleyi* seedlings planted along a waterway in Universiti Putra Malayisa Bintulu Sarawak Campus and (ii) to determine the effect of AMF on the growth of *P. motleyi* seedlings. A total of 30 seedlings were planted along a waterway stretch in UPMKB and 15 seedlings were treated with AMF while another 15 seedlings were left untreated. Parameters measured include plant height, collar diameter and leaf number were recorded for a period of 10 weeks. Leaf area and root morphology of *P. motleyi* seedlings were compared after the tenth week. AMF treated seedlings showed five times higher height and collar diameter growth than non-AMF treated seedlings while leaf number and leaf area were superior for all AMF treated seedlings. Roots of AMF treated seedlings were healthier with more fibrous and fine roots. AMF inoculation contributed to *P. motleyi* seedlings by forming mycorrhiza hyphae that helped the root system with the exploration and access to more soil nutrients from the surrounding area. Better nutrient uptake improved plant health including plant biomass. AMF treatment showed good potential in enhancing early growth performance of *P. motleyi* seedlings thus promoting better survival when being transplanted in the open field. Such conditions will benefit the rehabilitation activities of disturbed waterways.

Keywords: Arbuscular Mycorrhiza Fungi (AMF), growth, *Pentaspadon motleyi,* seedlings, waterway

INTRODUCTION

Riparian forests are ecotones delineating the transition from terrestrial ecosystems into aquatic ones. The high-water content in the soil resulted in unique vegetation and soil characteristics, differentiating it from the surrounding landscapes (Ring *et al*., 2018; Dufour *et al*., 2019). Vegetations in the riparian zones are more severely influenced by edaphic factors rather than the climate (van der Maarel *et al*., 2013). Riverine floodplains represent a challenging environment for plant to grow due to their heterogenous physico-chemical soil characteristics (Dai and Nimasow, 2024).

Pentaspadon motleyi of the Anacardiaceae family and a common species found in marshes at elevations of up to 200 m, extending from Sumatra to New Guinea and the Solomon Islands (Adnan *et al*., 2018). Also known as *Pelong* (Peninsular Malaysia); *Empelanjau, Pelajau* (Dayak, Sabah), *Lakacho*, *Plajau* and *Plasin Uping* (Sarawak); *Djuping, Empit*, *Empelanjau, Letjut, Panjau, Pelajau, Peladjau, Pelasit, Pilajau, Plajau, Planjau, Polajo, Praju, Tampison, Umpit* (Indonesia); *Ttoei-na*, *Oei-nam* (Thailand), *Vi hùng trung* (Vietnam) and *Ailala*, *Laleua*, *Laleva* (New Guinea). The main reason for the selection *P. motleyi* for waterway rehabilitation is related to its natural habitat which is commonly found in swamps, along streams and rivers (Adnan *et al*., 2018). The tree can grow to be quite large, reaching up to 36 m in height and 80 cm in diameter at breast height, with spreading buttresses and beautiful, fluffy crowns. Browne *et al*. (1955) noted that the trees can reach a height of 21 m after 11 years.

The tree has spirally organised compound leaves with 4 to 5 pairs of leaflets that are grouped at the ends of the twig tips and pinkish in colour when young. The leaves are 10 to 30 cm long and have 7 to 9 leaflets; the leaflets are normally opposite, pointed at the apex with a rounded base, and pinkish in colour when young (Kochummen, 1989). The blooms are yellowish white in colour and 4 mm in diameter. The sweeping canopies are loaded with flowers while in bloom. It blooms twice a year, from March to May and again from October to November and when in bloom, the trees stand out with full bloom and no leaves (Lim, 2012). Burgess (1966) and Wong (1975) recorded the bark of *P. motleyi* as grey, white with pink inner bark and pale sap. The wood is classified as a light hardwood with a pale yellow-green heart wood. When young, the sapwood is white with a green tint or light yellow with a pink tinge, 2 to 3 cm broad, and not usually clearly separated.

The wood is of good quality with straight stem which is quite strong and not cracked when used as building materials. The wood is simple to work with and is appropriate for general utility interior furnishing, panelling, partitioning, moulding, and other planking projects. Extractive compounds from the *P. motleyi* tree have been applied as medicinal components, including in the treatment of scabies (tinea) and malignant rashes (Heyne, 1987; Wiart, 2006). According to Yusro *et al*., (2009), past research indicated that wood extract of *P. motleyi* showed the ability to inhibit the development of fungus *Candida albicans* and *Trichophyton mentagrophytes. Pentaspadon motleyi* seeds (endocarp) can be consumed either eaten raw, fried, cooked, or roasted (Lim, 2012).

Soil consolidation influences structural stability, soil nutrient content, and microbial biomass (Lin *et al*., 2020). The potential of seedlings to grow well in the field can be influenced by many factors including soil compactness and erosion that can occur on the riverbank. Land along a waterway is commonly exposed to occasional short-term flood. Such situation can be detrimental to plants as flooding causes hypoxia or anoxia in soils creating low solubility and diffusion of oxygen in water while increased in the use of dissolved oxygen by microorganisms and roots (Fougnies *et al*., 2007). The survival of seedlings when planted in flooded and open field depends much on the ability and period required by the seedlings to adopt different physiological, morphological and biochemical strategies (Jia *et al*, 2021) to cope with the stress in the new environment.

The introduction of arbuscular mycorrhiza fungi (AMF) has been found crucial for plant growth and survival, enhancing nutrient acquisitions and tolerance to biotic and abiotic stresses (Dai and Nimasow, 2024). Inoculation of AMF on seedlings planted on flooded soil have shown variable effects with some plants recording improvement in growth (Miller and Sharitz, 2000), some showing decreased growth (Neto *et al*., 2006) while others failed to indicate any clear relationship (Hartmond *et al*., 1987). AMF have been reported to promote the development of a strong root system to help seedlings strive when a site is flooded (Fougnies *et al*., 2007) during heavy rainfall.

Research on the response of *P. motleyi* seedlings to AMF is lacking and this study can help in providing some insight into such relationship. The introduction of AMF to *P. motleyi* seedlings is expected to assist the initial growth when transplanted in the open field. Therefore, the objectives of this study are to compare the effect of AMF on the growth of *P. motleyi* seedlings when planted along the waterway in Universiti Putra Malaysia Bintulu Sarawak Campus (UPMKB). Changes in the plant height, stem diameter, leaf number, leaf area and root growth were evaluated and related to the potential of AMF in promoting *P. motleyi* seedlings growth.

MATERIALS AND METHODS

Study Site

The area along a waterway in Universiti Putra Malaysia Bintulu Sarawak Campus was selected for the project. The site is occasionally inundated with rainwater during heavy rain which will usually subside once the rain stopped. The area along the waterway was cleared during the construction of the waterway to reduce the incidence of flooding in the area. The soil was of the Bekenu series (Typic Paleudult) with a pH of 4.84 and undulating slopes. The soil nutrient contents were recorded as 0.48% N, 0.90 mg/kg

P, 102 mg/kg K, 17.2 cmol/kg exchangeable Ca and 27.0 cmol/kg exchangeable Mg.

Seedlings Source and Planting

Pentaspadon motleyi seedlings were obtained from UPMKB Forest Nursery and placed in an open area for a week for the hardening process in order to reduce stress on the seedlings when planted in the open field. Few days before planting, the seedlings were moved on site and kept under tree shade for further hardening while waiting for the planting holes to be ready. Fertilizers were added into the planting holes before planting. This was done to ensure that most of the fertilizer can reach the root system and did not escape due to water runoff. A single seedling was then planted in each hole which was then covered using soil. Each seedling was gently removed from the polybag to ensure that the roots were kept intact as much as possible to avoid root disturbance. A polyvinyl chloride (PVC) pipe was used to cover the seedling's stem in order to protect the stem from injury, particularly due to grass cutting activities. The PVC pipe was cut to approximately 45 cm in length and one vertical cut was made to ease the insertion of the seedling's stem into the pipe.

AMF Treatments

Fifteen seedlings were treated with AMF while another fifteen were left untreated. Thus 50% of the seedlings will be selected for AMF treatment while another 50% will be left untreated. Both treated and untreated seedlings were planted alternately in a row at a 8 m distance between seedlings on both sites of the waterway. AMF treatment was applied on *P. motleyi* seedlings by digging two holes on an opposite direction around the root of the treated seedlings. Each hole was added with 20 gm of the AMF inoculum (RHIZAgold®). The product is documented to contain a mixture of 12 AMF species (including the genera *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Sclerocystis*) and every 10 g of product has 250-300 mixed viable AMF spores. After addition of the AMF inoculum, the seedlings were left out in the field until the first measurement was conducted.

Data Collection

The first growth measurement was conducted one month after the application of the AMF inoculum. Measurements were conducted for a period of ten weeks from 14 September 2022 until 25 November 2022. Parameters included in determining the growth performance of *P. motleyi* seedlings were height, stem diameter and leaf number. Height growth was measured using a meter tape and measured from the root collar until the shoot tip of each seedling. Meanwhile, stem diameter was measured slightly above the root collar using a digital calliper. Data of height and diameter growth rate per week was conducted by deducting the measurement of height and diameter of the present week with the measurement of the past week. Leaf number was recorded based on manual counting. Recording of these parameters were conducted fortnightly.

Leaf area as well as root development of the seedlings were conducted during the last week of measurement using destructive methods. All leaves and roots from three random seedlings representing non-AMF and AMF treated seedlings were harvested. Photograph and leaf area were taken and determined using a web application software called Petiole®. The software is a mobile application designed for leaf area measurement that leverages on a smartphone's camera and a calibration plate to accurately determine leaf area. As to reduce destructive sampling, only two seedlings per treatment were dug out to sample to roots. Roots of the seedling were dug out carefully, and all soil and debris were washed with care under running water to avoid destruction to the root system. Photographs of the root system was taken to record the differences in root development due of the treatments.

Data Analysis

Data analysis was conducted using an independent T-test at 5% probability ($P < 0.05$) to determine the significant difference between the two-treatment means and performed using the Statistical Package for the Social Sciences (SPSS).

RESULTS

Growth Rate of *Pentaspadon motleyi* Seedlings

Table 1 shows the height growth rate of *P. motleyi* seedlings as recorded per week in terms of height growth. Height growth rates ranged from 0.13 to 0.93 cm in non-AMF treated seedlings and 1.13 to 3.27 cm for AMF treated seedlings. Comparison of height growth rate between the non-AMF treated and AMF treated seedings found significant differences in eight of nine weeks of measurements. Two weeks indicated highly significant differences ($P \leq 0.001$) between the two treatments, while the other two weeks and four weeks were significantly different at $p \leq 0.01$ and $p \leq 0.05$, respectively. Insignificant difference was detected only in week 7.

The cumulative height growth of *P. motleyi* seedlings shows the height growth of AMF treated seedlings were far superior to the non-AMF treated seedlings (Figure 1). The mean height of AMF treated seedlings increased by 17.12 cm from 133.00 cm during the first measurement to 150.12 cm during the last measurement. Meanwhile, the non-AMF treated seedlings observed only 3.69 cm increment from 130.00 cm to 133.69 cm. After nine weeks of measurements, the AMF treated seedlings were five times taller than the non-AMF treated seedlings.

Table 1. Height growth rate per week recorded for *P. motleyi* seedlings

Week	Height Growth Rate (cm) Per Week		P-value
	Non-AMF Treated	AMF Treated	
1	0.47 ± 0.17	1.20 ± 0.28	0.032
2	0.16 ± 0.12	2.33 ± 0.33	<0.001
3	0.33 ± 0.21	1.53 ± 0.34	0.005
4	0.80 ± 0.28	2.60 ± 0. 81	0.045
5	0.40 ± 0.21	3.27 ± 0.42	<0.001
6	0.20 ± 0.14	1.93 ± 0.53	0.004
7	0.93 ± 0.32	1.60 ± 0.47	0.246
8	0.13 ± 0.09	1.13 ± 0.48	0.049
9	0.27 ± 0.12	1.53 ± 0.52	0.023

Note: Significant difference in height increment between treatment means were conducted at $P \leq 0.05$.

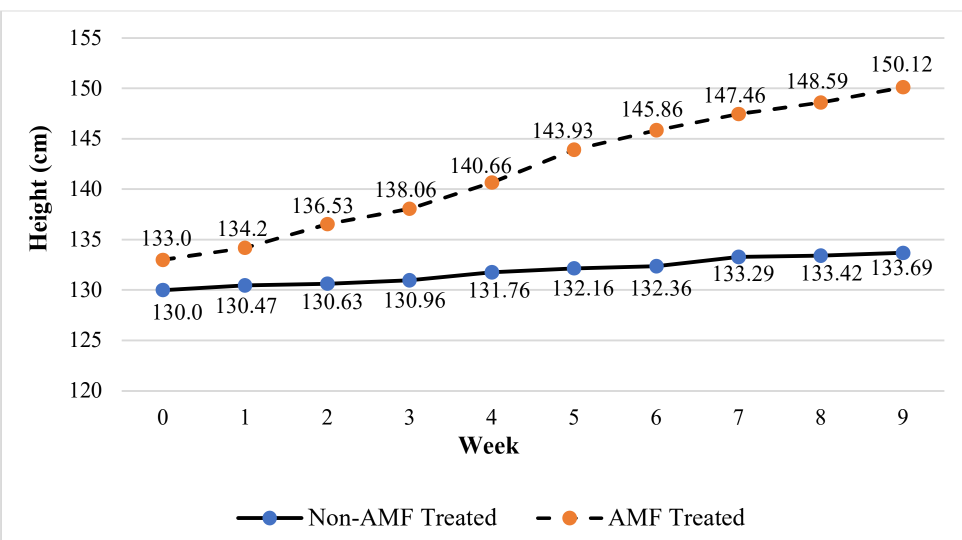

Figure 1. Cumulative height growth of *P. motleyi* seedlings

Diameter Growth Rate

Significant differences in the diameter growth rate per week for non-AMF and AMF treated *P. motleyi* seedlings were detected as shown in Table 2. Comparisons of treatments during weeks 1, 3, 4 and 6 were found to be highly significant ($P \leq 0.001$) while other weeks indicated significant differences at $P \leq 0.01$. Diameter growth rate per week ranged from 0.07 to 0.17 mm and 0.24 to 1.07 mm for non-AMF and AMF treated seedlings, respectively.

As indicated in Figure 2, the AMF treated seedlings showed superior cumulative diameter growth than the non-AMF treated seedlings. The non-AMF treated seedlings recorded an increase of 0.96 mm in total diameter growth while the AMF treated seedlings observed an increase of 4.36 mm to the initial diameter size. After nine weeks of measurement, AMF treated seedlings exhibited four to five times greater cumulative diameter growth than non-AMF treated seedlings.

Table 2. Diameter growth per week of *Pentaspadon motleyi* seedlings

Week	Diameter Growth Rate (mm) Per Week		P-value
	Non-AMF Treated	AMF Treated	
1	0.16 ± 0.05	1.07 ± 0.09	<0.001
2	0.17 ± 0.09	0.74 ± 0.19	0.012
3	0.07 ± 0.01	0.42 ± 0.07	<0.001
4	0.06 ± 0.01	0.52 ± 0.09	<0.001
5	0.14 ± 0.02	0.40 ± 0.10	0.013
6	0.08 ± 0.02	0.24 ± 0.03	<0.001
7	0.09 ± 0.01	0.36 ± 0.72	0.001
8	0.07 ± 0.01	0.24 ± 0.06	0.012
9	0.12 ± 0.02	0.37 ± 0.07	0.002

Note: Significant difference in diameter increment between treatment means were conducted at $P \leq 0.05$.

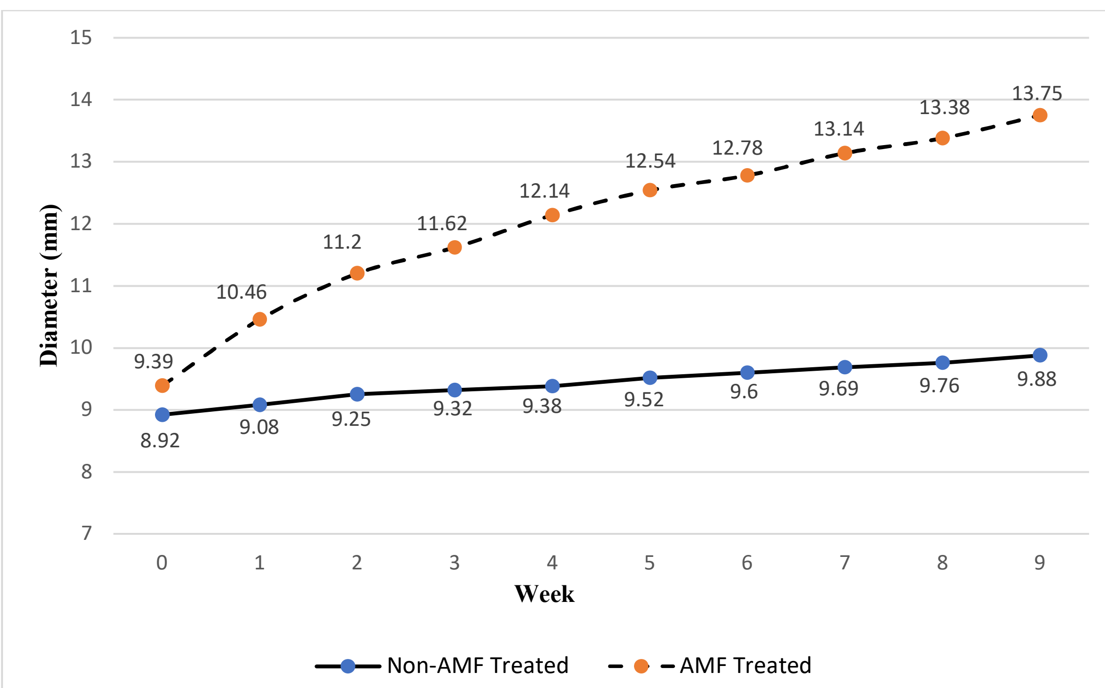

Figure 2. Cumulative diameter growth of *P. motleyi* seedlings

Leaf Number of *P. motleyi* Seedlings

Leaf number of *P. motleyi* seedlings also indicated significant difference between the non-AMF treated and AMF treated seedlings as shown in Figure 3. A decreased in leaf number was observed in non-AMF treated seedlings from 24 leaves during the beginning of the study to only 19 leaves at week 10. This is in contradiction with the number of leaves on AMF treated seedlings which showed an increase in numbers from 33 leaves at week 1 to 56 at week 10. Therefore, AMF treated seedlings has approximately 2 to 3 times more leaves than the non-AMF treated seedlings which were more obvious from week 2 onwards.

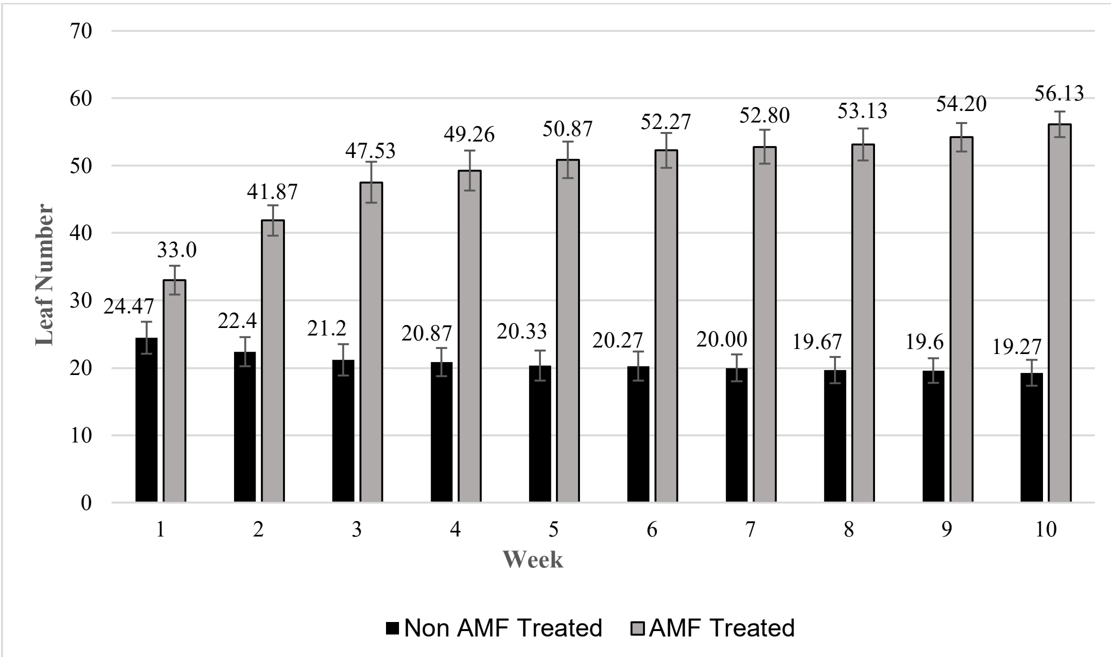

Figure 3. Cumulative leaf number of *P. motleyi* seedlings

Root growth and leaf area of AMF and non-AMF treated *P. motleyi* seedlings

Root growth of AMF treated *P. motleyi* seedlings were found better with more fibrous and fine roots as compared to the non-AMF treated seedlings after 10 weeks of transplanting (Figure 4). Besides that, the roots of AMF treated seedlings also looked darker and healthier. Higher leaf number and better leaf area expansion was observed in AMF treated seedlings. The leaf areas for non-AMF treated seedlings ranged from 21.10 cm^2 to 28.1 cm^2 with a mean value of 21.10 cm^2. Meanwhile, the mean leaf area recorded for AMF treated seedlings was 32.43 cm^2 with values ranging from 30.1 cm^2 to 35.1 cm^2.

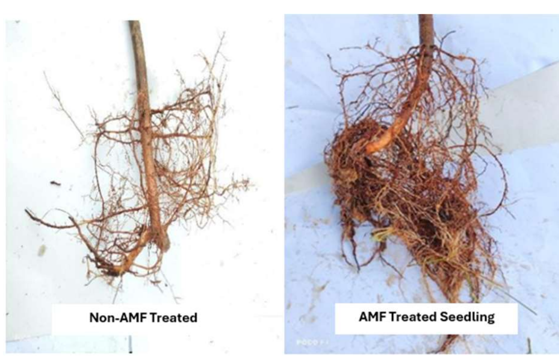

Figure 4. Comparison of root growth on AMF treated and non-AMF treated seedlings

DISCUSSION

As plants depend much on the root system for its physiological processes such as obtaining water and nutrients, plants need a well-developed root system to survive the harsh environment in the field (Rani *et al*. 2019). A good root system is vital for the survival of the seedlings after transplanting. As the research location involves a waterway which is occasionally affected by temporary flooding, the capability of young *P. motleyi* seedlings to overcome stress during the transplanting activities is an indication on the ability of the seedlings adapting to the new environment. In *Pterocarpus officinalis*, flooding has induced physiological and morphological changes in AMF inoculated seedlings making them more tolerant to flooding (Fougnies *et al*., 2007). Thus, AMF can help in improving soil characteristics and encourage continuous plant development even under stressful environment (Navarro *et al*., 2014; Alqarawi *et al*., 2014).

The enhancement of plant growth and budding potential are very important biological processes for rapid growth of plants, as well as improving the survival rate of seedlings (Morgan and Connolly 2013). AMF provides a lot of advantages to plants as young seedlings inoculated with efficient AMF will not only have stronger root system but have superior growth and can better establish themselves in the field (Navarro-Garcia *et al*., 2011).

AMF treated *P. motleyi* seedlings showed better vegetative growth in terms of plant height and collar diameter increment besides higher shoot, root, and total dry weights similar to the report by Kumar *et al*. (2017) as well as greater leaf length and leaf number by Mathur *et al*. (2016). Chinnathambi *et al*. (2024) reported that mycorrhizal plants showed greater shoot and root dry weight, leaf area and root length than non-mycorrhizal plants. Better growth performances demonstrated by the AMF treated *P. motleyi* seedlings indicated that the application of AMF has helped with the improvement and enhancement of the seedlings' growth rate. However, these findings must be interpreted with caution as this study has its limitations such as the short study duration (only 10-weeks), besides effect of other environmental factors that were not controlled during the data collection.

Plants with thriving mycorrhizal root systems can perform better in comparison to their non-inoculated counterparts (Smith and Smith, 2012). AMF enhances root growth of treated plants and assists plants in water and nutrient uptake (Kim *et al*., 2017). Better root growth can be observed in AMF inoculated *P. motleyi* seedlings where finer roots, better root spread, and more root hairs can be observed (Morgan and Connolly 2013). Similarly, high flooding tolerance by AMF inoculated *P. officinalis* seedlings was found to be attributed to the production of adventitious roots (Fougnies *et al*., 2007). AMF inoculation dramatically increase root biomass by increasing the number of 1st and 2nd order lateral root, root total length, root surface area and root volume in *Prunes persica* seedling under flooding (Zheng *et al*. 2020). Improvement of plant growth and nutrient accumulation is the effect of AMF inoculation that extends the absorption area to the root system of the host plant (Bourles *et al*., 2020).

AMF inoculated plants can explore more soil volume for available nutrients and water (Kumar *et al*. 2017). AMF plant roots can significantly improve the access of roots to a large soil surface area through the formation of hyphal network (Bowles *et al*., 2016). AMF improves plant nutrition acquisition by increasing the availability as well as translocation of various nutrients (Rouphael *et al*., 2015). The fungal hyphae can help in accelerating the decomposition process of soil organic matter (Paterson *et al*., 2016) and improves the quality of soil by influencing its structure and texture (Zou *et al*., 2016; Thirkell *et al*., 2017). In this study, quantitative root parameters such as root length, surface area, and biomass were not assessed. To strengthen the understanding of the root system's role in influencing nutrient uptake, these factors should be incorporated in future research.

The leaf blade is an important organ particularly for plant photosynthesis, respiration, and transpiration. The leaf size and shape can influence photosynthetic efficiency and are closely related to plant growth potential, nutrient supply, yield, quality, and resistance (Nicotra *et al*., 2011). In cotton seedlings, AMF supports better nutrient uptake thus promotes good vegetative growth by improving photosynthesis rate, CO_2 concentration, transpiration and energy use efficiency (Peng *et al*., 2024). AMF also contributes to an increase in chlorophyll a, photosynthetic rate, stomatal conductance, and transpiration rate (Mathur *et al*., 2016) that leads to higher plant photosynthates production and biomass development.

In the present study, AMF treated *P. motleyi* seedlings showed larger leaf area, that may contribute to better light reception by the seedlings' blade, allowing the seedlings to photosynthesize better and produce more food stock through rapid photosynthesis. Plants inoculated with AMF has been known to enhance plant growth as indicated by the increase in leaf area as well as N, P, Ca, and K contents (Balliu *et al*., 2015). According to Begum *et al*. (2019), increased in photosynthetic activities and other leaf functions are directly linked to the uptake of N, P, and carbon that improved growth of AMF inoculated plants.

AMF inoculation provides plenty of advantages to plants particularly to young seedlings (Navarro-Garcia *et al*., 2011). AMF assist plants during the initial growth by enhancing plant growth performances. AMF treatment assists growth promotion not only by improving water and mineral nutrient uptake from the soil but has also been found to safeguard the plants from fungal pathogens (Smith and Read, 2008; Jung *et al*., 2012). Similarly, long term association with AMF is speculated to enhance the survival and establishment of *P. motleyi* seedlings by improving nutrient acquisition, water relations and soil structure thus facilitating successful establishment in waterway rehabilitation.

CONCLUSION

Significant differences were observed between the AMF treated and non-AMF treated seedlings with better growth performance observed from *P. motleyi* seedlings inoculated with AMF. Plant height and collar diameter of AMF treated seedlings observed five times higher values than non-AMF treated seedlings. Leaf number and leaf area also observed similar superior trends while root morphology of AMF treated seedlings were found to be better developed and look heathier.

AMF treatment supports good root growth among *P. motleyi* seedlings. AMF that colonizes the plant root system, provide better plant growth by increasing nutrient accumulation as the inoculated roots can extend the root absorption area. In addition, AMF inoculation promotes the development of mycorrhizal hyphae that contribute to better soil exploration and nutrient uptake by the root system. Better nutrient uptake by the root system eventually enhances photosynthate production by the leaves contributing to other vegetative growth and biomass accumulation such as diameter, height, and leaf number.

Better growth performance by AMF treated *P. motleyi* seedlings is an indication that inoculated seedlings were stronger and performed better when planted in the open field. The well-developed root system of AMF treated seedlings promotes the establishment of the seedlings in the fields. This study indicates that the inoculation of *P. motleyi* seedlings with AMF can enhance early growth of the seedlings which can be a determining factor in the ability of seedlings to survive the harsh environment such as along waterways which was exposed to direct sunlight and flood.

However, it must be highlighted that this study was conducted within a short period of 10-weeks using a very small sample size thus require caution when interpreting the results. Longer monitoring period with larger sample size may be required to confirm the broader applicability of these findings. Nonetheless, this study still provides some insights into the potentials of using AMF inoculated *P. motley*i seedlings to rehabilitate disturbed waterways by promoting better early growth when being transplanted in the open field.

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