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# Effectiveness of Mycorrhizal Soils from Miombo Woodland on the Growth and Survival of *Pinus* Seedlings in a Nursery

**Chikumbutso Manyalo**
Department of Forestry, Faculty of Natural Resources, Lilongwe University of Agriculture and Natural Resources, P.O. Box 219, Lilongwe, Malawi, East Africa

**Gift Kamanga-Thole**
Department of Forestry, Faculty of Natural Resources, Lilongwe University of Agriculture and Natural Resources, P.O. Box 219, Lilongwe, Malawi, East Africa

**Edward Missanjo***
Department of Research, Malawi Assemblies God of University, P.O. Box 184, Lilongwe, Malawi, East Africa
Email: edward.em2@gmail.com

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## Abstract

Pine seedling production is challenged by in availability of pine plantations, where mycorrhizae soils would be collected. Pine plantations are found in distant sites from farmers due to different silvicultural zones. This might hinder the success of afforestation and reforestation programmes. This study was carried out to find out the effectiveness of using mycorrhizae soils from miombo woodlands in enhancing survival and growth of pine seedlings. Seedlings were subjected to four different mycorrhizae to ordinary soil ratios treatments which were completely randomized in four replicates, namely: T1 (100% mycorrhizae soil from old pine plantations); T2 (100% mycorrhizae soil from miombo woodlands); T3 (1:10-mycorrhizae soil from miombo woodland to ordinary soils); and T4 (1:10-mycorrhizae soil from old pine plantations to ordinary soils) as a control. The results indicate that there were significant ($P$<0.05) differences among treatments on mean height, root collar diameter and survival of Pinus seedlings after 12 weeks of pricking out. Pinus seedlings subjected to T1 and T2 produced higher height, root collar diameter growth and higher survival rate than those subjected to T3 and T4. There were no significant ($P$>0.05) differences between T1 and T2 on Pinus seedlings height, root collar diameter growth as well as survival rate. However, seedlings from T2 had higher height (14.09±0.19cm) and root collar diameter (0.34±0.01cm) growth than seedlings from T1 (13.86±0.14, 0.33±0.02cm) respectively. On the other hand, seedlings from T1 had higher survival rate (100%) than those from T2 (90±5.06%). Therefore, the study recommends that farmers to adopt the use of 100% miombo woodland soil in raising *Pinus* seedlings as an alternative, where there is scarcity of old pine plantation soils.

**Keywords:** Afforestation; Reforestation; Height; Root collar diameter; Scarcity.

## 1. Introduction

Mycorrhiza is an anatomical structure that results from a symbiotic association between a soil fungus and plant roots. It is recognized that mycorrhizae association play a key role in the nutritional status and growth of forest trees more especially pines [1]. According to the tree species and the growing practices and conditions, mycorrhizae provide different benefits to the plants and the environment. Its association promotes seedling survival, growth through enhanced nutrient uptake during nursery stage [2]. It also reduces nutrient additions within the nursery management programs and protects the roots against soil borne pathogens [3].

There is a symbiotic relationship between pine seedlings and mycorrhizal fungi. The fungi produce thin, hair-like threads of cytoplasm called hyphae, with a hyphal tip at each end. One tip enters a plant root and the other tip explores the soil matrix [4]. The mycelial network can extend across many hectares although the hyphae are small in diameter. These networks extend from the root system into the bulk soil, well beyond the zone occupied by the roots and root hairs. According to Marx, *et al.* [4], the absorptive area of mycorrhizal hyphae is approximately 10 times more efficient than that of root hairs and about 100 times more efficient than that of roots. However, the mycorrhizal relationship benefits both the host plant and the fungi. The plant provides the fungi with carbohydrates and in exchange, the fungi increase the plants ability to take up phosphorus and micronutrients from the soil and provide protection from certain root diseases. It also improves the plants ability to access water from the surrounding soil, which helps to keep the plant hydrated in the dry soil conditions.

Furthermore, networks of fungal hyphae also provide an important first step for the polymerization of plant sugars derived from photosynthesis leading to the formation of humus, a high molecular weight gel-like substance that holds four to twenty times its own weight in water. Humic substances significantly improve soil structure, porosity, cation exchange capacity and plant growth. This also improves aggregate stability, enhance soil structure and build stable soil carbon.

Six types of mycorrhizae have been classified, but they are categorized into two groups: the ectomycorrhizae and the endomycorrhizae [5]. Ectomycorrhizae coat the outside of roots and infect the cell walls of root tissue. They

*Corresponding Author

associate with woody plants and produce fruiting bodies. Many of our edible forest mushrooms are ectomycorrhizal fungi. This is extremely more diverse group of fungi, but associates with only a minority of the plant species hence used in miombo woodlands and pine plantations.

Endomycorrhizal fungi actually penetrate into root cells and form tree like structures called arbuscules, which are thought to aid in nutrient exchange. They are also called arbuscular mycorrhizal fungi (AM fungi). These are different division, or phyla, of fungi and do not produce mushrooms. Instead they have tiny underground fruiting bodies that are rarely seen by people. Endomycorrhizal fungi are far less diverse than ectomycorrhizae and comprise of only six genera. They however associate with plants both woody and herbaceous. Arbuscular mycorrhizal fungi have the potential to influence the economic benefits of agricultural systems through both direct and indirect processes related to plant nutrition [6], access to moisture in water-limiting situations [7], building soil structure [5], protection of soil carbon in aggregates [8] and strengthening plant resilience to disease [9].

Miombo woodland is a significant biome covering about 10% of the African landmass [10, 11]. Miombo woodlands are found in most countries of Southern and Central Africa and are the dominant forest component of Angola, Malawi, Mozambique, Tanzania, Zambia and Zimbabwe [12]. Miombo trees are dominated by generas; *Brachystegia, Julbernardia* and *Isoberlina (Fabaceae,* subfamily *Caesalpinioideae)* but many other species also occur.

Reak, *et al.* [13], indicated that pine seedlings need mycorrhiza to survive. Pine seedling production is challenged by availability of pine plantations, where mycorrhiza would be collected. This affects afforestation programs negatively in Malawi. Pine plantations are found in distant sites from farmers due to different silvicultural zones. However, miombo woodlands, another source of mycorrhiza is commonly found in Malawi. Perhaps mycorrhiza from miombo woodlands would substitute that from pine plantations and help increase pine seedling production. Unless the effectiveness of miombo woodland mycorrhiza is deduced, farmers will continue expecting the use of mycorrhiza from pine plantation which will continue discouraging them to raise pine seedlings. It is against this background that the study was carried out to find out the effectiveness of using mycorrhizal soils from miombo woodlands in enhancing growth and survival of pine seedlings. The information will be used in promoting production of pine seedlings for reforestation programs in Malawi and nearby countries, in places away from pine plantations.

# 2. Materials and Methods

## 2.1. Study Site

The study was conducted in Malawi at Bunda Forestry Department nursery where pine seedlings were raised. Soil analysis and identification of mycorrhiza fungi was done in soil and plant pathology laboratory at Lilongwe University of Agriculture and Natural Resources (LUANAR), located in the central region of Malawi which lies at the coordinates of $14^0$ 35'S and $33^0$ 50' E, and 1200 m above sea level. The area receives an annual rainfall of 875 mm to 1000 mm. Soil types vary from clay loam to sandy loam textural classes [14].

## 2.2. Seed Acquisition and Soil Collection

*Pinus oocarpa* seeds were supplied by Forestry Research Institute of Malawi (FRIM) and soils were collected from three different sources: Mycorrhizal soils from old pine plantation in Chongoni and miombo woodland in Bunda forest; and ordinary soil from Bunda farm. The soils were collected at a depth of 0-30 cm. The characteristics of the soil collected are presented in Table 1.

**Table-1.** Characteristics of the soil collected

| Soil property | Mycorrhizal soil- Pine plantation | Mycorrhizal soil - Miombo woodland | Ordinary soil- Bunda Farm |
|---|---|---|---|
| Organic matter (%) | 5.55 | 6.93 | 2.86 |
| Phosphorus (mg $kg^{-1}$) | 60.90 | 144.36 | 80.64 |
| Zinc (mg $kg^{-1}$) | 0.75 | 1.37 | 0.78 |
| Iron (mg $kg^{-1}$) | 36.62 | 17.69 | 10.80 |
| Manganese (mg $kg^{-1}$) | 16.50 | 13.20 | 17.26 |
| pH ($H_20$) | 5.82 | 6.72 | 5.52 |
| Number of colonies | | | |
| • White | 6 | 60 | 2 |
| • Orange | 16 | 4 | 2 |

## 2.3. Experimental Design and Treatments

Seeds of *Pinus oocarpa* were sown in one tray of sand on February 11, 2019. Sand was used in this case to promote easy germination. After successful germination, on February 25, 2019, a total of 1200 seedlings were pricked out (transferring of the seedlings from a seedbed into black polythene tubes) into black polythene tubes of size 0.48 $m^3$. The purpose of this operation was to allow the seedlings to grow singly per tube. This helps to produce healthy seedlings that grow vigorously because of the removal of competition, and it was done under a shade in order to avoid death of the seedlings due to direct sunlight that could have caused high transpiration. The seedlings were subjected to four different mycorrhizae to ordinary soil ratios treatments which were completely randomized in four replicates. Each treatment had 75 seedlings. The treatments were:

T1: 1:0 (100% mycorrhizae soil from old pine plantation)
T2: 1:0 (100% mycorrhizae soil from miombo woodland)
T3: 1:10 (miombo woodland mycorrhizae soil to ordinary soil)
T4: 1:10 (old pine plantation mycorrhizae soil to ordinary soil) as a control

Watering was done regularly twice a day, in the morning hours and at sun set, to maintain adequate moisture necessary for seedling growth as recommended by Ingram and Chipompha [15].

## 2.4. Data Collection and Analysis

Height, root collar diameter and survival rate measurements for each seedling was recorded once every week for a period of twelve weeks from the date of pricking out. Analysis of variance (ANOVA) for height, root collar diameter and survival rate were performed according to the model shown in Table 2 to test the significance of soil ratios and time effects. Soil ratios and time were both considered as fixed effects. Variance components for the sources of variation were also estimated. Statistical analysis was performed using GenStat 18.1 for Windows.

**Table-2.** Model used in analysis of variance

| No. | Source of Variation |
|---|---|
| 1 | Soil ratios (S) |
| 2 | Time (T) |
| 3 | S x T |
| 4 | R/T/S |

# 3. Results

## 3.1. Seedlings Height and Root Collar Diameter Growth

Summary of the results on seedling height and root collar diameter growth are presented in Tables 3 and 4 as well as in Figure 1. The results indicate that soil ratios, time and the interaction between soil ratios and time significantly ($P<0.05$) influenced seedling height growth. However, time was highly ($P<0.001$) significant and contributed the highest (93.62%) of the total variation. On the other hand, only soil ratios and time significantly ($P<0.05$) influenced root collar diameter growth. Contrary to seedling height growth, soil ratios contributed the highest (50.06%) of the total variation on root collar diameter growth.

There were no significant ($P>0.05$) differences on seedling height growth between 100% mycorrhizae soils from pine plantation and miombo woodland. However, seedling height growth from miombo woodland was slightly higher (14.09±0.19 cm) than seedling height growth from pine plantation (13.86±0.14 cm). Mixture of pine and ordinary soil (1:10) and a mixture of miombo and ordinary soil (1:10) had the least mean seedling height growth.

The 100% mycorrhizae soils from both miombo and pine plantation gave the highest seedlings mean height and root collar diameter growth which increased rapidly between week 3 and 9, then increased at a decreasing rate between week 9 and 12. A similar trend was also observed for the other treatments, but with lowest values of mean height and root collar diameter growth (Figure 1).

**Table-3.** Mean height, root collar diameter and survival rate of Pinus seedlings at 12 weeks after pricking out

| Treatment (Mycorrhizae to ordinary soil ratio) | Height (cm) | Root collar diameter (cm) | Survival (%) |
|---|---|---|---|
| 1:0 (100% MS from old pine plantation) | $13.86\pm0.14^{a}$ | $0.33\pm0.02^{a}$ | $100\pm0.00^{a}$ |
| 1:0 (100% MS from miombo woodland) | $14.09\pm0.19^{a}$ | $0.34\pm0.01^{a}$ | $90\pm5.06^{a}$ |
| 1:10 (miombo woodland MS to ordinary soil) | $7.97\pm0.23^{b}$ | $0.21\pm0.02^{b}$ | $50\pm4.08^{b}$ |
| 1:10 (old pine plantation MS to ordinary soil) | $8.61\pm0.19^{b}$ | $0.23\pm0.02^{b}$ | $55\pm2.89^{b}$ |

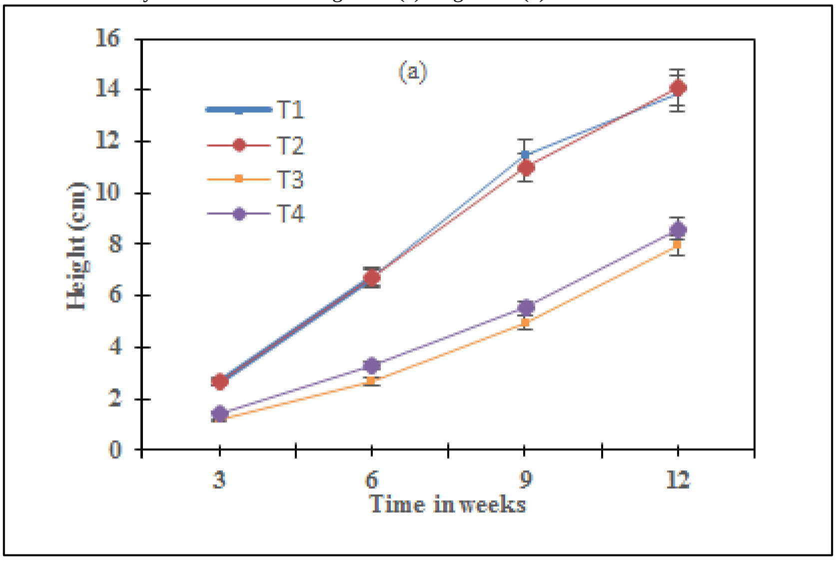
MS=mycorrhizae soil; Mean values are followed by standard errors; Means with different superscript within a column are significantly different ($P<0.001$)

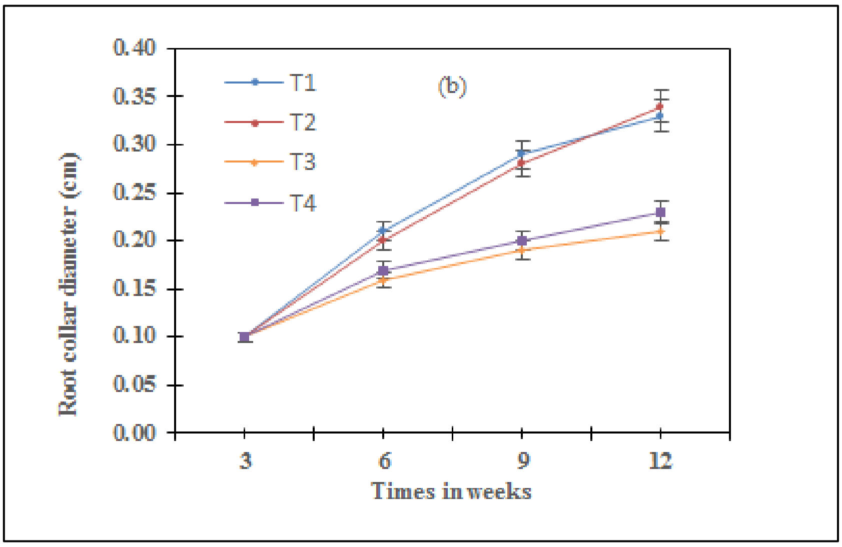
**Table-4.** Variance components for height, root collar diameter and survival rate

| Source of variation | *df* | Height | | Root collar diameter | | Survival rate | |
|---|---|---|---|---|---|---|---|
| | | ***P*-value** | **Var %** | ***P*-value** | **Var %** | ***P*-value** | **Var %** |
| Soil ratios (S) | 3 | <0.001 | 4.03 | 0.010 | 50.06 | <0.001 | 61.22 |
| Time (T) | 3 | <0.001 | 93.62 | <0.001 | 46.03 | <0.001 | 20.92 |
| T x P | 9 | 0.006 | 2.03 | 0.709 | 2.17 | <0.001 | 9.64 |
| R/T/S | 304 | | | | | | |

df degree of freedom; var variance (%)

**Figure-1.** Effect of mycorrhizae soil on the growth (a) height and (b) root collar diameter of Pinus seedlings




### 3.2. Seedlings Survival

Summary of the results on the survival rate of Pinus seedlings are presented in Tables 3 and 4. The results indicate that soil ratios, time and the interaction between soil ratios and time significantly ($P$<0.001) influenced the survival rate of the Pinus seedlings. However, soil ratios contributed the highest (61.22%) of the total variation. Pine soils had the highest survival rate of 100% which was not significant to miombo soil with survival rate of 90±5.06%. It is also evident from the results that the survival rate decreased with an addition of ordinary soil to pine and miombo soils.

## 4. Discussion

### 4.1. Seedlings Height and Root Collar Diameter Growth

The findings in the present study indicates that mycorrhizae inoculation of seedlings results into rapid seedling growth. This is in agreement with findings by Simard and Durall [16]. Simard and Durall [16] reported that mycorrhizae fungal hyphae explores nutrients from soil not yet colonized by feeder roots and increase absorptive surface area for nutrients and water there by necessitating seedling growth. Due to high organic matter percentage of 6.93 (Table 1) in miombo woodland soil attributed to soil characteristics of good aeration, structure, drainage, moisture holding capacity and nutrient availability directly. Humus has an effect on the soil structure. The aggregates which were sustained by roots and hyphae of ectomycorrhiza fungi were held together by bonds of organic compounds which reduces surface crusting, enabling water to enter the soil in the tubes more easily and percolate downward through the soil improving drainage and ease of root penetration [17].

Furthermore, water holding capacity of soil also increased due to the presence of organic matter. The capacity to store water was improved by water absorbing materials such as mycorrhiza (ectomycorrhiza fungi) as collected from the forest floor, through their relationship with the tree seedlings. Most soil nitrogen and phosphorus of 144.36ppm in miombo woodland and that in other soil sources (Table 1) is organic and other elements such as sulfur are associated with humus as such, mycorrhiza in the soil increases availability and absorption of these nutrients [16]. In addition, the buffering capacity of miombo soil with pH of 6.72 (Table 1) increases with an increase in organic matter thus a soil high in organic matter will not be susceptible to sudden changes in acidity [17]. This result in good performance of seedlings under miombo woodland soils.

### 4.2. Seedlings Survival

The highest survival rate was recorded in mycorrhizae soil from old pine plantation seconded by, that from miombo woodland and the lowest mean from the ratio of miombo to ordinary soil (Table 3). This indicates that seedlings inoculated with 100% mycorrhizae soils have high chances of establishing in the nursery. This is in agreement with the findings of Wang, *et al.* [18]; Missanjo and Thole [19] who reported that pines need mycorrhizae to survive. Similarly, Lee and George [20] reported that mycorrhizal hyphae had also a significant contribution in uptake of phosphorus and zinc by inoculated cucumber seedlings, which resulted in an increased concentration of those nutrients in the plant shoots, hence higher survival rate of seedlings in the nursery. Therefore, the amount of phosphorus and zinc in different mycorrhiza soil sources affect the survival rate of the seedlings.

Seedlings grown on pure miombo woodland soil demonstrated a higher mean height and root collar diameter growth and higher survival rate which were not significantly different from old pine plantation soil. Therefore, the study recommends that farmers to adopt the use of 100% miombo woodland soil in raising Pinus seedlings as an alternative, where there is scarcity of old pine plantation soils.

## 5. Conclusion

The study has revealed that Pinus seedling height and root collar diameter growth as well as survival rate was influenced by different mycorrhizae to ordinary soil ratios. Pinus seedlings subjected to 100% mycorrhizae soils produced higher height, root collar diameter and higher survival rate than those subjected to the practiced ratio of 1:10 (mycorrhizae to ordinary soils). There were no significant differences between 100% mycorrhizae soils from miombo woodland and old Pinus plantation on Pinus seedlings height, root collar diameter as well as survival rate. However, seedlings from 100% mycorrhizae soils from miombo woodland had higher height and root collar diameter growth than seedlings from 100% mycorrhizae soils from old Pine plantations. On the other hand, seedlings from 100% mycorrhizae soils from old Pine plantation had higher survival rate than those from 100% mycorrhizae soils from miombo woodlands. Therefore, the study recommends that farmers to adopt the use of 100% miombo woodland soil in raising *Pinus* seedlings as an alternative, where there is scarcity of old pine plantation soils.

## 6. Data Availability

The data that support the findings of this study can be obtained from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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