

Establishment of Africa Red Mahogany (*Khaya anthoteca*) Pre-Inoculated with Arbuscular Mycorrhizae Fungi (AMF) and Compost Application on an Ex-Coal Mined Site

Philip Worlanyo Dugbley^{1,*}, Irdika Mansur^{1,2}, Basuki Wasis¹

¹Department of Silviculture, Faculty of Forestry, Bogor Agricultural University, Bogor-Indonesia

²SEAMEO Biotrop, Jalan Raya Tajur 6K, Bogor, Indonesia

Email address:

philidug87@yahoo.com (P. W. Dugbley), irdikam@gmail.com (I. Mansur)

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Abstract: Coal mining provides a means for creating wealth and significantly contributes to export earnings, economic activity and employment whilst supporting regional development. However, coal mining is one of the most severe disturbances in terrestrial ecosystems. Thus, the removal of the natural vegetation and upper soil horizons for mining exploration hinders the establishment and survival of plant and soil microbial communities. Revegetation of ex-coal mined lands is therefore required to enable the recover, as close as possible, to its previous integrity. The establishment of tree species capable of protecting the underlying soil and its micro-fauna and flora is one way of achieving this aim. This study therefore aims to investigate the effect of arbuscular mycorrhizae fungi (AMF) pre-inoculation and compost application on the growth performance of the Africa red mahogany, *Khaya anthoteca* on an ex-coal mined site. The field design for this study was the completely randomized design (CRD) in factorial experiment. Four (4) levels of each factor namely compost and AMF were used with sixteen treatment combinations and each treatment replicated four times giving sixty four (64) experimental units. The results indicated that compost has significant effect ($P < 0.001$) on height, diameter and leaf increment with steady increment during this study. There was no significant effect of mycorrhizae treatment as well as interaction between both factors (AMF and compost) on the growth of *K. anthoteca*. However, compost composition from a mixture of *Salvinia natans* and that prepared from the paddy husk (C3) recorded the highest increment in height of 9.31 cm while compost from *S. natans* only (C1), rice hull compost; herein known as paddy husk compost (C2) and control (C0) recorded increments of 9.00 cm, 5.78 cm and 4.47 cm respectively. The arbuscular mycorrhizae fungi played a role in the survival of the species on the field. There was percentage difference of between 18.5-37.5% over the control treatment. AMF from *Glomus manihotis* had the highest percentage survival of 81.25% while the control treatment of mycorrhizae had the lowest percentage of 43.75%. Plants are also able to withstand harsh environmental conditions through fungi-plant symbiosis enhancing the chances of survival on the field and thus, aiding the plant establishment. The study concludes that AMF and compost applications are feasible and sound technologies for the establishment of *K. anthoteca* on ex-coal mined sites.

Keywords: Coal Mining, *Khaya anthoteca*, Plant Growth, Arbuscular Mycorrhizae Fungi (AMF), Compost

1. Introduction

Coal mining provides means for creating wealth and enhances export earnings, economic activities as well as employment whilst supporting regional development. For example, the mining sector of Indonesia contributes to the nation's economy currently, for about 11.54% of total GDP [23]. However, coal mining is one of the most severe disturbances in terrestrial ecosystems. It causes large-scale

deforestation and land degradation with complete loss of topsoil. Thus, the removal of the natural vegetation and upper soil horizons for exploration and mining that hinders the establishment and survival of plants and soil microbial communities [7]. Mining also results in the formation of artificial habitats that are microbiologically poor, requiring human intervention for their proper restoration [27].

Revegetation of coal mined lands is therefore required to enable the re-use of such lands for other purposes. The establishment of tree species capable of protecting the underlying soil and its micro-fauna and flora is one way of achieving this aim.

Khaya anthoteca commonly referred to as the Africa red mahogany is a species belonging to the family Meliaceae. This species is heavily exploited, particularly in East and West of Africa. The species is used in high class cabinetwork and for the production of veneers and any application where good quality, medium weight hardwood is needed [13]. It also weathers well and is resistant to borers and termites. The dense crown makes it suitable as a shade tree and also popular for windbreaks as well as for aesthetic purposes. It has successfully been introduced in South Africa, Cuba and Puerto Rico and on a limited scale in Indonesia and Peninsular Malaysia where it has been used in plantations and also in Taungya systems.

Mycorrhizal fungi have over the years played critical roles in nutrient cycling and the functioning of ecosystems. According to [29], mycorrhiza fungi are the main pathway through which most plants obtain mineral nutrients and as such, are critical in terrestrial ecosystem functioning. In this mutualistic symbiosis, plants exchange photosynthates, not only for mineral nutrients, but also for increased resistance to disease, drought and extreme temperatures. Thus, plants are able to withstand harsh environmental conditions through fungi-plant symbiosis. It also been reported that mycorrhizal fungi are removed entirely in newly graded lands and always requires inoculation if the objective is a functional terrestrial ecosystem. Eroded land is also in nearly the same condition. The arbuscular mycorrhizal fungi (AMF) can therefore be integrated in soil management. These are structures resulting from the symbiosis between these fungi and plant roots, occurring in most soils and colonize roots of many plant species and directly involved in plant mineral nutrition. The symbiotic root-fungal association increases the uptake of less mobile nutrients [21], essentially phosphorus (P) but also of micronutrients like zinc (Zn) and copper (Cu), the symbiosis has also been reported as influencing water uptake. AMF can also benefit plants by stimulating the production of growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses as well as increasing resistance to pests and soil borne diseases [1] and benefits have mainly been attributed to improved phosphorous nutrition.

Furthermore, the addition of composted residue is an effective treatment for increasing rhizosphere aggregate stability. Mycorrhiza is increasingly important for improving the growth of seedlings following the addition of composted residue to soil under severe climatological conditions as reported by [6]. They also reported that high proportion of stable aggregates of soil is mainly attributed to a higher microbial activity of root biomass and particularly to the presence of vesicular arbuscular mycorrhiza in the rhizosphere aggregates. At the same time, reforestation techniques based on the addition of composted residue and

mycorrhizal inoculation in the nursery could be used as a tool for improving soil structure, with subsequent improvement in plant growth. This study therefore aims to investigate the effect of arbuscular mycorrhizae fungi (AMF) pre-inoculation and compost applications on the growth performance on vegetatively propagated *K. anthoteca* (Africa red mahogany), on an ex-coal mined site located in South Sumatra of Indonesia.

2. Materials and Methods

2.1. Study Area

This research was conducted in a coal mining site namely; PT. Bukit Asam located in South Sumatra of Indonesia. South Sumatra falls within coordinates 2°45'S 103°50'E of Indonesia. The area is characterized by a bimodal rainfall pattern with the major wet season between May and July. This area experiences a short dry season in August and a long one between December and March. The annual rainfall ranges between 2000 mm and 3000 mm. The study was conducted from August to December, 2014.

2.2. Research Materials and Tools

Materials and tools used for the study included; record log sheets, clip board, pencil, tape measure, electronic caliper, meter rule, analog weighing scale, non-poisonous marker, Microsoft excel and Minitab Version 16. Three-month old *K. anthoteca* seedlings were obtained from Carita, Banten province of West Java, Indonesia.

2.3. Experimental Design and Layout Procedures

The design for this field research was the Factorial experiment in Completely Randomized Design (CRD). Two factors; mycorrhizae and compost with four levels of each factor were used giving a total of sixteen (16) treatment combinations and each treatment combination replicated four (4) times. In total, sixty-four (64) experimental units were used for this study. The specified treatments (M) for mycorrhizae were as follows; M₀ (control; without mycorrhiza inoculation); M₁ (±50 single spores of *Gigaspora margarita* mycorrhizae); M₂ (±50 spores of *Glomus manihotis* mycorrhizae); M₃ (±25 spores each of *Gigaspora margarita* and *Glomus manihotis* mycorrhizae). The treatments (C) for compost were; C₀ (control; no compost); C₁ (±5 Kg of *Salvinia natans* compost); C₂ (±5 Kg of paddy husk compost); C₃ (±2.5 Kg each of paddy husk and *Salvinia natans* composts). Planting space of 4 x 4 m (625 plants/ha) was used and the various treatment combinations were randomly allocated to the plots with each plot having one of the treatment combinations. Reference points of 5 cm above the soil surface of the seedlings were marked with non-poisonous indelible ink marker to provide consistency at the point of height and diameter measurements.

2.4. Data Collection and Analysis

The plant parameters measured were the height (cm), stem diameter (mm), leaf counts and percentage survival. According to [17], height and stem diameter are some of the frequently used methods of measuring the growth of multicellular living systems and also often advantageous to use several characteristics for the same system. An indelible ink was used to mark each plantlet 5cm above the soil, where the diameter and height readings were taken since irregularity of the soil around the seedlings can affect the recording. Initial measurements of plant parameters were recorded two days after transplanting and fortnightly thereafter over sixteen weeks (4 months) of the field research. Data on percentage survival was collected after the field experiment. Data on plant parameters were subjected to a two-way analysis of variance (ANOVA) at a significance level of 5% ($\alpha \leq 0.05$). Microsoft excel was used in data entry and computation for the increment of the various plant parameters measured (data management). All statistical analyses were performed using the Minitab statistical analysis package V.16. (Minitab, Inc.). Tukey's Honest Significant Difference test was used for multiple comparison tests where treatment means differed significantly. The mathematical model for the research design is as follows;

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk}$$

Where:

Y_{ijk} = the (ijk)th observation for $i=1, 2, 3$ and $j=1, 2, 3$ and $k=1, 2, 3$ and 4 ; and $k=1, 2, 3$ and 4

μ = parameter common to all treatments (the mean)

τ_i = the i th treatment effect of factor A (mycorrhizae)

β_j = the j th treatment effect of factor B (compost)

$(\tau\beta)_{ij}$ = the (ij)th interaction effect of factor A and B

ϵ_{ijk} = random error component with normal distribution

3. Results and Discussion

3.1. Plant Growth

Growth is the irreversible increase in the size of a plant and has also been considered as the product of its physiological processes. Plants have indeterminate growth, thus, they have the capacity to grow from the apical meristem indefinitely. Growth generally occurs in cycles such as seasonally or daily. Factors affecting the growth of plants are broadly categorized into genetic and environmental factors as well as the interaction between these factors.

3.2. Growth of *K. anthoteka* on an Ex-Coal Mined Site

The growth of plants depends on the availability of nutrients from the soil. Thus, it is important that the soil should continuously provide nutrients for the growth and development of plants [25].

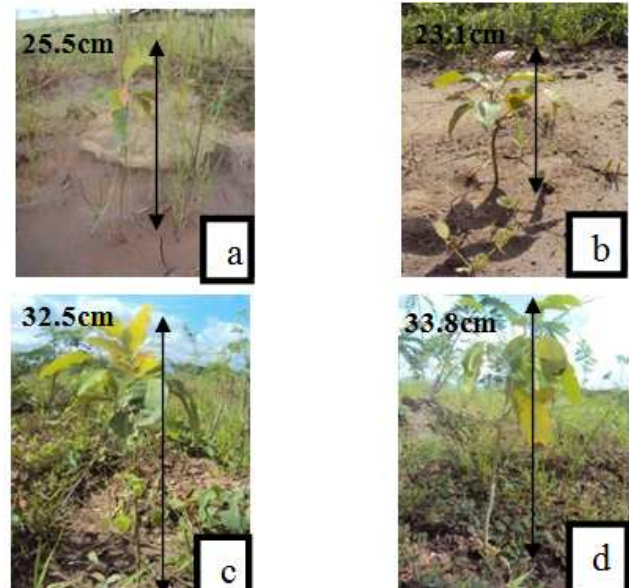


Figure 1. Growth of *Khaya anthoteka* on the field: a) Paddy husk compost b) Without Compost c) *Salvinia natans* compost d) Mixed compost.

Compost application to *K. anthoteka* had a significant effect on the height growth of the species. Analysis of variance (ANOVA) tested at $\alpha \leq 5\%$ showed a significant effect ($P < 0.001$) of compost on the mean height increment. There was no significant effect ($P > 0.05$) of mycorrhizae treatment as well as the interaction between both factors (Table 1). Compost composition from a mixture of *S. natans* and that prepared from rice hull (herein known as paddy husk compost) recorded the highest increment in height of 9.31 cm while compost from *S. natans* only (C1), paddy husk compost (C2) and control (C0) had increments of 9.00cm, 5.78 cm and 4.47 cm respectively. However, there was no difference between increments of C3 and C1 (Table 4). An increment of between 29.31-100.22% of the compost treatment over the control was recorded. The use of compost to enhance the growth of plants has been reported and figure 1 shows the final heights of the plants at the time of termination of this study with the various compost treatments. Compost application significantly enhances mitigate the negative effect of a delay in sowing. The use of compost, therefore, is a sound technology for combating soil degradation and often leads to humus accumulation in the soil and thereby supplying nutrients in several binding forms to the soil matrix. Compost also has dynamic impact on exchange processes between root system and sorption complex. This has mainly been attributed to the humified OM and the colloid properties of the humic substances [22].

However, the nutrient effect of organic composts depends largely on the transformation processes that take place in the soil after their application. This applies especially to nitrogen (N), for which its plant availability is tightly related to microbial transformations. The results of this study are in conformity to earlier reports that, compost application significantly enhances the growth of plants with respect to height. Effect of compost prepared from organic material,

thus, *S. natans* at a rate of 3.13 tons/ ha on the increment of *K. anthoteca* height was significantly different from that observed both in the control and compost from a less organic material such as paddy husks (rice hull) compost treatments. Laboratory analysis of paddy husk compost (C2) and composite soil of the research site showed low levels of organic carbon and nitrogen of 5.23%; 1.05% and 0.39%;

0.07% respectively. On the other hand, compost prepared from *S. natans* had 15.94% of organic carbon and 1.15% of total nitrogen. According to the European Commission, (2001), the degradability and C/N ratio of the organic matter determines whether it comes to a net mineralization or immobilization of N after the application of an organic fertilizer.

Table 1. Analysis of composts

No	Parameter	<i>Salvinia natans</i> compost	Rice hull (paddy husk) compost ^b
1	Moisture Content (%)	78.27	40.28
2	pH	6.96	6.55
3	Ash content (%)	49.61	72.35
4	Organic material (%)	19.32	5.34
5	Organic carbon (%) ^a	15.94	5.23
6	C/N	13.86	4.98
7	N (%) ^a	1.15	1.05
8	P (mg/100g)	19.34	11.84
9	K (mg/100g)	835.84	158.67
10	Mn (ppm) ^a	914	50.32
11	Fe (ppm) ^a	12349	

^aSample analyzed at SEAMEO Biotrop. ^bSample analyzed by PT. Bukit Asam (South Sumatra)

Again, phosphorus (P), another important and macro nutrient for plant growth responsible for photosynthesis and energy transfer was relatively high (19.34 mg/100 g) in compost from *Salvinia natans* as compared to that of community compost from paddy husk of 11.84 mg/100 g (Table 1). Also, according to the [30], compost products contain a considerable variety of macro and micronutrients. Although often seen as a good source of nitrogen, phosphorous, and potassium, compost also contains micronutrients essential for plant growth. Since compost contains relatively stable sources of organic matter, these nutrients are supplied in a slow-release form. It also improves the cation exchange capacity of soils, enabling them to retain nutrients longer. It allows tree crops to more effectively utilize nutrients while reducing nutrient loss by leaching.

Table 2. Two-way analysis of variance for height increment (cm)

Source	Df	SS	MS	F	P-Value
Mycorrhiza	3	3.66	1.22	0.45 ^{ns}	0.72
Compost	3	272.71	90.90	33.50*	0.00
Interaction	9	22.09	2.45	0.90 ^{ns}	0.52
Error	48	130.26	2.71		
Total	63	428.72			

* Significant at alpha (α) \leq 5%^{ns} not significant

Microorganisms can also promote root activities as specific fungi work symbiotically with plant roots, assisting them in the extraction of nutrients from soils. In a symbiotic association, fungus colonizes plant root hairs through the cortex cells and acts as an extension of the root system. This type of association is characterized by the formation of arbuscles (finely branched hyphal structures) in the region of the root cortex that may function as nutrient organs or nutrient exchange sites between the symbionts as well as fungal multiplication. Effect of AMF inoculation to *K. anthoteca* for height increment was not significantly different from the control treatment although the means of the inoculated propagules were relatively higher compared to the control. *Glomus manihotis* inoculation recorded a height increment of 7.51 \pm 0.62 cm while *Gigaspora margarita*, mixed culture and the control treatment recorded increments of 7.18 \pm 0.69 cm, 7.06 \pm 0.646 cm and 6.84 \pm 0.70 cm respectively. According to [8] the AMF genera *Gigaspora* and *Scutellospora* produce only arbuscules with extensive intraradical and extraradical hyphal networks whereas *Glomus*, *Entrophospora*, *Acaulospora*, and *Sclerocystis* in addition, also produce vesicles. Arbuscules are usually short-lived and begin to collapse after a few days while vesicles are storage structures and can remain in roots for months or even years [4].

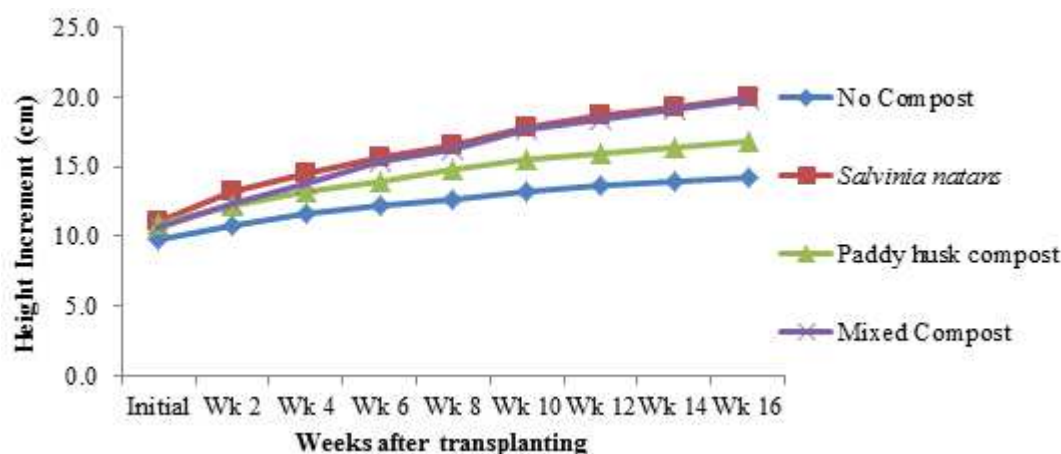


Figure 2. Effect of compost treatment on height increment (cm).

3.3. Diameter Increment

Increase in plant diameter or girth has often been considered as a secondary growth. However, with the addition of organic materials this process can be hastened. Analysis of Variance (ANOVA) tested at $\alpha \leq 5\%$ showed a significant effect ($P \leq 0.000$) of compost on the mean plant diameter increment with steady increment for the study period. There was no significant effect ($P = 0.148; 0.188$) of mycorrhizae treatment as well as the interaction between both factors on diameter increment (Table 3). In terms of diameter increment, compost from *S. natans* recorded the highest mean increment of 3.61 ± 0.238 mm although there was no significant difference between the means of *S. natans* (C1) and mixture compost (C3) according to Tukey's Honest Significant Difference (HSD) method at 95.0% confidence (Table 5). There was however percentage increment of between 9 and 48% of compost treatment over the control experiment.

Although soil may vary considerably in structure and in physical, chemical and biotic properties, the rate of growth of a seedling is influenced by those properties of the soil. From the soil, the plant derives its nutrients and it is a storehouse for water and oxygen, all of which are necessary for the physiological processes associated with growth. Hence the relative abundance of these factors in a particular soil, determine the rate at which the seedling will grow [3]. Plants need a number of essential elements to enable them to grow and reproduce. Plant macronutrients (primary nutrients) including Nitrogen (N), Phosphorus (P) and Potassium (K). A laboratory test of the composts used in this study had varied levels of these nutrients (Table 1) and they are needed in relatively large quantities for plants metabolic processes.

The formation of mycorrhizae induces great changes in the physiology of the roots, in the internal morphology of the plant and in the mycorrhizosphere, thus, the soil surrounding the roots [16]. The symbiotic association of AMF and plant roots has been considered to be the oldest symbiosis of plants and is suspected to ecologically be the most important symbiotic relationship between microorganisms and higher plants [24]. The fungi of vesicular-arbuscular mycorrhizae

colonize considerable portions of the root system and in spite of the carbon drain they impose on the host plant, their presence within the root tissues can positively influence several aspects of the host plant's physiology. In the majority of cases, improved phosphate uptake is the primary cause of growth and yield enhancements in the mycorrhizal plants. Although according to this study mycorrhizae treatment did not significantly affect the diameter growth of *K. anthoteca*, *Glomus* Spp recorded the highest increment and this is due to the fact that mycorrhizae produced by *Glomus* Spp often result in simultaneous growth in 2 directions. Also, the mycorrhizal roots have different phosphate absorption kinetics and lower threshold values than non-mycorrhizal roots. The external hyphae developing around mycorrhizae explore a large volume of soil and absorb available phosphate beyond the depletion zone at the root surface. Phosphate accumulating in the external fungal hyphae is translocated to the internal mycelium by a well-developed transport system and transferred to the host tissues mainly across the intracellular arbuscules [11]. Certain specialized enzyme activities are specifically associated with this alternative pathway of phosphate nutrition in mycorrhizal plants. Improved phosphate nutrition is not always sufficient to explain the observed effects of vesicular-arbuscular mycorrhizae on the host plant's physiology [11].

Table 3. Two-way analysis of variance for diameter increment (mm)

Source	Df	SS	MS	F	P-Value
Mycorrhizae	3	3.11	1.04	1.86 ^{ns}	0.15
Compost	3	17.44	5.81	10.44 *	0.00
Interaction	9	7.34	0.82	1.46 ^{ns}	0.19
Error	48	26.72	0.56		
Total	63	54.61			

* Significant at alpha (α) $\leq 5\%$ ^{ns} not significant

According to [4], the primary aim of mycorrhizal inoculation is to increase the yield of plants grown for plantation forestry. These growth responses however, depend on the mycorrhizal dependency of the host plant species, soil properties; especially the availability of nutrients such as P and the capacity of the fungi to provide benefits to the host

plant. Since there is relatively no difference between the amount of phosphorus in the soil and applied composts the effect of mycorrhizae on the diameter increment of *K. anthoteca* may not be remarkable. Although there was no significant difference among the means of the mycorrhizae treatment on diameter increment of *K. anthoteca*, *Glomus* spp had the highest mean increment of 3.25 ± 0.17 mm compared to *Gigaspora* spp, mixed culture and non-mycorrhizal plants recording increment of 2.65 ± 0.12 mm. Aside the factors mentioned above, soil temperature and moisture contents are other factor limiting the full potential of AMF [4]. The study site is quite critical with some extreme environmental conditions such as high temperatures leading to low soil moisture due to high evaporation rates even after watering. According to [20] of all the factors controlling seedling growth, water is the most critical. Water is the vehicle for all physiological and biochemical processes through which life is maintained. In the plant, opposing effect of transpiration and water absorption controls water. Whenever transpiration is greater than absorption, the plant becomes dehydrated. A decrease in hydration of protoplasm of cells in the meristematic tissues usually results in cessation or checking of cell division or cell enlargement or both. There is considerable variation between and within species of fungi in their response to those factors. The capacity of fungi to produce soil hyphae is thought to be a major determinant of mycorrhizae fungus benefits to their host and would result from inherent properties of the fungi and interactions with soil conditions [5].

3.4. Leaf Count

Unlike animals, plants only have 3 organs namely; the roots, the stems and the leaves with the stems and leaves together forming the shoot of the plant. Leaves differ from stems in that, they do not have an apical meristem, thus, leaves are determinate (limited in their growth), while stems are indeterminate (theoretically capable of growing forever). In this study, analysis of variance of compost application showed a significance effect (Table 4). The same was observed for both height and diameter increments with significant differences among the various compost types

(Tukey HSD, $p < 0.05$). The growth of plants depends on the availability of nutrients from the soil. Prolonged uptake of nutrients by either growing plants or excavation of the land for any purposes depletes soil of vital nutrients, adversely affecting the growth of plants [25]. In this study, plant biomass (leaf count) was substantially increased when green waste compost (*S. natans*) was used as the organic matter component instead of paddy husk. It has been shown previously that increasing the organic fraction of a substrate increases plant growth [19]. Compost application in the research showed a significant increment in the number of leaves (Fig. 3). However, compost prepared from green waste such as *S. natans* was significantly different from the other compost (Table 5).

Table 4. Two-way analysis of variance for leaf increment

Source	Df	SS	MS	F	P-Value
Mycorrhiza	3	19.81	6.60	2.60 ^{ns}	0.06
Compost	3	135.06	45.02	17.71*	0.00
Interaction	9	13.06	1.45	0.57 ^{ns}	0.81
Error	48	122.00	2.54		
Total	63	289.94			

* Significant at alpha (α) $\leq 5\%$ ^{ns} Not significant

As indicated in other plant parameters earlier, mycorrhizae effect in this study did not significantly affect the growth of *K. anthoteca* on the field. In recent times, there have been a number of cases which indicate that AM colonization does not result in any increases in growth or in total plant P, and sometimes the AM plants are smaller than the non mycorrhizal controls [15, 28]. There is therefore a continuum of responses from strongly positive to negative, indicating considerable ‘functional diversity’ in AM symbioses [14]. The AM-responsiveness in terms of plant growth is determined by properties of the plant genome such as development of extensive root systems and long root-hairs that enhance P uptake by the plant when it is non-mycorrhizal, the AM fungal genome, thus, inherent extensiveness of external hyphae and other features and plant-fungus genomic interactions [28, 29].

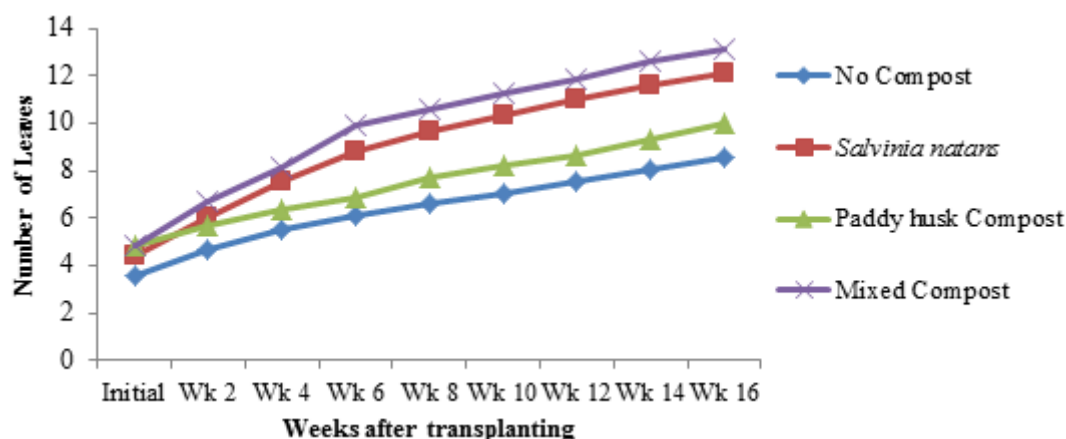


Figure 3. Effect of compost treatment on leaf counts

Table 5. Grouping information for compost treatment means for measured plant parameters using Tukey's HSD method at 95% confidence.

Compost Treatments	N	Mean \pm SE Mean		
		Height (cm)	Diameter (mm)	Leaf Count
C0	16	4.47 \pm 0.22 ^b	2.44 \pm 0.15 ^b	5.00 \pm 0.29 ^b
C1	16	9.00 \pm 0.45 ^a	3.61 \pm 0.24 ^a	7.69 \pm 0.45 ^a
C2	16	5.78 \pm 0.42 ^b	2.53 \pm 0.19 ^b	5.19 \pm 0.32 ^b
C3	16	9.31 \pm 0.47 ^a	3.42 \pm 0.20 ^a	8.25 \pm 0.51 ^a

Means that do not share a letter in the same column are significantly different at a confident of 95% ($\alpha \leq 5\%$)

3.5. Survival of *Khaya anthoteka* under Field Conditions

After four months of transplanting vegetatively propagated *K. anthoteka* to the field, non-mycorrhizal (NM) treatment recorded the highest mortality of 56.25% with only 43.75% plants surviving while *K. anthoteka* propagules pre-inoculated with *Glomus manihotis* had the highest percentage survival of 81.25%. There were significant differences between NM plants and AMF inoculated plants as well as between the various AM treated plants on the percentage survival of *K. anthoteka* according to a t-test analysis for independent samples. However, there were no significant differences between compost and mycorrhizal applications on the percentage survival of *K. anthoteka* P ($T \leq t$, 0.90) although the mean percentage survival of mycorrhizal treatment was higher (64.06%) than the application of compost treatment (62.5%). Association of fungi with plant roots has long been documented and they function as plants root hair and acting as an extension of the root system [18]. The beneficial effects of AM fungi result from one or several mechanisms. With mycorrhizal colonization in the roots, there is increased absorption surface area, greater soil area exposed for longevity of absorbing roots, better utilization of

low-availability nutrients and better retention of soluble nutrients, thus reducing reaction with soil colloids or leaching losses [18, 26]. The arbuscular mycorrhizae fungi increase establishment, nodulation and atmospheric nitrogen fixation capacity in [30]. Mycorrhizae influence the colonization of roots by other microorganisms, and reduce the susceptibility of roots to soil-borne pathogens such as nematodes or phyto-pathogenic fungi [26]. The study area for this research is characterized by high temperatures with low soil moistures hence propagules with AMF inoculation were more tolerant as compared to NM. According to [18] AM also modify soil-plant-water relations, thus promoting better adaptation of plants to adverse conditions, such as drought, salinity or heat stress. At elevated heavy metal concentrations in soils, mycorrhizal fungi have been shown to detoxify the environment for plant growth. Many studies have again, indicated that AM symbioses can significantly alter plant water relations, but the reported effects have not been consistent between different investigations and mechanisms are not clear. Nonetheless, an extensive review by [2] covering hundreds of studies, highlights a number of trends in AM compared to non-mycorrhizal (NM) plants growing under water restrictions in pot experiments. These studies include increased drought tolerance, greater depletion of soil water, higher stomatal conductance and transpiration, better supply of diffusion-limited nutrients in dry soil and lower drought stress (assessed as reduced concentrations of xylem abscisic acid in AM plants). Such differences suggest that AM plants are under less stress in dry conditions than NM controls [9]. Some studies have also indicated that, improved nutrition of AM compared with NM plants may be the main basis for improved drought tolerance. About 80% of the studies assessed by [2] also revealed AM plants grow better under drought stress than NM plants.

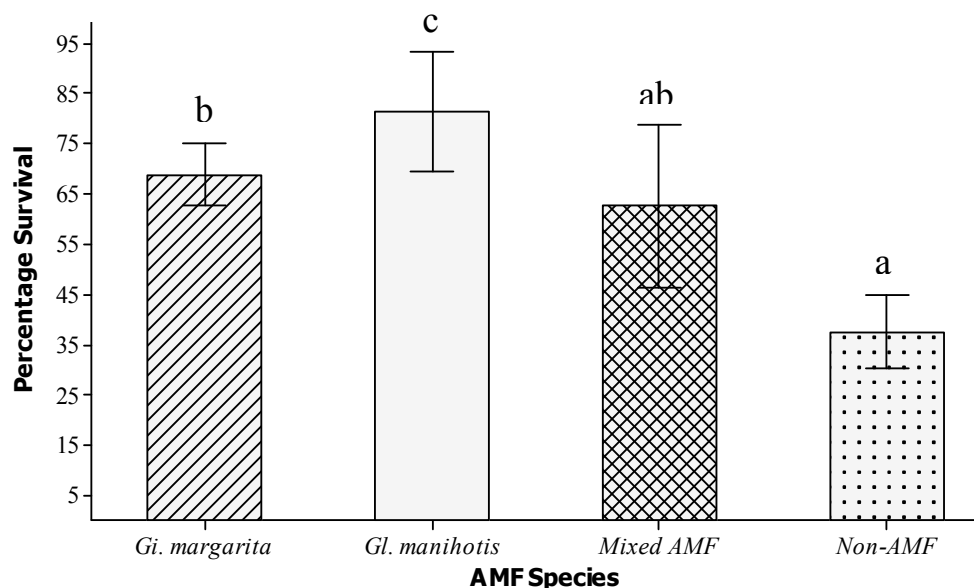


Figure 4. Effect of mycorrhizae treatment on percentage survival of *Khaya anthoteka*

In this study, *Glomus manihotis* inoculation recorded a higher percentage survival compared to *Gigaspora margarita* and mixed inoculums (*Glomus manihotis* and *Gigaspora margarita*) inoculations. A study conducted by [12] on the effect of inoculation of two arbuscular mycorrhizal fungi, alone and in combination on the establishment and growth of *Acacia auriculiformis* in a wasteland soil, studied under nursery and field conditions showed that *Acacia auriculiformis* exhibited a maximal mycorrhizal dependency of 79.6% on dual inoculation. Mycorrhizal dependency differed with AM fungal isolates and age of the plant. Under field conditions, AM colonization of *A. auriculiformis* enhanced tree survival rates (85%) after transplanting. Arbuscular mycorrhiza-colonized plants showed significant increase in height, biomass production and girth as compared to non-mycorrhizal plants. The study concluded that, mycorrhizal symbiosis play vital role in helping *A. auriculiformis* to establish and thrive in alkaline wasteland soils. Hence, the nursery inoculation programs with selected AM fungal species or combination of synergistically interacting species may be helpful to produce vigorous seedlings to survive under wasteland stress soil [12]. In this study however, the species used for the pre-inoculation were from two different genera, thus, *Glomus* and *Gigaspora* and a mixture of these two microorganisms therefore may therefore not be synergic and competing for the same resources under critical field conditions.

4. Conclusions

Mycorrhizal fungi play critical roles in nutrient cycling and terrestrial ecosystem functioning. Mycorrhizae improve plant growth through increase supply of nutrients to plant, enhance water uptake efficiency. Again, some of the benefits of inoculating with mycorrhizal fungi include; improved plant establishment rates, increased drought resistance, decreased transplant shock and improved survival. Furthermore, the addition of composted residue is an effective treatment for increasing rhizosphere aggregate stability. *K. anthoteca* is an important tropical tree species with high economic value and this study has shown that the species can be established on ex-coal mined sites. Base on the outcome of this study therefore, stem cutting of *K. anthoteca* pre-inoculated with AMF and compost applications can be established on ex-coal mined sites. Compost made from organic materials such as *S. natans* is preferable for the growth and development of the plants with application rate of 5 Kg/plant and the propagules at heights of at least 30 cm tall. Nonetheless, other aquatic weeds for composting and subsequent application should also be experimented. Again, the results of this study should be ascertained through the establishment of *K. anthoteca* in other mining sites to widen its application for revegetation. Finally, it must however be emphasized that, *K. anthoteca* can also be established on the field (ex-coal mined sites)

without mycorrhizae inoculations. On the other hand, inoculation will help facilitate the reintroduction of these soil microorganisms into the ecosystem.

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