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Farmyard manure and arbuscular mycorrhiza fungi inoculation enhanced growth and fruit fibre productivity of *Calotropis procera* in semi-arid eastern Kenya

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Abstract

A greenhouse experiment was conducted at the South Eastern Kenya University (SEKU) to determine the effects of farmyard manure (FYM) and arbuscular mycorrhiza fungi (AMF) inoculation on growth, vigor, shoot nutrient uptake (SNU) and AMF root colonization percentage (RC%) of potted *Calotropis procera* (Calotropis) seedlings. A field trial was laid out to determine the effects of the treatments on growth and fruit fibre productivity of the transplanted seedlings. The experiments were laid out in a split-split-plot design with a 2*2*2 factorial arrangement and 3 replications. The main plot factor constituted 2 Kenyan *Calotropis* provenances, Kibwezi and Tharaka. The sub-plot factor comprised of FYM and without FYM application while the sub-sub-plot factor involved inoculation with mixed strains of commercial AMF and without inoculation. Farmyard manure (FYM) and the integration of FYM and AMF inoculation (FYM*AMF) significantly improved Calotropis seedlings' growth, vigor, SNU and AMF RC% in the greenhouse. However, FYM*AMF was comparatively superior to solely FYM and AMF. There were positive correlations of AMF RC% with growth, shoot dry weight (SDW) and SNU of the seedlings at the end of the greenhouse experiment. Under field conditions, FYM and FYM*AMF significantly enhanced growth and fruit fibre productivity of Calotropis plants than the controls, barring a few cases. The study recommends the use of FYM and FYM*AMF in Calotropis seedlings' production in the drylands of Kenya. Future research should investigate the persistence of the introduced commercial AMF strains in the soil using molecular techniques.

Keywords: *Calotropis procera*; Arbuscular mycorrhiza fungi inoculation; Farmyard manure; Fruit fibre productivity

1. Introduction

Calotropis procera (Ait.) R. Br. (*Calotropis*) is a multipurpose, drought-tolerant wild shrub in the family Asclepiadaceae [1]. The species originates from the tropical Africa and Asia, and it is widely distributed in the tropical and subtropical regions of the world. Calotropis is adapted to a wide range of environmental conditions. It mainly occurs in disturbed landscapes and isolated areas such as roadsides, overgrazed rangelands, abandoned farmlands, disturbed urban regions and mined areas in the Arid and Semi-Arid Lands (ASALs) and at the Coast [2].

Calotropis is a multipurpose species [1, 3-6]. The species has been identified as a potential cash crop for fibre production, and a source of fuelwood, fodder and medicine in the drylands of Kenya [2, 7]. In the year 2017, a new market for export of Calotropis fruit fibre to China emerged [2]. However, collection of the fibre from the wild was inadequate in terms of meeting the quality and quantity demands for the fibre [7]. Thus, domestication of Calotropis in the ASALs of Kenya is paramount to meet the growing human and industrial demand for the species. Moreover, domestication of Calotropis can contribute toward the realization of Kenya's Vision 2030 [8] and the constitutional

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requirement of 10% forest cover [9]. It can also augment the resilience of the dryland communities to climate change impacts through diversification of livelihood options and enhancement of households' incomes through the sale of Calotropis products. Furthermore, Calotropis substantially promotes soil carbon sequestration [10] and acts as a soil binder [11] and a source of green manure [11, 12]. Hence, cultivation of the species could help in the mitigation of climate change impacts and soil fertility improvement. However, adoption of Calotropis on farms is hampered by inadequate information on its cultivation requirements [2]. Thus, there is a need for research intervention to develop sound on-farm production techniques for the species.

Production of high-quality seedlings is a prerequisite for any successful tree domestication program [13]. Seedlings' quality correlates positively to their survival, growth and productivity in the field. Improving seedlings' quality at the nursery is, therefore, inevitable for excellent survival and productivity in the field. The present study elucidates the effects of commercial AMF inoculation and FYM on growth, vigor, nutrient uptake and AMF RC% of potted Calotropis seedlings in the greenhouse, and the subsequent effects of the treatments on growth and fruit fibre productivity of the out-planted seedlings in a semi-arid area of eastern Kenya. The information availed in this paper is fundamental in guiding future research and the use of commercial AMF and FYM in Calotropis production in the study area.

2. Material and methods

2.1. Study Area Location and Description

The study was conducted at SEKU in Kitui County ($37^0$45' & $39^0$0'E: $0^0$3.7' & $3^0$0'S). The climate of the study area is semi-arid with a bimodal annual rainfall of 500-1050 mm and average temperatures of 16-34 °C [14].

2.2. Experimental Design and Treatments

The experimental design used was a split-split-plot with a 2*2*2 factorial arrangement and 3 replications. The main plot factor constituted two Kenyan Calotropis provenances, Kibwezi (KP) and Tharaka (TP). The sub-plot and sub-sub-plot factors comprised 2 rates of FYM (with FYM - 2:1:1- soil:sand:manure and without FYM - 2:1:0 - soil:sand:manure) and 2 levels of commercial mixed (*G. mosseae, G. etunicatum, G. claroideum and G. intraradices*) AMF inoculum (with and without inoculation), respectively.

2.3. Greenhouse Experiment

2.3.1. Establishment and Management

The greenhouse experiment was conducted following the standard procedures [15]. Forty (40) pots were included in each experimental unit (EU) giving a total of 960 (24 EUs * 40) pots. Each pot was sawn with 2 Calotropis seeds and thinned to one seedling after emergence. The pots were weeded regularly by handpicking and watered to field capacity early in the morning carefully, to avoid cross-contamination.

2.3.2. Sources of Calotropis Seeds, Farmyard Manure and Arbuscular Mycorrhiza Fungi Inoculum

Calotropis seeds were obtained from ICRAF, Kenya; FYM was sourced from SEKU farm; and AMF inoculum was bought from the Dudutech IPM Solutions, Flamingo Horticulture Limited in Naivasha, Kenya.

2.3.3. Chemical Analysis of the Potting Soil and FYM

Soil and manure sub-samples were air-dried at room temperature and ground to pass through a 2 mm sieve. The soil sub-sample was analyzed for pH at a ratio of 1:2.5 soil:water and electrical conductivity (EC) using the potentiometric method [16], available phosphorus (P) by Bray-1 extraction followed by molybdenum blue colorimetry [17], total nitrogen (N) by micro-Kjeldahl digestion method [18] and organic carbon (C) by the Walkley and Black method [19]. Exchangeable potassium (K) and extractable iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were extracted in 1M ammonium acetate at pH 7 and measured using a flame photometer (K) and atomic absorption spectrophotometer (Fe, Mn, Zn, and Cu) [20].

Farmyard manure (FYM) sub-sample was analyzed for pH, Organic C, total N, total P, K, Mg (magnesium), Ca (calcium) and sodium (Na) following the standard procedures [21].

2.3.4. Determination of Arbuscular Mycorrhiza Fungi Diversity and Abundance in the Potting Soil

A trap culture of the experimental soil was set up in a glasshouse using sorghum as the bait plant. The experiment was laid in a Completely Randomized Design (CRD) with 5 replications. The soil sub-sample was thoroughly homogenized with coarse sand (1:1, v/v) and sterilized twice using an autoclave at 121 °C for 1 hour, with intervals of 24 h between autoclaving. Disinfected 250-cm^3 plastic pots (having drainage perforations at the bottom) were filled ¾ full with the autoclaved soil/sand mixture. Each pot was added 200 g of the soil and topped up with the sterilized soil/sand mixture, forming a layer of soil 2 cm below the surface. The growing medium in each pot was wetted and sawn with seeds of a mycotrophic species, *Sorghum bicolor* (Sorghum). Sowing was done at 2 cm depth and the seeds covered with the sterilized soil/sand mixture. Each pot was sawn with 3 seeds and watered to field capacity using tap water. After emergence, the pots were thinned to 2 seedlings per pot. The experiment was maintained for 3 months after which watering was minimized to allow the pots to dry slowly to stimulate sporulation by AMF. At the end of the 4^{th} month, the plants were cut near the base, and the cultures were left to dry undisturbed. From each pot, 100 g of soil was sampled using a soil core at a depth of 0–15 cm for AMF spore extraction and characterization. Arbuscular mycorrhiza fungi (AMF) spores were extracted from the soil sample by wet-sieving and decanting method [22]. The spores were isolated based on their morphotypes and identified according to their genera with reference to standard monographs and descriptions [23]. Spore density per genus was computed as follows:

$$\text{Spore density} = \text{number of spores per 100 g of dried soil}$$

$$\text{Number of spores g} - 1 \text{ of dried soil} = \text{number of spores per 100 g of dried soil}/100$$

2.3.5. Sampling Procedures

Fifteen (15) seedlings were randomly selected from each EU and tagged for the assessment of the seedlings' growth, vigor, SNU and AMF RC%. Growth was monitored on weekly basis beginning from the second week after emergence (WAE) for 12 weeks (3 months), after which the seedlings were destructively harvested and partitioned into root and shoot components. The root components were thoroughly washed free of soil and separated into upper, middle and lower root systems. Lateral root samples of each part were randomly excised from the main root, cut into 1-cm fragments and stored under 70% ethanol for determination of AMF RC% [24]. The shoot components were weighed fresh and oven-dried at 65 °C to constant weights for SDW and SNU determination [25]. Dried shoot samples were weighed and taken to the laboratory at KEFRI, Nairobi for nutrient concentration analysis. Root fragments from each treatment were transferred to the Mycology Laboratory of the NMK for AMF colonization assessment.

2.3.6. Determination of Seedlings' Growth

Growth of seedlings was assessed based on shoot height (SH), root collar diameter (RCD) and the number of leaves per seedling (LN). Shoot height (SH) was measured from the growing media surface to the highest growing tip using a 100 cm ruler (1 mm accuracy) whereas RCD was measured twice in opposite directions by using a caliper (to the nearest 0.01 mm) just below the cotyledons [26]. The number of leaves per seedling (LN) was determined by counting and recording the total number of leaves on each seedling.

2.3.7. Determination of Seedlings' Vigor

The vigor of the seedlings was evaluated based on the seedlings' SH, RCD, sturdiness quotient (SQ) and SDW at the end of the experiment. Shoot height (SH) and RCD were determined as explained in section 2.3.6. Sturdiness quotient (SQ) was computed as a ratio of SH to RCD [27] while SDW was determined by measuring using an electronic balance (0.01 g accuracy) after oven drying at 65 °C to constant weights [25, 28].

2.3.8. Determination of Seedlings' Shoot Nutrient Uptake

The seedlings' SNU was determined at the end of the experiment. The oven-dried seedlings' shoots were ground to pass through a 2 mm sieve, sub-sampled and analyzed for total N, P and K concentrations [16]. Shoot nutrient uptake (SNU) of each seedling was computed as a product of the seedling's SDW and shoot nutrient (N, P and K) concentration (SNC), mathematically expressed as follows [29]:

$$SNU = SDW * SNC/100$$

Where; SNU = shoot nutrient uptake (kg); SDW = shoot dry weight (kg); SNC = shoot nutrient concentration (%)

2.3.9. Determination of Seedlings' Arbuscular Mycorrhiza Fungi Root Colonization

Thirty (30) randomly selected fine-root tissue fragments of approximately 1 cm each were processed following the standard procedures developed by McGonigle, Miller, Evans, Fairchild and Swan [30]. The fragments were examined under a compound microscope at a magnification of x40. The percentage of the fragment covered by hyphae, arbuscules, vesicles and intraradical spores was determined as the measure of the intensity of AMF colonization on the roots. Arbuscular mycorrhiza fungi (AMF) RC% was calculated using the formulae developed by McGonigle et al. [30] as shown below.

$$RC\% = \frac{Number\ of\ root\ segments\ colonized\ by\ AMF}{Total\ number\ of\ root\ segments\ observed} * 100$$

2.4. Field Experiment

2.4.1. Initial Experimental Site Characterization

A composite soil sample was collected from the out-planting site and analysed for physico-chemical properties at the Soil Laboratory in KEFRI, Nairobi.

The soil sub-sample was air dried at room temperature, ground to pass through a 2 mm sieve and analyzed for texture by hydrometer method, moisture content (MC) by gravimetric method [31], available P by the Olsen's method [32] and determined calorimetrically by the ascorbic acid-molybdate blue method [33], pH and EC by potentiometric methods (1:2.5 soil:water ratio) using conductivity meter and pH meter, respectively [34], bulk density by core method [35], organic C by the Walkley-Black oxidation (Loss on Ignition) method [36], total N by the Kjeldahl distillation method [18] and exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+} and Cu^{+2}) by extraction in 1M ammonium acetate at pH 7 and measuring using a flame photometer (K^{+}) and atomic absorption spectrophotometer (Ca^{2+}, Mg^{2+} and Cu^{2+}) [38].

2.4.2. Field Trial Establishment and Management

The experimental site was cleared off the vegetation cover and leveled using a tractor. Twenty-four (24) experimental plots (EPs), each measuring 6 m by 6 m (36 m^2) were demarcated, leaving a distance of 4 m and 2 m between main plots and sub-plots, respectively. Sixteen (16) planting holes (45 cm x 45 cm) were dug in each EP at 2*2 m espacement. Each planting hole was planted with 1 Calotropis seedling according to Mbora et al. [15], giving a total of 16 seedlings per plot. A guard row constituting Calotropis plants was established around the Eps, 2 m away from the experimental plants to eliminate external influences. Foreign growths around the plants were removed manually using a jembe.

2.4.3. Plant Sampling

Twelve (12) randomly selected plants in each EP were tagged for the assessment of growth and fruit fibre productivity, leaving the outer bounder plants as guard rows. Growth of plants was monitored on each tagged plant in each EP on monthly basis beginning from the 1st month after transplanting (MAP), for 12 months (1 year).

2.4.4. Determination of the Effects of Treatments on Calotropis Growth and Fruit Fibre Productivity

Growth of Calotropis plants in the field was measured based on the plants' diameters and heights. Diameter measurements were taken (in cm) at the soil line using a veneer caliper. When the plants grew big, the veneer caliper was replaced with a diameter tape. Height measurements were taken (in m) on the leader stem using a ruler, and replaced with a height rod when the plants grew big,

Calotropis fruit fibre productivity was assessed on each tagged plant in each EP for 1 year beginning from the 1st to the 12th MAP. Fibre productivity was determined at the fruits' physiological maturity which was marked by the browning of the fruit covers. All the mature fruits on each plant were harvested and their numbers noted. Fibre was extracted from all the fruits and weighed on an electronic balance. The mean fruit fibre weight per plant was computed. The number of plants and the fruit fibre productivity per hectare were computed using the formula shown below [39].

$$Number\ of\ plants\ per\ ha = 10000\ m^2 / (\text{row spacing} * \text{spacing in the rows})$$

$$= 10{,}000\ m^2 / (2\ m * 2\ m)$$

$$= 2{,}500\ plants/ha$$

Fruit fibre productivity (kg/ha) = number of plants per hectare * number of mature (harvested) fruits per plant * mean fruit fibre weight (g) / 1000

2.4.5. Statistical Analysis

Data collected were compiled using Microsoft excel software and subjected to ANOVA of the GenStat software (15th edition) to test the significance of treatment means and their interactions. Significant means were separated using Tukey's LSD at p = 0.05. Pearson's correlation analyses were conducted to determine the relationships between dependent and independent variables.

3. Results and discussion

3.1. Greenhouse Experiment

3.1.1. Characterization of Soil and Farmyard Manure Used in the Greenhouse Experiment

Table 1 Chemical properties of soil used in the greenhouse experiment

Soil property	Concentration
pH in water	6.20
EC (mS/c)	0.003
Organic C (%)	0.78
Total N (%)	0.24
Available P (ppm)	4.50
K (ppm)	584.81
Mn (ppm)	141.69
Zn (ppm)	9.24
Fe (ppm)	40.94
Cu (ppm)	1.93

Table 2 Chemical properties of farmyard manure used in the greenhouse experiment

Chemical properties	Concentration
pH	7.30
Total N (%)	2.52
Organic C (%)	16.40
Total P (%)	0.56
Total K (%)	4.67
Total Mg (%)	4.90
Total Ca (%)	10.30
Total Na (%)	0.13

Table 3 Arbuscular mycorrhiza fungi spore diversity and abundance in soil used in the greenhouse experiment

AMF diversity		**AMF spore density**	
Family	Genera	No. of spores per 100 g of dried soil	No. of spores g^{-1} of dried soil
Gigasporaceae	*Scutellospora*	30	0-1
	Gigaspora	5	0-1
Glomeraceae	*Glomus*	12	0-1
Acaulosporaceae	*Acaulospora*	28	0-1
Total		75	0-1

3.1.2. Effects of Treatments on Calotropis Seedlings' Growth in the Greenhouse

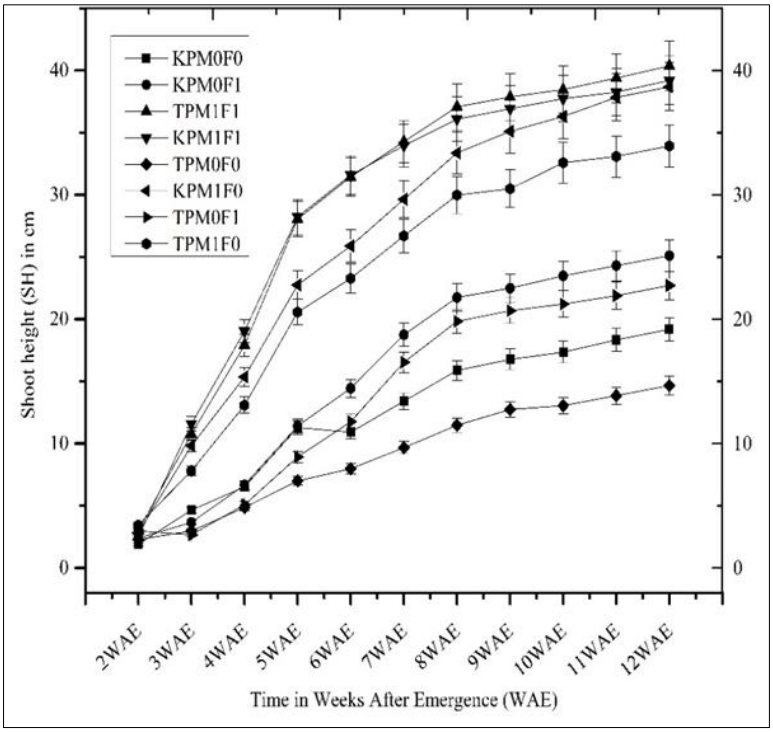

Figure 1 Treatments' effects on Calotropis seedlings' shoot height growth for 12 weeks (3 months) under greenhouse conditions. Error bars represent standard error

The study portrayed insignificant differences between Calotropis provenances (KP and TP) with regard to growth (SH, RCD and LN) in the greenhouse (Figures 1 - 3). The study also depicted insignificant effects of AMF inoculation on the seedlings' SH and LN increments throughout their growth period, and on their RCD increments in the first (4 WAE) and third (12 WAE) months of their growth in relation to the control treatments (Figures 1 – 3). The lack of growth response due to AMF inoculation was attributed to the low fertility of soil used in the experiment (Table 1). AMF colonization is inhibited under low soil fertility especially, P deficiency. The soil used in the experiment had 4.5 ppm P (Table 1) which is below the threshold of 5-10 ppm P for effective AMF colonization [24]. Under conditions of low soil P, slight supplementary additions of P are paramount to promote AMF colonization hence growth of the host [40, 41].

Farmyard manure (FYM) and the integration of FYM and AMF inoculation (FYM*AMF) had significant positive effects on the seedlings' SH and RCD increments throughout the growth period, and on the seedlings' LN increments in the first (4 WAE) and the second (8 WAE) months of growth compared to the control treatments (Figures 1 – 3). Notably, the integration of FYM and AMF inoculation was invariably superior to the other treatments with respect to SH and RCD growth across the entire monitoring period (Figures 1 – 3). These results corroborate the findings of El Kinany et al. [42] who documented significant improvements in growth of micropropagated date palm plantlets in substrates amended with organic fertilizer (compost) and those supplemented with a mixture of organic fertilizer and AMF. The significant growth observed in FYM treatments with respect to the control treatments in this study was ascribed to the

enhanced nutrition of the plants through FYM mineralization and the improved physico-chemical and biological properties of the soil used in the experiment due to FYM amendment [43-45]. Moreover, FYM could have promoted the effectiveness of the native AMF in colonizing the host, and consequently improved the seedlings' growth. On the other hand, the significantly superior growth registered in AMF*FYM treatments was due to the augmented positive effects of both FYM and AMF [42, 46]. Farmyard manure (FYM) and AMF complement each other in enhancing soil fertility and plant growth [47, 48].

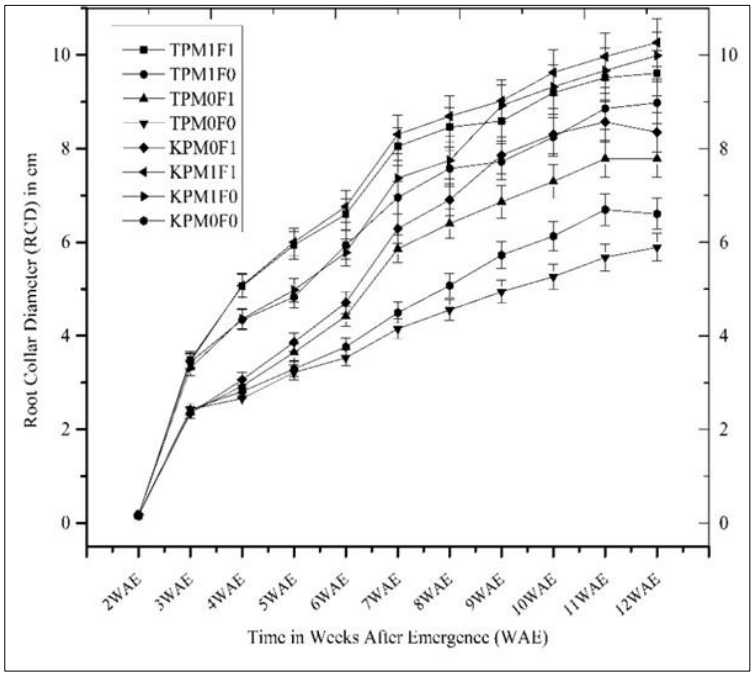

Figure 2 Treatments' effects on Calotropis seedlings' root collar diameter growth for 12 weeks (3 months) under greenhouse conditions. Error bars represent standard error

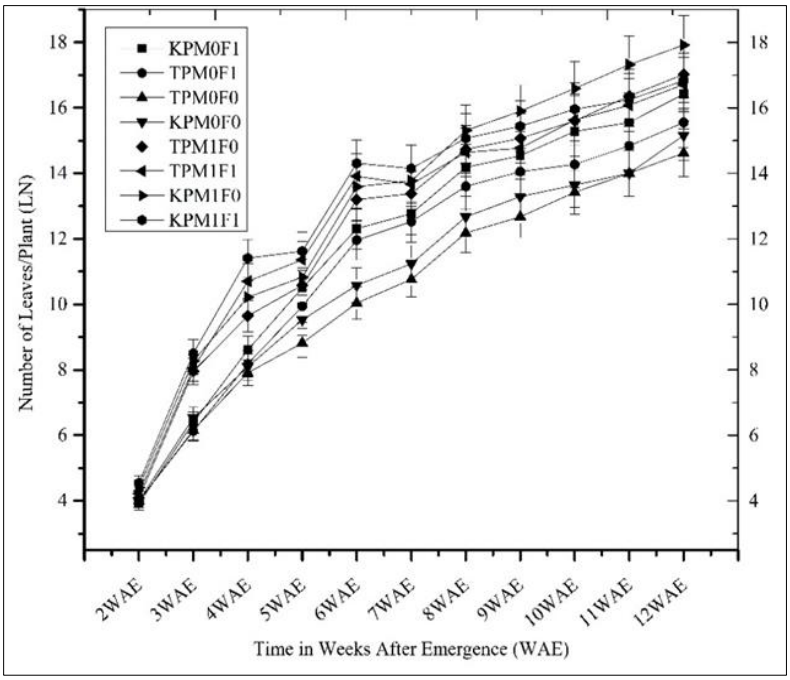

Figure 3 Treatment effects on Calotropis seedlings' leaf numbers for 12 weeks (3 months) under greenhouse conditions. Error bars represent standard error

3.1.3. Effects of Treatments on Calotropis Seedlings' Vigor

Shoot Height, Root Collar Diameter and Sturdiness Quotient

The size of a seedling during transplanting is crucial in predicting its performance in the field [49, 50]. Both SH and RCD show seedling's size [51]. Large seedlings, generally portray better performance in the field than small seedlings [51]. Ivetić, Devetaković and Maksimović [52] found a positive correlation between the size of seedlings at transplanting and their subsequent growth in the field. Previous studies have recommended large seedlings for afforestation purposes [49, 50].

In this study, there were no statistical differences between Calotropis provenances (TP and KP) with regard to the seedlings' SH and RCD across all the treatments at the end of the experiment (Table 4). Arbuscular mycorrhiza fungi (AMF) inoculation had also insignificant effects on the seedlings' SH and RCD. These results agree with the findings of El Kinany et al. [42] who observed insignificant effects of inoculation with commercial strains of AMF on growth of micropropagated date palm plantlets under greenhouse conditions. The lack of significant effects of AMF inoculation on growth of Calotropis seedlings in this study was attributed to low fertility of the soil used in the experiment (Table 1). Hence, a slight amendment with fertilizer could enhance growth of the seedlings.

Farmyard manure (FYM) and the integration of FYM and AMF inoculation (FYM*AMF) yielded significant increments in the seedlings' SH and RCD relative to the control treatments (Table 4). The percentage SH increments in FYM treatments (M1F0) relative to the control treatments (M0F0) were 56.78 and 50.43 in TP and KP, respectively while the percentage SH increments in the combined FYM and AMF inoculation treatments (M1F1) relative to M0F0 were 63.65 and 51.07, respectively. On the other hand, the percentage RCD increments in M1F0 relative to M0F0 were 33.84 and 34.38 in KP and TP, respectively whereas the percentage RCD increments in M1F1 relative to M0F0 were 35.65 and 38.69 in KP and TP, respectively. The same way, Trisilawati, Hartoyo, Bermawie and Pribadi [53] reported significant improvements in growth of Centella plants due to manure amendment while El Kinany et al. [42] recorded significant growth in shoot heights of micropropagated date palm plantlets grown in substrate amended with organic fertilizer (compost) and those applied with a mixture of organic fertilizer and AMF.

Noticeably, the integration of FYM and AMF inoculation (FYM*AMF) gave the tallest (40.36 cm) and the thickest (10.27 cm) seedlings in TP and KP, respectively at the end of the experiment (Table 4). Likewise, El Kinany et al. [42] observed the highest growth (SH and RCD) in treatments applied with a mixture of AMF and organic fertilizer than solely AMF and organic fertilizer while Schüßler et al. [46] found superior growth in native potential tree seedlings treated with AMF in combination with a low dosage of slow-release fertilizer.

The Calotropis seedlings' SQ values in this experiment were within the acceptable range of 2.50 and 4.21 (Table 4) [54, 55]. These SQ values implied that the seedlings were robust with greater chances of growth and survival under unfavorable conditions [56]. The seedlings were also resistant to damage from handling during transplanting and early management in the field [27].

Shoot Dry Weight

The results of this study depicted statistical similarities in SDW of Calotropis provenances at the end of the experiment (Table 4). AMF inoculation, also, had insignificant effect on SDW with respect to the control treatments. The same observation was made by Chareesri, De Deyn, Sergeeva, Polthanee and Kuyper [57], who found no significant effect of commercial AMF inoculation on shoot dry biomass of rice (*Oryza sativa* L.) under greenhouse conditions.

In this study, FYM and FYM*AMF significantly enhanced SDW above the control treatments (Table 4). The percentage SDW increments in FYM treatments were 40.07 and 49.19 in KP and TP, respectively while the percentage SDW increments in FYM*AMF treatments were 31.90 and 39.93 in KP and TP, respectively. Similarly, El Kinany et al. [42] observed significant improvement in shoot biomass of micropropagated date palm plantlets treated with both organic fertilizer (compost) and commercial strains of AMF under greenhouse conditions. On the other hand, Abdullahi, Lihan, Edward and Demie [58] observed significant improvements in SDW of maize treated with poultry manure and the combination of poultry manure and AMF while Schüßler et al. [46] recorded significantly superior above-ground biomass of native potential tree seedlings treated with both AMF and a low dosage of slow-release fertilizer under greenhouse conditions.

The significant increase in SDW in the FYM and FYM*AMF treatments relative to the control treatments in this study was ascribed to the improved nutrition of the plants through FYM mineralization [59, 60] and the enhanced nutrients' absorptive potential of the plants due to their high AMF RC% (Table 4) [61, 62]. Farmyard manure (FYM) enhances nutrients' supply to plants through mineralization hence growth and biomass accumulation in the plant tissues [63, 64]. Arbuscular mycorrhizal fungi (AMF) colonization, on the other hand, promotes plants' nutrients absorptive potentials via extended extraradical hyphae networks which increase the roots' absorbing areas, aiding plants to exploit greater

soil volume [65-67]. This claim is supported by the positive correlation (r = 0.66) between Calotropis seedlings' AMF RC% and SDW in the present investigation. Abdullahi et al. [58] had the same observation on maize.

3.1.4. Effects of Treatments on Calotropis Seedlings' Shoot Nutrient Uptake

There were no significant differences between Calotropis provenances with regard to SNU (Table 4). Arbuscular mycorrhiza fungi (AMF) inoculation had also no significant effect on SNU in relation to the control treatments (Table 4). However, FYM and FYM*AMF treatments significantly enhanced the SNU than the control treatments. The percentage SNU increments in the FYM treatments were 82.96 (N), 96.88 (P) and 59.90 (K) in KP and 79.77 (N), 97.73 (P) and 61.55 (K) in TP while the percentage SNU increments in the FYM*AMF treatments were 85.41 (N), 98.06 (P) and 62.25 (K) in KP and 86.50 (N), 98.68 (P) and 67.21 (K) in TP.

The significant improvements in SNU in FYM and FYM*AMF treatments were attributed to the availability of nutrients in the soil due to FYM mineralization [68] and the enhanced absorptive potentials [69] of the plants due to their high AMF RC%. Farmyard manure (FYM) supplies nutrients to plants gradually and in balanced quantities through the mineralization process which enhances their uptake by plants [59]. Moreover, the humic substances, such as fulvic acids produced through FYM decomposition adsorb free cations from the soil solution and avail them for uptake by plants [60]. Farmyard manure (FYM), also, stimulates plants' AMF colonization which subsequently enhances the uptake of nutrients by plants [69]. Besides, both FYM and AMF improve soil structure which encourages growth of plant roots aiding their absorption of nutrients from the soil [70].

3.1.5. Effects of Treatments on Calotropis Seedlings' Arbuscular Mycorrhiza Fungi Root Colonization

The results of this study exhibited AMF colonization in both AMF inoculated and non-inoculated pots (Table 4). This implied the presence of infective AMF propagules in the soil used in the experiment. The initial characterization (trap culture) results revealed the presence of four AMF genera (*Scutellospora, Gigaspora, Glomus* and *Acaulospora*) in the potting soil (Table 3). Notably, the genera *Acaulospora*, *Glomus* and *Scutellospora* were found associating with *C. procera* in the dry land localities of India [71].

Table 4 Treatment effects on Calotropis seedlings' shoot heights, root collar diameters, leaf numbers, sturdiness quotients, arbuscular mycorrhiza fungi root colonization percentages, shoot dry weights and shoot nutrient uptake at the end of the greenhouse experiment

Treatment	Growth			SQ	RC %	SDW (g)	SNU		
	SH (cm)	RCD (cm)	LN				P-uptake	N-uptake	K-uptake
TPM0F0	14.67d	5.91c	14.62b	2.50c	6.73b	19.77c	0.40c	17.72e	37.87c
KPM0F0	19.18cd	6.61bc	15.16ab	2.89bc	9.33b	24.47c	0.60c	19.74de	45.85c
TPM0F1	22.71cd	7.78abc	15.55ab	2.91bc	30.00ab	24.89c	6.11c	49.77cde	67.15bc
KPM0F1	25.11bc	8.35abc	16.42ab	3.00bc	32.00ab	24.88c	6.28c	57.96cd	66.17bc
TPM1F0	33.94ab	8.98ab	17.02ab	3.76ab	26.67ab	38.91ab	17.62b	87.61bc	98.48ab
KPM1F0	38.69a	9.99a	17.92a	3.87ab	31.44ab	40.83a	19.25b	115.88ab	114.35a
TPM1F1	40.36a	9.61a	16.71ab	4.211a	58.66a	32.91b	30.32a	131.27a	115.50a
KPM1F1	39.20a	10.27a	16.83ab	3.82a	64.33a	35.93ab	30.99a	135.33a	121.46a
LSD	6.063	1.547	1.765	0.544	27.2	4.738	4.837	23.48	21.59
p value	0.013	<0.009	0.044	0.002	0.018	<0.001	<0.001	<0.001	<0.001

Means in a column followed by the same letters are not significantly different using Tukey's LSD at 5% level. SH = shoot height, RCD = root collar diameter, LN = leaf numbers, SQ = sturdiness quotient, RC% = root colonization percentage, SDW = shoot dry weight, SNU = shoot nutrient uptake, P = phosphorus, N = nitrogen, K = potassium, TP = Tharaka provenance, KP = Kibwezi provenance, M0 = without farmyard manure, M1 = with farmyard manure, F0 = without Arbuscular Mycorrhiza Fungi inoculum, F1 = with Arbuscular Mycorrhiza Fungi inoculum, LSD = Least Significant Difference.

Provenance types exhibited statistical similarities with regard to their levels of colonization by AMF (Table 4). Arbuscular mycorrhizal fungi (AMF) inoculation and FYM had insignificant effects on the seedlings' AMF RC% in comparison with the control treatments (Table 4). However, AMF inoculation and FYM relatively improved the seedlings' AMF RC% above the control treatments. The lack of significant response of Calotropis seedlings to AMF inoculation in this study was attributed to low fertility of the potting soil. Several authors have documented inhibition

of AMF colonization by low levels of soil fertility especially, P deficiency [24, 41, 72, 73]. On the contrary, high P concentration hinders mycorrhizal colonization [74, 75]. The optimal level of soil P for effective AMF colonization is 5-10 ppm P [24]. Under conditions of low soil P, Abbott and Robson [40] and Bolan et al. [41] recommended slight supplementary additions of P to promote AMF extra-radical hyphae growth and improve AMF colonization. Moreover, a balanced state of macro and micronutrients in soil is vital for optimal AMF colonization. Nyamwange et al. [73] and Mukhongo et al. [72] underlined the need for starter nutrients' amendments to enhance AMF colonization in low fertile soils.

The integration of FYM and AMF significantly improved Calotropis seedlings' AMF RC% with respect to the control treatments. Other workers have also reported significant positive effects of combining AMF and organic inputs on AMF colonization [48, 58, 76, 77]. The elevated AMF RC% in AMF*FYM treatments in this study was attributed to the augmented positive effects of both FYM and AMF [57, 69, 72, 73, 78, 79].

3.2. Field Experiment

3.2.1. Initial Experimental Field Soil Characterization

Table 5 Physico-chemical properties of the experimental field soil

Soil property	Concentration
pH in water (1:2.5 – soil:water)	6.80
EC (mS/c)	0.002
Organic C (%)	1.87
Total N (%)	0.25
Available P (ppm)	8.10
K (ppm)	300.71
Mg (ppm)	196.00
Ca (ppm)	104.00
Mn (ppm)	128.36
Zn (ppm)	7.95
Fe (ppm)	50.02
Cu (ppm)	1.88
MC (%)	1.60
Bulk Density (g/cm^3)	1.10

3.2.2. Effects of Treatments on Calotropis Growth and Fruit Fibre Productivity

Growth

Calotropis provenances were invariably statistically similar in terms of diameter (Figure 4) and height (Figure 5) growth throughout the monitoring period in the field, barring a few cases.

Arbuscular mycorrhizal fungi (AMF) inoculation significantly improved diameter growth of Calotropis TP plants in the 7th and 9th MAP and KP plants from the 7th to 12th MAP relative to the control treatments. Arbuscular mycorrhizal fungi (AMF) inoculation, also, had significant positive effects on the height growth of Calotropis KP plants in the 7th and 8th MAP. In line with these findings, Ouahmane et al. [80] reported significant improvement in growth of AMF pre-inoculated *Cupressus atlantica* seedlings one year after transplanting in the field. Other workers have also reported significant improvements in growth of plants due to AMF inoculation under field conditions [39, 81, 82]. The observed significant improvements in growth of Calotropis in AMF inoculated treatments in this study were attributed to the improved AMF RC% of the plants during transplanting which enhanced the plants' nutrients and moisture absorptive potentials in the field [61, 62] leading to the improvement in growth [63, 64].

Farmyard manure (FYM) significantly improved height growth in Calotropis KP plants from the 1st to 6th MAP and in TP plants from the 1st to 7th MAP. Farmyard manure (FYM) also significantly enhanced diameter growth of Calotropis TP and KP plants throughout the growth period. The same way, Switzer and Nelson [50] reported an increase in growth of Loblolly pine plants in the field through nursery soil fertilization.

The integration of farmyard manure and AMF inoculation (FYM*AMF) had significant positive effects on height growth of Calotropis KP plants from the 1st to 4th MAP and of TP plants from the 1st to 7th MAP. The integration of farmyard manure and AMF inoculation (FYM*AMF), also, significantly enhanced diameter growth in TP and KP plants throughout the observation period. These results are similar to those reported by Abdullahi et al. [81], who realized significant improvements in growth of maize plants through application of poultry manure and the combined application of poultry manure and AMF inoculation in the field.

The significantly higher growth attained in the FYM and FYM*AMF treatments relative to the control treatments in this study were attributed to the improved vigor [25, 49, 50] and enhanced nutrients and moisture absorptive potentials of the seedlings due to their high AMF RC% at transplanting (Tables 4) [61, 62]. The effect of mycorrhizal fungi on plant growth depends on early colonization [46, 83-85]. High mycorrhizal colonization of seedlings during transplanting is associated with better growth of the seedlings in the field [25]. Furthermore, several authors have demonstrated a close relationship between the nutritional status of seedlings before transplanting and their post-planting growth performance [50, 51, 86]. In this study, FYM and FYM*AMF seedlings registered significantly higher SNU than their corresponding control counterparts during transplanting (Table 4).

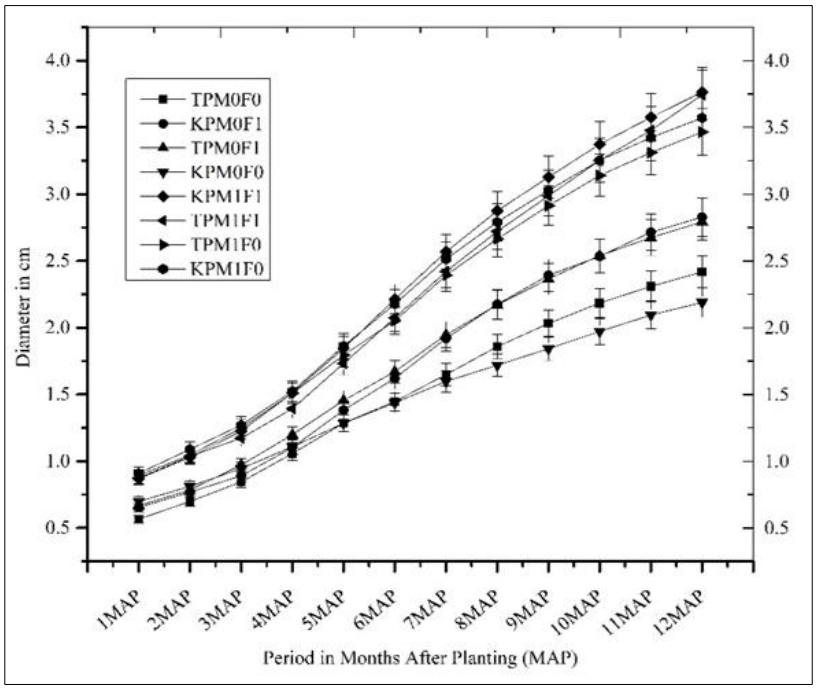

Figure 4 Effects of treatments on Calotropis diameter growth for 12 months in the field

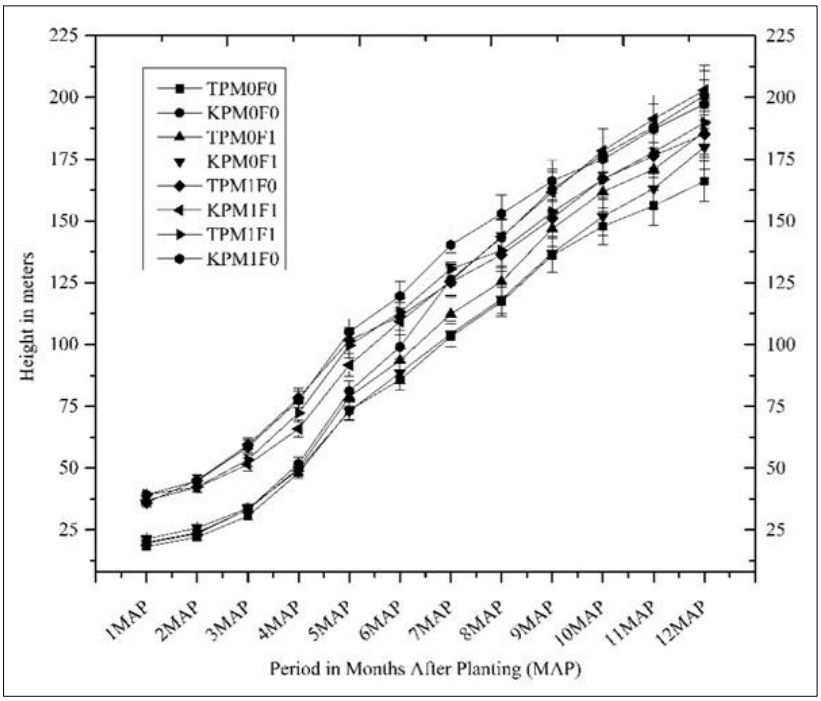

Figure 5 Effects of treatments on Calotropis height growth for 12 months in the field

Fruit Fibre Productivity

There were no significant differences between Calotropis provenances with regard to fruit fibre productivity. Arbuscular mycorrhiza fungi (AMF) inoculation had also insignificant effect on the fruit fibre productivity while FYM and FYM*AMF enhanced the fruit fibre productivity significantly (Table 6). Correspondingly, Juntahum et al. [68] did not observe a significant increment in cane yield with AMF inoculation but they observed significant yield gain of the same with both 100% fertilization and the combination of 50% fertilization and AMF inoculation after 12 months in the field. These results suggested that soil fertility had a substantial influence on yield.

Table 6 Effects of treatments on Calotropis fruit fibre productivity

Treatments	**Calotropis fruit fibre productivity (Kg/ha)**		
	1 - 6 MAP	**7 - 12 MAP**	**Overall at 12 MAP**
TPM0F0	10.69c	416.25b	433.40b
KPM0F0	10.97c	390.28b	407.80b
TPM0F1	12.92bc	492.08b	499.40b
KPM0F1	12.92bc	510.69b	523.60b
KPM1F0	16.25ab	721.25a	748.60a
TPM1F0	16.53ab	700.97a	723.80a
TPM1F1	16.94a	824.17a	843.90a
KPM1F1	18.89a	759.86a	776.00a
P value	<0.001	<0.001	<0.001
LSD	2.41	102.52	97.5

During the 1st to 6th MAP, FYM improved the fruit fibre productivity in TP and KP significantly by 35.33% and 32.49%, respectively whereas AMF*FYM enhanced the same significantly by 36.90% and 41.93%, respectively (Table 6). During the 7th to 12th MAP, FYM improved the fruit fibre productivity in TP and KP significantly by 40.62% and 45.89%, respectively while AMF*FYM improved the same significantly by 49.50% and 48.64%, respectively (Table 6). At 12 MAP,

FYM significantly enhanced the overall fruit fibre productivity in TP and KP by 40.12% and 45.53%, respectively while AMF*FYM improved the same by 48.64% and 47.45%, respectively (Table 6).

The significant increase in fruit fibre productivity in FYM and FYM*AMF treatments above the control treatments in this study was ascribed to the improved vigor of the seedlings [25, 49, 50] and their increased nutrients and moisture absorptive potentials due to their significantly high AMF RC% during transplanting (Tables 4) [61, 62]. Furthermore, the FYM and FYM*AMF seedlings exhibited significantly higher SNU than their control counterparts during transplanting (Table 4). Shoot nutrient uptake (SNU) is a crucial indicator of seedlings' quality and their performance in the field [25, 51, 56].

4. Conclusion

The amendment of potting soil with FYM in greenhouse significantly improved Calotropis seedlings' growth, vigor, nutrient uptake and AMF RC%. However, the combination of FYM and AMF inoculation gave a comparative advantage over solely FYM and AMF inoculation. Furthermore, both FYM and FYM*AMF recorded significantly higher growth and fruit fibre productivity of Calotropis plants in the field than their control counterparts, barring a few cases. Hence, FYM and FYM*AMF are recommended technologies for raising quality Calotropis seedlings, and improving growth and fruit fibre productivity of Calotropis in the study area.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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