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Growth performance of *Pterocarpus angolensis* seedlings in mycorrhizae colonized and uncolonized soils from high rainfall area of Zambia

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ABSTRACT

Objectives: *Pterocarpus angolensis* trees are being harvested at an alarming rate in Zambia because of its high value timber. Considering the low number of seedlings that recruit into adults in forests, efforts have been devoted without success to propagate trees in nurseries. This study aimed at formulating a suitable growth substrate to foster its propagation in nurseries.

Methodology and results: Soils underneath mature trees were collected and examined for the presence of beneficial microorganisms. Laboratory analysis confirmed mycorrhizal contamination. Then, four subset soil treatments were constituted and amended with chicken manure to enhance plant growth. They were (1) sterilized soil without chicken manure in which mycorrhizae were destroyed, (2) sterilized soil with manure, (3) unsterilized soil without manure and (4) unsterilized soil with manure used in a completely randomized experiment with four replications to grow *P. angolensis* seeds. Seed germination was assessed, and plant height, diameter, taproot length and biomass were recorded 30 and 70 days after germination. There was no difference in seed germination rate between soil treatments. However, significant statistical differences were found between soil treatments ($P \leq 0.05$) for all the plant growth parameters assessed. Mycorrhizae-colonized soils amended with chicken manure consistently resulted in significantly enhanced seedling growth and profuse plant biomass production. Seedlings in mycorrhizae-colonized soil with chicken manure had 7.94% and 5.94% overall increment in height and stem collar diameter, respectively compared to 5.64, 5.28 and 6.85% height increment and between 3.33 and 4.64% collar diameter increment for the other treatments.

Conclusion and application of findings: The growth of *P. angolensis* seedlings in nurseries can be improved by adding chicken manure and mycorrhizal inocula to the soil substrate. This silvicultural management is cost-effective, as no fertilizers are needed since mycorrhizae increase considerably plant uptake of nutrients mainly phosphorous and nitrogen. The enhanced seedling growth achieved necessitates expanded field testing of the technology to ensure the feasibility of wider afforestation and reforestation programs that have great potential for *P. angolensis* conservation in Zambia and within the region.

Key words: *Pterocarpus angolensis*; mycorrhizae; chicken manure; biodiversity conservation, Zambia

INTRODUCTION

Pterocarpus angolensis DC [Family: Fabaceae Syn Papilionaceae] is an important forest tree species that is found in the miombo woodlands of Zambia and also in the dry evergreen and dry deciduous forests (Storrs, 1995). It is also common in Angola, Botswana, Democratic Republic of Congo, Malawi, Mozambique, South Africa, Swaziland, Tanzania and Zimbabwe (Aubrey, 2003; Caro *et al*., 2005; Chisha-Kasumu *et al*., 2006). *P. angolensis* grows in mixed stands with several other tree species such as *Brachystegia speciformis*, *Julbernardia paniculata*, *J. globiflora*, *Parinari curatellifolia*, *Syzygium guineense*, *Uapaca* species and *Isoberlinia angolensis* (Chisha-Kasumu *et al*., 2007). All these species play an important role in the miombo ecosystem by providing high value timber and non-timber forest products that are at the core of local livelihoods (Ng'andwe *et al*., 2007). Moreover, they constitute a habitat for wildlife (Aubrey, 2003).

This tree species is highly sought after by furniture makers for its high value timber *(*Aubrey, 2003; Mwitwa, 2004*)*. The reason behind its heavy commercial exploitation is the quality of wood which is easy to work on when making carvings, furniture, joinery, veneer and as multipurpose timber *(*Aubrey, 2003; Hengari *et al*., 2004). Furthermore, it has medicinal properties and is able to cure ringworms, ulcers, eye infections, malaria and stomach ache, and to increase blood and the supply of breast milk (Storrs, 1995; Aubrey, 2003). However, the rate at which *P. angolensis* trees are being harvested is alarming considering the low rate at which seedlings are recruited into adult populations in nature (Caro *et al*., 2005). Due to its high value timber, loggers increasingly harvest even small-sized immature trees in order to satisfy the expanding market demand (Stahle *et al*., 1999).

Nursery propagation of *P. angolensis* has not been successful (Hengari *et al*., 2004) and attempts to grow the species commercially have also been unsuccessful (Caro *et al*., 2005). In addition, natural regeneration of *P. angolensis* remains a problem due to failure of the seedlings to survive during establishment (Chisha-Kasumu *et al*., 2007). Recent studies of the seed bank structure of *P. angolensis* in the Copperbelt Province of Zambia found no seeds of the species up to a depth of 20 cm and with only 95 saplings per ha in disturbed miombo woodlands (Mimba, 2008; Musaba, 2008). This low survival rate has been attributed to several factors including adverse environmental and physical conditions of the miombo woodlands that are characterized by long dry seasons, continuous fire outbreaks and browsing by animals in some areas (Stahle *et al*., 1999; Aubrey, 2003; Mwitwa, 2004) as well as recurrent annual dieback of seedlings, competition from other trees mainly for light and delayed seed production (Caro *et al*., 2005). The study by Hengari *et al*. (2004) in South Africa showed that *P. angolensis* trees in nature grow in association with arbuscular mycorrhizal fungi (AMF) that improve uptake of minerals such as phosphorus, zinc, copper and iron required for plant development. AMF also boost plant tolerance to stresses, enhance photosynthesis, stimulate production of plant growth hormones, and induce production of antimicrobial compounds as plant defense mechanism (Mishra & Mishra, 2004; Arpana & Bagyaraj, 2007; Javaid *et al*., 2007).

According to Mwitwa (2004), the low rate of natural regeneration dynamics of *P. angolensis* and the inexistence of appropriate silvicultural technology for seedlings management in the nursery constitute major problems in the propagation of this species. In South Africa, Hengari *et al*. (2004) studied a soil with mycorrhizal colonization levels of 45 and 75% and found that *P. angolensis* seedlings with high AMF colonization levels had fewer and smaller leaves, produced less biomass and were less vigorous than the seedlings with lower mycorrhizal colonization levels. These observations indicated there is need to determine the most suitable environment for improved symbiosis between *P. angolensis* and mycorrhizal fungi.

Thus, this study was undertaken to ascertain the nature of mycorrhizal association

with *P. angolensis* in forests in Zambia and to formulate a suitable growth substrate for nursery beds to enhance propagation of seedlings at large scale commercial level to support the trees afforestation and reforestation programs in the country.

MATERIALS AND METHODS

Soil collection and sterilization: The soil used for the study is yellowish red, fine loamy and well drained chromi-haplic acrisol and was collected underneath mature *P. angolensis* trees in Mwekera National Forest Reserve No. 6 (12°45' and 12°52'S; 28°16' and 28°30'E; 1200 to 1300m above sea level) situated 20 km South-east of Kitwe District in Copperbelt Province, Zambia. The province is in the Agroecological Region III and experiences three seasons annually, i.e. a warm and rainy period from November to April, a cold and dry period from May to August, and a hot and dry period from September to October. The annual rainfall ranges between 1300 and 1800 mm with high temperatures oscillating between 27-33°C and minimum temperatures between 9-14°C. The soil was obtained at depth of between 10 and 20 cm which has high levels of mycorrhizal activities (Negi, 1996). A subset soil sample was wetted and steam-sterilized for 1 h at 120°C and 28.9 psi to destroy soil microbial activity.

***P. angolensis* seed sowing and germination**: Seeds of *P. angolensis* were purchased from the Zambia National Tree Seed Centre and sown at Copperbelt University (CBU) nursery in Kitwe. A portion of the sterilized and the unsterilized soil samples were separately amended with chicken manure, while other portions were not amended. Chicken manure was used to improve plant growth as it has been shown to increase plant nutrient uptake (Aryantha *et al*., 2000; Röper *et al*., 2005). The treated soils were thoroughly mixed with manure, and both treated and untreated soils were wetted and filled in 10 cm diameter and 15 cm length polythene pots. Prior to sowing, seeds were carefully nicked by removing a portion of the seed coat to break seed dormancy (Chisha-Kasumu *et al*., 2007) and planted 1 seed per pot at 2-3 cm depth. The pots were then watered twice daily, in the morning and afternoon, to facilitate seed germination and plant growth. Germination was recorded starting when the first seed emerged from soil, which was considered as day 1.

Experimental layout and soil treatments: The experiment consisted of four soil treatments, i.e. (1) sterilized soil (mycorrhizae-uncolonized) without chicken manure, (2) sterilized soil (mycorrhizae-uncolonized) with manure, (3) unsterilized soil (mycorrhizae-colonized) without manure and (4) unsterilized soil (mycorrhizae-colonized) with manure. After assessment of seed germination, 36 pots with the most vigorously emerging seedlings were chosen from each treatment to have in total 144 plants. They were split into four replicates in a complete randomized block design with 9 seedlings per replication. In this design, the treatments are compared within blocks without interference of block-to-block variations (Clewer & Scarisbrick, 2001).

Mycorrhizae cultures and identification: The remaining soil without manure was filled into pots and used to grow maize seeds for in-vivo culture of mycorrhizae (Brundrett *et al*., 1996; Wright & Upadhyaya, 1996). To ensure effective soil mycorrhizal colonization, additional inoculum in small root pieces of 2-months old maize was applied to the unsterilized soil treatments. Other roots were gently removed from pots by washing under running tap water and processed as described by Hengari *et al*. (2004). They were stored in 50% ethanol for 1 day, rinsed with sterilized water and soaked in 10% KOH solution before autoclaving for 1 h at 90°C and 17.5 psi while in the solution for delignification and making plant cells flexible for free entry of water and dye. They were again rinsed with sterilized water, immersed in 1% HCl solution for 10 min and autoclaved as describe above. After the last rinsing with sterilized water, they were stained with 0.05% aniline blue solution and autoclaved for 1 h. From the autoclave, they were kept in staining solution for 3 days after which they were distained in 25 ml sterilized water and 475 ml lactic acid solution for 4 days. Thin root slices (about 20-30 µm thick) were then cut with razor blades, placed on microscope slides and observed under light microscope for possible modification of the roots as a result of the presence of fungal mantle on the surface, and development of arbuscules and vesicles inside root cells. Further observations were done under a compound microscope for additional fungal structures to identify mycorrhizae.

Determination of mycorrhizal colonization of maize roots: Counting of the roots colonized by mycorrhizae was done by observing fine maize roots, of 3 sub-sample plants, from the distaining solution under dissecting microscope using the gridline intercepts as

described by Brundrett *et al*. (1996). The formula of Dubey & Maheshwari (2002) was used to calculate the mycorrhizae colonization percentage (%MC) as:

$$\% \text{ MC} = \frac{\textit{Total number of root segments showing Mycorrhizae association}}{\textit{Total number of observed root segments}} \text{ X } 100$$

Plant growth performance: Seedlings germination rate was recorded 24 days from sowing when the first plant emergence occurred and assessed for a period of 23 days after which no additional emergence was observed. Plant health was evaluated for prevalence of aphid, and for incidence of leaf spots and root rot. At 30 and 70 days after seed germination, plant height, stem collar diameter and taproot length were recorded and dry weight biomass productivity for both above and below ground plant parts were determined. Plant height was measured from the stem-root collar to the highest terminal bud using a ruler whereas the stem-root collar diameter was recorded using vernier calipers adjusted to the nearest 0.1 mm. Taproot length was measured from the stem-root collar site to the tip of the taproot also using a ruler. Seedling biomass was assessed by collecting roots from the pots after rinsing with running tap water, dividing them into shoot and root parts, oven-drying at 80°C for 24 h and estimating the plant dry weight using an electronic beam balance.

Statistical analyses: Recorded data were subjected to one way analysis of variance (ANOVA) using GenStat Discovery Edition 3 software (VSN International Ltd, UK). Fisher's least significance difference (*LSD*) test was then used to compare the treatment means when *F*-statistic values were significant.

RESULTS

Microscopic observations of maize roots showed that they were colonized by VAM (Fig. 1) and the presence of fungal hyphae, arbuscules and vesicles was apparent in the roots as indicated by the aniline blue stained areas in root cells (Fig. 2). Nearly all the root cells were colonized by aseptate fungal hyphae in the form of bundles of fine threads, intracellular branched haustoria like arbuscules and round to oval thick walled vesicles. Overall, the colonization of maize roots observed amounted to 49.3%, thus confirming the presence of VAM in the forest soil used for the study.

Seed germination started 24 days after sowing when the first seedling emerged from soil and it varied among the different soil treatments. The germination ranged between 57.8% for soil without mycorrhizae but with chicken manure and 76.6% in soil without mycorrhizae and manure. The germination pattern of *P. angolensis* seeds for the various soil treatments is shown in Fig. 3. Neither damage from aphids nor leaf spot symptoms were observed on the seedlings. However, 5.6% of the seedlings grown in soil without mycorrhizae but with chicken manure developed root rot symptoms (Table 1).

ANOVA of plant height and stem collar diameter data at 30 and 70 days after seed germination revealed statistically significant differences ($P \leq 0.05$) in both plant growth parameters between the soil treatments (Table 2). Since trends of data after ANOVA were similar at 30 and 70 days, only the outcomes of the analysis at 70 days are shown in Table 2. Though there was an increase in plant height and stem collar diameter in all the soil treatments tested, mean height and collar diameter of the seedlings grown in mycorrhizae-colonized soil amended with chicken manure was significantly greater ($P \leq 0.01$ and $P \leq 0.001$, respectively) than the height and collar diameter of seedlings in the other soil treatments (Table 1). Seedlings in mycorrhizae-colonized soil with chicken manure had 7.94% increment in height 70 days after sowing as compared to 5.64, 5.28 and 6.85% increment for seedlings in mycorrhizae-uncolonized soil without manure, mycorrhizae-uncolonized soil with manure and mycorrhizae-colonized soil with no manure, respectively. Similarly, seedlings growing in mycorrhizae-colonized soil with chicken manure had the highest collar diameter increment (~ 5.94%) 70 days post emergence compared to between 3.33 and 4.64% for the other treatments (Table 1).

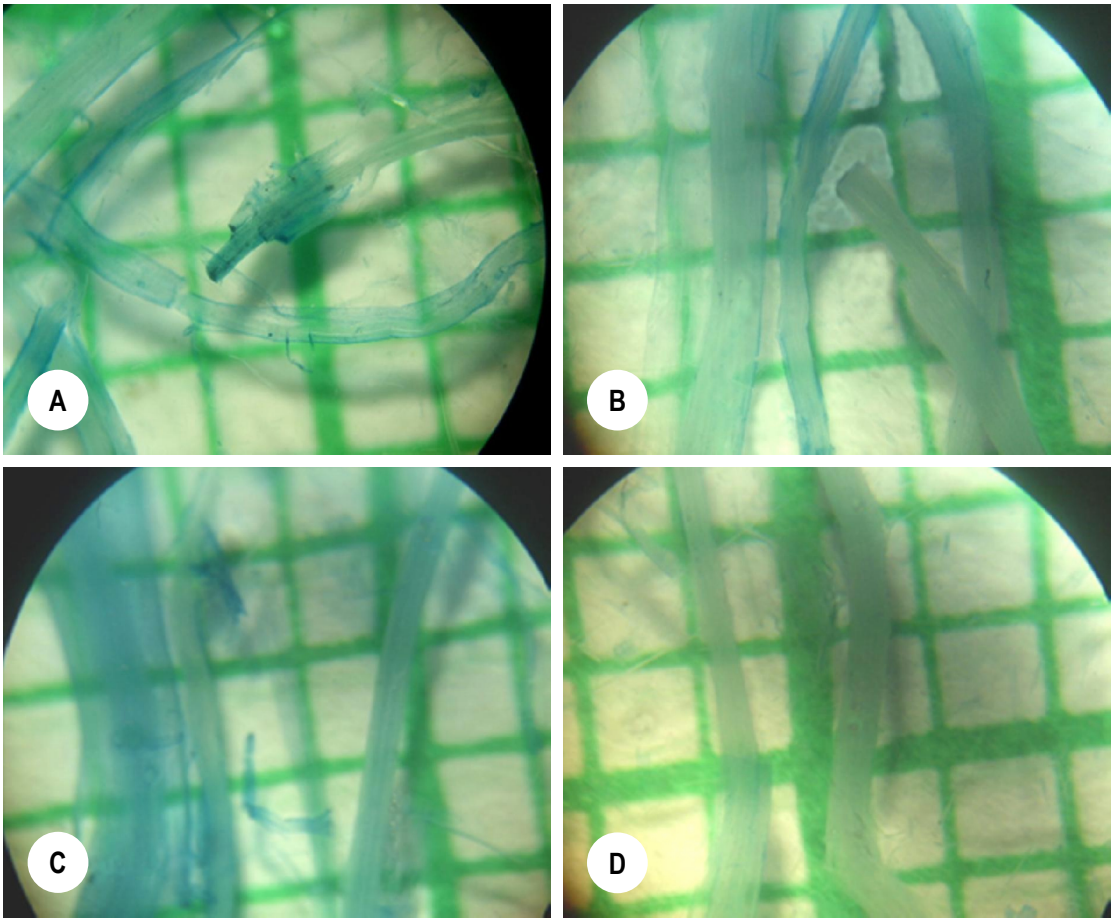

Figure 1: External morphology of roots of maize grown in soil collected beneath mature *Pterocarpus angolensis* from Mwekera Nation Forest Reserve in Kitwe, Zambia. (A) Blue stain between root epidermal and cortex tissues showing inside presence of mycorrhizal colonization; (B) Untransformed roots despite mycorrhizal colonization; (C) Normal maize roots showing blue staining due to mycorrizal colonization and (D) Non-modified normal roots without blue staining indicating lack of mycorrhizal colonization

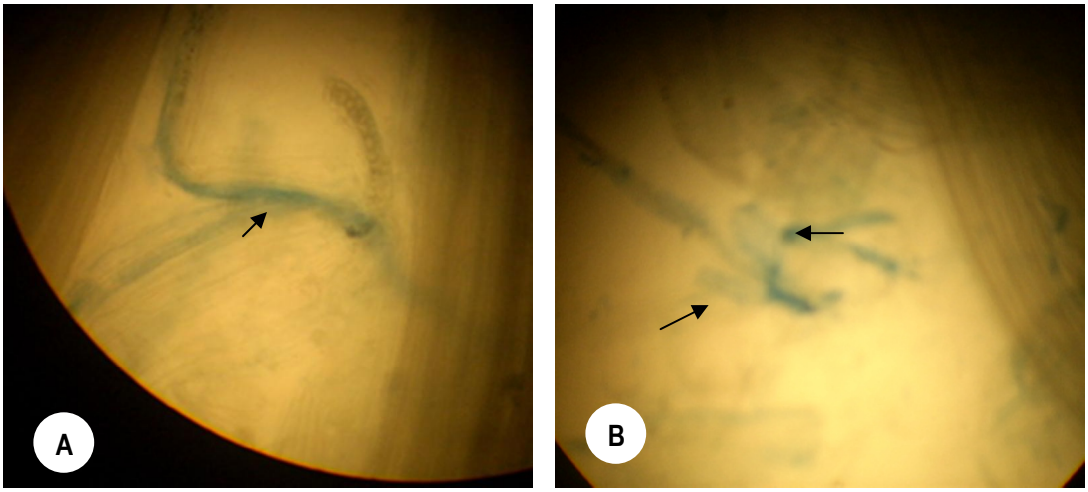

Figure 2. Growth and development of fungal hyphae inside roots of maize grown in soil collected beneath mature *Pterocarpus angolensis* from Mwekera Nation Forest Reserve in Kitwe, Zambia. (A) Blue staining showing vesicles and bundles of fungal hyphae inside root cells and (B) Arbuscules at root tips.

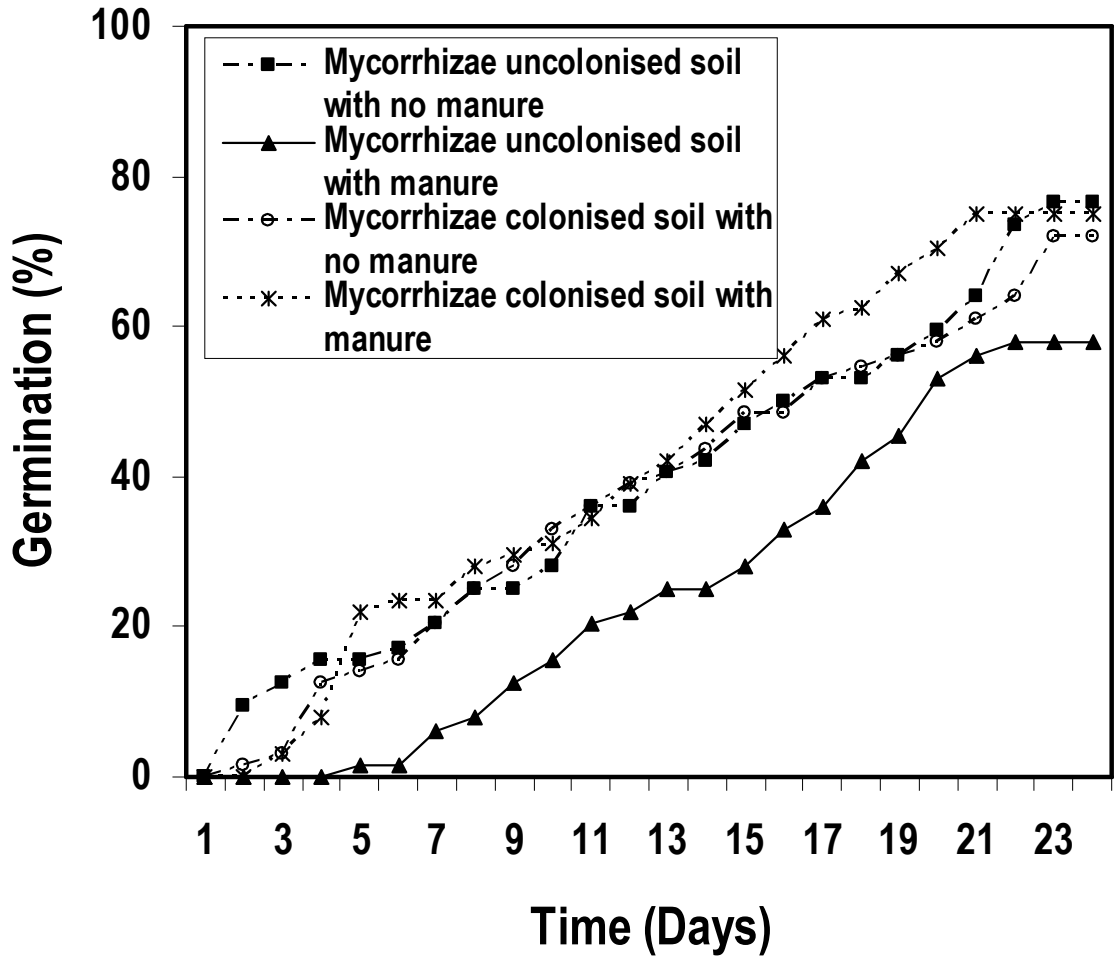

Figure 3. Germination pattern of *Pterocarpus angolensis* seeds sown in mycorrhizae-colonized and non-colonized soils from high rainfall area of Zambia.

Table 1: Growth and health status of *Pterocarpus angolensis* seedlings grown in mycorrhizae-colonized and non-colonized soils collected beneath mature *P. angolensis* in Mwekera Nation Forest Reserve in Kitwe, Zambia.

Soil Treatments[a]	% incidence Root Rot (%)[b]	Plant Height (cm)[c]		% Increase[d]	Collar Diameter (cm)[e]		(%) Increase
		30d	70d		30d	70d	
Mycorrhizae-uncolonized soil with no chicken manure	0.00	6.74	7.12	5.64	0.18	0.17	3.33
Mycorrhizae-uncolonized soil with chicken manure	5.64	5.87	6.18	5.28	0.16	0.17	3.25
Mycorrhizae-colonized soil with no chicken manure	0.00	6.86	7.33	6.85	0.18	0.18	4.63
Mycorrhizae-colonized soil with chicken manure	0.00	7.68	8.29	7.94	0.21	0.22	5.94
Average	1.41	6.79	7.23	6.48	0.18	0.19	5.56
CV (%)		7.90	8.20		6.30	6.70	…
LSD ($P \leq 0.05$)		0.86	0.95	…	0.02	0.02	…

[a]Soil obtained from Mwekera National Forest Reserve underneath mature *P. angolensis* trees
[b]Incidence determined as percentage of the number of roots with rot over the total number of seedling roots in each treatment
[c]Height measured from the stem-root collar to the highest terminal
[d]Percent increment plant height estimated 70 days after seed germination
[e]Diameter measured at root collar area

Table 2: Analysis of variance for growth parameters of *Pterocarpus angolensis* seedlings grown in mycorrhizae-colonized and non-colonized soils from Mwekera Nation Forest Reserve in Kitwe, Zambia, amended with or no chicken manure.

Source of Variation	df	Mean Squares[a]				
		Plant Height (cm)	Collar Diameter (cm)	Taproot Length (cm)	Plant Biomass (g) Dry Weight	
					Above Ground	Below Ground
Replication	3	0.34	0.040	0.59	0.05	0.013
Soil Treatment	3	2.98**[b]	0.217***	3.60***	45.96***	239.800***
Error	9	0.35	0.016	0.27	0.03	0.008
Total	15	0.87	0.061	1.00	9.33	48.000

[a]Mean squares of *Pterocarpus angolensis* growth parameters 70 days after seed sowing. Diameter and above and below ground biomass values have been multiplied by 100, 1000 and 10,000 respectively.
[b]** and *** statistically significant at $P \leq 0.01$ and $P \leq 0.001$, respectively

There were also statistically significant differences ($P \leq 0.001$) for taproot development and plant biomass between soil treatments (Table 2). The average taproot length of 12.22 cm for seedlings grown in mycorrhizae-colonized soil with chicken manure was significantly longer ($P \leq 0.05$) than the length of 10.44, 10.06 and 10.68 cm for seedlings grown in mycorrhizae-uncolonized soil without manure, mycorrhizae-uncolonized soil with manure and colonized soil without manure, respectively (Table 3). The mean dry weight of biomass above and below ground for seedlings grown in mycorrhizae-colonized soil amended with chicken manure was similarly significantly greater ($P \leq 0.05$) than that of seedlings in the other treatments (Table 3). The greatest weight of biomass above and below ground of 0.40 and 0.29 g, respectively, was for seedlings grown in mycorrhizae-colonized soil with manure; while the lowest was for seedlings grown in mycorrhizae-uncolonized soil with manure, which were 0.15 g and 0.12 g, respectively.

DISCUSSION

Overall, germination of *P. angolensis* seeds was low across the treatments and this could be due to several factors such as poor storage of the seeds before purchase and unfavorable temperature at the time of sowing that might have affected seed viability. According to Dzwonko & Gawronski (2002), temperature plays an important role in seed germination; with very low temperature delaying or reducing germination of seeds in certain tree species. In this study, seeds were sown during the month of August with the prevailing average temperature of about 20°C which is mild. In an earlier study by Chisha-Kasumu *et al*. (2007), germination of *P. angolensis* seeds ranged between 30-70% over 3 weeks to 6 months from sowing and varied considerably with seed source. This corroborates our observation whereby 57.8% germination occurred within 3 weeks in mycorrhizae-uncolonized soil with chicken manure and 76.6% in mycorrhizae-uncolonized soil with no manure. It was observed that seed germination was often low in mycorrhizae-uncolonized soil amended with chicken manure possibly due to presence of antimicrobial compounds from microorganisms in the manure that could have suppressed or inhibited seed germination (De Brito Alvarez *et al*., 1995). On the other hand, seeds sown in mycorrhizae-colonized soils with or without chicken manure had high germination rates mainly due to the influence of mycorrhizae on seed germination as has been previously reported (Takawira-Nyenya, 2005).

In this study, maize was used to culture mycorrhizal inocula *in-vivo*, which were used to ascertain fungal association types in soil. Laboratory analysis of maize roots confirmed the presence of VAM in the soil used mainly because of the absence of root surface modification by a fungal mantle that is characteristic of ectomycorrhizae (ECM), and also the presence of arbuscules, vesicles and fungal hyphae inside the root cells (Brundrett *et al.,* 1996; Tate, 2000). This finding is important for our study as it concurs with results by Hengari *et al.* (2004) in South Africa showing that mycorrhizae present underneath mature *P. angolensis* are VAM. VAM forms an association between fungi and plant root cells in which fungi live (Coleman & Crossley, 2003). They penetrate the host intracellularly to form terminal vesicles and highly-branched hyphal arbuscules. The later grow and ramify in a tree-like pattern inside the root cells (Högberg, 1992; Coleman & Crossley, 2003) and act as an interface for transfer of soil nutrients from the fungus to the host and for carbon compounds from the host to the fungus (Högberg, 1992; Farahani *et al*., 2008). Vesicles on the other hand constitute the storage structures for oil products (Högberg, 1992; Coleman & Crossley, 2003).

Table 3: Root development and biomass of *Pterocarpus angolensis* seedlings grown in mycorrhizae-colonized and non-colonized soils from Mwekera Nation Forest Reserve in Kitwe, Zambia, amended with or no chicken manure.

Soil Treatments[a]	Taproot Length (cm)[b]	Plant Biomass[c] (g) for		
		Above Ground	Below Ground	Total[d]
Mycorrhizae-uncolonized soil with no chicken manure	10.44	0.30	0.25	0.55
Mycorrhizae-uncolonized soil with chicken manure	10.06	0.15	0.12	0.27
Mycorrhizae-colonized soil with no chicken manure	10.68	0.32	0.25	0.57
Mycorrhizae-colonized soil with chicken manure	12.22	0.40	0.29	0.69
Average	10.85	0.29	0.23	0.52
CV (%)	4.80	1.80	0.40	…
LSD ($P \leq 0.05$)	0.83	0.01	0.001	…

[a]Soil obtained from Mwekera National Forest Reserve underneath mature *P. angolensis* trees
[b]Length from stem root collar sites to tips of taproots estimated 70 days after seed germination
[c]Biomass on basis of shoot and root dry matters 70 days after seed germination

[d]Total of above and below ground plant biomass

The chopped maize roots were mixed together with soil and added to unsterilized soils, already colonized by mycorrhizae, to enhance the colonization of the roots and boost *P. angolensis* seedlings growth and development. In addition, chicken manure was applied to the potting mix and this considerably enhanced plant growth especially in mycorrhizae-colonized soils as suggested by Brundrett *et al*. (1996). They observed that this often enhances mycorrhizal colonization and improves growth of the inoculated seedlings. Plants like maize, sorghum, onions and other grasses are able to culture mycorrhizae in roots (Brundrett *et al*., 1996; Wright & Upadhyaya, 1996). This is mainly because of the fibrous root characteristics which can capture massive fungal hyphae, numerous spores and other soil-borne mycoflora. Generally, the *P. angolensis* root system is coarse with few lateral hairs and low surface area for fungal hypha entanglement (Hengari *et al*., 2004). However, several studies have revealed that *P. angolensis* grows in association with VAM in natural habitat (Hengari *et al*., 2004; Takawira-Nyenya, 2005), an association which assists the trees in getting essential nutrients for growth, e.g. phosphorous. As a result of hyphal growth and elongation beyond the immediate root zone of the host plant, mycelia channel and concentrate soil nutrients from far away for the plant (Högberg & Piearce, 1986). Furthermore, Brundrett *et al*. (1996) observed that addition of manure to soil with mycorrhizae enhances colonization of mycorrhizal fungi on the host roots. As reported by Buee *et al*. (2000), chicken manure contains microbial populations some of which are parasites to plants. When these parasitic microbes attack plant roots, the roots react by secreting profuse exudates in the rhizosphere which in turn activate mycorrhizal germination, growth towards the fine roots and transcriptional activity often with inhibitory effects on potential host pathogens and beneficial impacts on the plant growth.

Consistently, significant differences ($P \leq 0.05$) were recorded in plant height, collar diameter, taproot length and plant biomass between seedlings grown in mycorrhizae-colonized soils and more so when chicken manure was added. The increase in all the plant growth parameters in these soil treatments could have been attributed to an increase in activity of meristematic cells influenced by mycorrhizal associations (Mishra & Mishra, 2004; Selvaraj & Chellappan, 2006). Moreover, chicken manure has been shown to stimulate root growth, increase nutrient uptake and provide a rich substrate for soil microbes (Reed, 1999; Aryantha *et al*., 2000). Nevertheless, the low plant biomass recorded for seedlings grown in mycorrhizae-uncolonised soil with chicken manure could be due to the reduction in seedling growth caused by chicken manure-borne microbes which might have become parasitic in the absence of mycorrhizae. About 5.6% of the roots of seedlings grown in this soil treatment developed root rot symptoms which could be attributed to the absence in the soil of antagonistic microorganisms such as endospore-forming bacteria and actinomycetes as a result of soil sterilization (Craft & Nelson, 1996; Reed, 1999; Aryantha *et al*., 2000). That there was no root rot infection in the other soil treatments strongly supports the view by Reed (1999) that microbial antagonism effects through addition of chicken manure to the soil offer a considerable protection to the plant roots against soil-borne pathogens. Hence, microbial imbalance in the mycorrhizae-uncolonized soils could have resulted into an increased utilization of the essential soil nutrients needed for plants growth by parasitic microorganisms (De Brito Alvarez *et al*., 1995), resulting in low seedling growth and biomass.

From our findings, we conclude that the growth performance of *P. angolensis* seedlings can be considerably improved by addition of chicken manure and mycorrhizal inoculum to the soil substrate in nursery beds. This silvicultural management is cost-effective as no fertilizers are needed since mycorrhizae increase considerably plant uptake of several nutrients; particularly phosphorous and nitrogen. With the achieved improved growth of *P. angolensis*, it is necessary to expand first field testing of the technology to ensure the feasibility of wider afforestation and reforestation programs of the species on a commercial level that have great potential for its conservation in Zambia and within the region.

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REFERENCES

Arpana J. and Bagyaraj DJ, 2007. Response of kalmegh to an arbuscular mycorrhizae fungus and a growth promoting rhizomicroorganism at two levels of phosphorus fertilizer. American-Eurasian Journal of Agriculture and Environmental Science 2: 33-38.

Aryantha IP, Cross R, Guest DI, 2000. Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures. Phytopathology 90: 775-782.

Aubrey A, 2003. *Pterocarpus angolensis* DC distribution. Witwatersrand National Botanical Garden, January 2003. South Africa National Biodiversity Institute, South Africa. http://www.sanbi.org/*Pterocarpus angolensis* distri.htm.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N, 1996. Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research. Pierrie Printers, Canberra, Australia. 374 pp.

Buee M, Rossignol MJ, Jaunean A, Ranjeva R, Becard G, 2000. The pre-symbiotic growth of Arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant exudates. Molecular Plant-Microbe Interact 13: 693-698.

Caro TM, Sungula M, Schwartz MW, Bella EM, 2005. Recruitment of *Pterocarpus angolensis* in the wild. Forest Ecology and Management 219: 169-175.

Chisha-Kasumu E, Price HA, Woodland S, 2006. In-vitro shoot multiplication and rooting from seedling explants of *Pterocarpus angolensis* in Zambia. Southern African Journal of Forestry 208: 31-37.

Chisha-Kasumu E, Woodward S, Price HA, 2007. Comparison of the effects of mechanical scarification and gibberellic acid treatments on seed germination in *Pterocarpus angolensis*. Southern Hemisphere Forestry Journal 69: 63-70.

Clewer AG. and Scarisbrick DH, 2001. Practical Statistics and Experimental Design for Plant and Crop Science. John Wiley & Sons (Eds.), West Sussex, England. 346 pp.

Coleman DC. and Crossley Jr, 2003. Fundamentals of Soil Ecology, Elsevier Science, Massachusetts, USA. 205 pp.

De Brito Alvarez M, Gagne AG, Antoun H, 1995. Effects of compost on Rhizosphere microflora of the tomato and on the incidence of plant growth promoting Rhizobacteria. Applied and Environmental Microbiology 6: 194-199.

Dubey RC. and Maheshwari DK, 2002. Practical Microbiology. S.Chand and Company Limited, New Delhi, India. 173 pp.

Dzwonko Z. and Gawronski S, 2002. Influence of litter and weather on seedling recruitment in a mixed Oak Pine woodland. Annals of Botany 90: 245-251.

Farahani HA, Lebaschi HM, Hamidi A, 2008. Effect of arbuscular mycorrhizal fungi, phosphorus and water stress on quantity and quality characteristics of coriander. Advances in Natural and Applied Sciences 2: 55-59.

Hengari SN, Valentine AJ, Theron JM, 2004. The effect of the colonization of arbuscular mycorrhizal fungi on the growth of pot grown *Pterocarpus angolensis* seedlings. Southern African Forestry Journal 202:13-20.

Högberg P, 1992. Root symbiosis of trees in African dry tropical forests. Journal of Vegetative Science 3: 393-400.

Högberg P. and Piearce GD, 1986. Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation types and successional patterns. Journal of Ecology 74: 775-785.

Javaid A, Riaz T, Nawazkhan S, 2007. Mycorrhizae status of *Narcissus papyraceus* Ker-Gawl Co-cultivated with *Cynodon dactylon* Pers. International Journal of Agriculture and Biology 9: 901-904.

Killham K, 1994. Soil Ecology. University of Cambridge, Cambridge, United Kingdom. 242 pp.

Mimba MM, 2008. Seedling recruitment in *Pterocarpus angolensis*: A case study of Mwekera National Forests. BSc. Degree unpublished thesis. School of Natural Resources, Copperbelt University. Kitwe, Zambia. 50 pp.

Mishra BB. and Mishra SN, 2004. Mycorrhizae and its significance in sustainable forest development. Orissa Review 11: 52-55.

Musaba M, 2008. The soil seed bank structure of a population of *Pterocarpus angolensis* DC. Family Fabaceae Syn. Papilionaceae. BSc. Degree unpublished thesis. School of Natural Resources, Copperbelt University. Kitwe, Zambia. 64 pp.

Mwitwa JP, 2004. Growth and physiological parameters related to shoot die back in *Pterocarpus angolensis DC* seedlings. PhD thesis. Stellenbosch University, South Africa. 169 pp.

Negi SS, 1996. An Introduction to Forest Pathology. International Book Distributors, Delhi, India. 238 pp.

Ng'andwe P, Muimba-Kankolongo A, Banda MK, Mwitwa JP, Shakashite O, 2007. Forest Revenue, Concession systems and the Contribution of the Forestry Sector to Poverty Reduction and Zambia's National Economy. An analytical report to FAO/nfp and Forest Department, Ministry of Tourism, Environment and Natural Resources. Lusaka, Zambia. 103 pp.

Reed M, 1999. Cultivating Australian Native plants: Achieving results with small research grants. A report for the Rural Industries Research and Development Corporation. RIRDC Publication No 99/7. School of Biological sciences, Macquarie University. Sydney, Australia. http://www.aff.org.au/Malcolm_Reed's_report_to_RIRDC.pdf .

Röper H, Khan S, Körner I, Stegmann R, 2005. Low-technique options for chicken manure treatment and application possibilities in agriculture. In: Cossu, R., Stegmann, R. (Eds.), Proceedings of Sardinia 2005 - Tenth International Waste Management and Landfill Symposium. 3 - 7 October 2005, S. Margherita di Pula, Cagliari, Italy.

Selveraj T. and Chellappan P, 2006. Arbuscular mycorrhizae: A diverse personality. Journal of Central European Agriculture 1: 349-358.

Stahle DW, Mushove PT, Cleveland MK, Roig F, Haynes GA, 1999. Management implications of annual growth ring in *Pterocarpus angolensis* from Zimbabwe. Forest Ecology and Management 124: 217-229.

Storrs AEG, 1995. "Know your Trees": Some of the Common Trees found in Zambia. Regional Soil Conservation Unit, Nairobi, Kenya. 380 pp.

Tate RL, 2000. Soil Microbiology. Second edition. John Wiley and Sons, Inc, New York, USA. 508 pp.

Takawira-Nyenya R, 2005. *Pterocarpus angolensis* DC. In: Jansen, P.C.M., Cardon, D. (Eds). Prota 7: Timbers, Wageningen, Netherlands. http://www.Prota.org/*Pterocarpus angolensis*_En.htm.

Webster J, 1993. Introduction to Fungi. 2nd edition. Cambridge University Press. Cambridge, UK. 669 pp.

Wright SF. and Upadhyaya A, 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science Journal 161: 575-586.