PRELIMINARY ASSESSMENT OF EXPONENTIAL NUTRIENT LOADING AND ARBUSCULAR MYCORRHIZAL INOCULATION ON THE PHYSICAL GROWTH OF *Acacia mangium* AND *Khaya senegalensis* SEEDLINGS IN TROPICAL FOREST NURSERY

Jeyanny, V.*, Wan Rasidah K., Lee S.S., Ghazali M.H. and Fauzi M.S.
Forest Research Institute Malaysia, 52109, Kepong, Selangor, WEST MALAYSIA.
Tel: 6(03) 62797136; Fax: 6(03)-62731427; Email: jeyanny@frim.gov.my

ABSTRACT

Acacia mangium and Khaya senegalensis have the potential to yield high quality timber for various wood products, namely, sawn timber, plywood, furniture and decorative works. Both species are popular for forest plantation in Malaysia. In order to enhance the growth and yield of these species, various methods involving soil enhancement has been introduced in plantation nursery. Exponential fertilization (EF) and arbuscular mycorrhizal (AM) inoculation were reported to initiate superior growth via improved mineral nutrition. Thus, the combined use of AM inoculation and EF on the selected species was further assessed in this study. The effects of the treatment on the physical growth of *A. mangium* and *K. senegalensis* seedlings were investigated. Pot trials of uninoculated control with standard fertilization (control, F1M1), uninoculated with EF (120 mg/kg P) [F2M1], uninoculated with EF (240 mg/kg P) [F3M1] were administered. The same fertilization treatments were done for inoculated seedlings as F1M2, F2M2 and F3M2 treatments. The experiment was laid out in a complete randomized design. Seedling growth was monitored for five months in the nursery. Shoot height, stem diameter and number of phyllodes/leaves were recorded monthly. The combined effects of standard fertilization with inoculation improved the overall seedling growth of *A. mangium* (stem diameter and phyllodes number) and *K. senegalensis* seedlings (stem diameter, mean height and leaf number).

Keywords: mycorrhizas, exponential fertilization, seedling establishment, nursery, forest plantation, yield

INTRODUCTION

The alarming rate of deforestation has prompted afforestation and reforestation efforts to compensate the timber source from the dwindling natural forests. The global timber industry is in need of species that yield high quality timber and fetched favourable price in the global market. Two of the species which have the promising potential for timber production is *Khaya senegalensis* (African Mahogany) and *Acacia mangium*. A member of the *Leguminosae*, *A. mangium* was first introduced to Malaysia in the early 1960s for the use of pulp and paper, sawn timber, plywood, wood chips and furniture. Its timber is priced at USD 73 m^{-3} (Malaysian Timber Industry Board 2007). *Khaya senegalensis* originates from Africa and was introduced to Malaysia in the 1950’s (Arnold 2004). This exotic and

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fast growing species from the *Meliaceae* family has been reported to have multiple uses, especially for the furniture industry to produce panels, cabinets, superior joinery and also other decorative works (Malaysian Timber Industry Board 2007).

The success of forest plantations starts from the early stages in nursery. Adequate environmental conditions and nutrient supply usually promises healthy seedlings for field outplanting. Thus, the key to expedite a seedling's growth is the ability to maintain its growth at a high exponential rate, provided with optimum light, temperature, moisture and nutrients (Timmer & Armstrong 1987). The concept of steady- state nutrition, whereby nutrients are applied at exponentially increasing rates that match early plant growth rates were formulated by Ingestad & Lund (1986). This condition is obtained when the relative uptake rate of nutrients is controlled by a numerically equal and constant relative addition rate (Ingestad & Agren 1995). It provides stable internal nutrient supply as opposed to conventional fertilization, where nutrient concentrations decline due to growth dilution (Timmer 1996). Furthermore, conventional method may trigger nutrient stress from excess fertilization at the initial growth stage and possible under-fertilization at the end. Exponential fertilization (EF) initiated superior growth due to retranslocation of nutrient reserves to active growth sinks (Boivin et al. 2004), and was reported to improve performances of *Picea mariana* (Close et al. 2005), mesquite (Imo & Timmer 1992) and Chinese fir seedlings (Xu & Timmer 1999). Improved outplanting responses of exponentially loaded over conventional fertilization on variable sites (Timmer & Munson 1991; Quoreshi & Timmer 2000) and on simulated soil fertility gradients (Xu & Timmer 1999; Salifu & Jacobs 2006) were also well documented. To date, EF has not been tested in species such as *Acacia* and *Khaya* which are widely used for plantation purposes.

Besides EF, some workers have also tested the ability of mycorrhizal inoculation combined with fertilization to enhance seedling growth. Quoreshi & Timmer (2000) provided some insights of the improved growth of *Picea mariana* (black spruce) inoculated with *Laccaria bicolor*, an ectomycorhizal fungus combined with EF. After transplanting, nutrient loaded and inoculated seedlings outperformed control by 45-92 % for dry weight (Quoreshi & Timmer 2000) and increased N, P and K nutrients (Quoreshi & Timmer 1998). Likewise, arbuscular mycorrhizas (AM) are also known to improve mineral nutrition from soil, particularly phosphorus (P) and increase plant yield (Mosse 1981). These microbes have been reported to improve plant resistance to drought (Sieverding 1991) and plant water retention (Kyllo et al. 2003). Initial investigations in Malaysia were mooted by Norani (1989) and Azizah & Kamal (1987), where AM inoculation on *Parkia speciosa*, *Albizia falcataria and Theobroma cacao* increased plant growth and nutrient uptake. Following suit, combined treatments of AM inoculation and fertilization were able to enhance growth of *A. falcataria*, *P. speciosa* (Norani 1989), *Hopea* spp. (Lee & Alexander 1994; Mohd. Ghazali 2004), *Azadirachta excelsa* (Ong et al. 2002) and *Acacia* spp. (Lee et al. 2006). However, to date, EF and AM inoculation has not been tested for

A. mangium and *K. senegalensis*. Gradual acclimatization of these seedlings to higher nutrient inputs exponentially may be conducive to increasing fungal tolerance to nutrient loading treatments. Furthermore, healthy and vigorous seedlings may perform better in variable sites of outplanting. The purpose of this study was to evaluate and report if mycorrhizal inoculation and EF would be an added advantage to promote the physical growth and development of *K. senegalensis* and *A. mangium* seedlings.

MATERIALS AND METHODS

Growing conditions and seedling establishment

The study was conducted at the FRIM shade house at Kepong (03°13’58 N: 101°38’06 E). The shade house temperature ranged between 29 and 32 ºC, had light penetration of 32% and relative humidity of between 80-82%. Polyethylene bags (15 cm x 20 cm) were filled with 2.0 kg of a 3:2:1 ratio of unsterile soil:organic matter:sand prior to transplanting. It is common practice to use unsterile soil for planting seedlings in Malaysia. The unsterile soil consisted of Rengam series (*Typic Paleudult*) top soil. The soil mixture contained 0.14% N, 2.8% organic C, 35.5 mg kg $^{-1}$ available P, 0.89 cmol kg $^{-1}$ Ca, 0.14 cmol kg $^{-1}$ Mg, 0.29 cmol kg $^{-1}$ K initially. The pH, cation exchange capacity (CEC) and electrical conductivity (EC) of the media were 5.0, 7.9 cmol kg $^{-1}$ and 0.02 mS cm $^{-1}$ respectively. *Acacia mangium* seeds were purchased from the FRIM Seed Technology Laboratory. The seeds were collected from Karak, Pahang and *K. senegalensis* seeds were collected from Kangar, Perlis. Seeds were germinated on river sand beds for 1 month before transplanting. The nutrient content of seedlings prior to experiment was determined only for P which was 0.5% for *K. senegalensis* and 0.4% for *A. mangium* seedlings.

The treatments for the pot trials were as follows:

F1M1: With standard fertilization, without AM inoculation (control)
F2M1: With exponential fertilization (120 mg P kg $^{-1}$ soil), without AM inoculation
F3M1: With exponential fertilization (240 mg P kg $^{-1}$ soil), without AM inoculation
F1M2: With standard fertilization, with AM inoculation
F2M2: With exponential fertilization (120 mg P kg $^{-1}$ soil), with AM inoculation
F3M2: With exponential fertilization (240 mg P kg $^{-1}$ soil), with AM inoculation

Mycorhizal inoculation and exponential fertilization

Seedlings were inoculated using Rhizagold^{TM} for +AM treatments by adding 50g/polybag to seedling roots during transplanting. Rhizagold^{TM} (Biotrack Technology Sdn Bhd, Serdang, Malaysia) is commercially produced native inoculum which consists of 12 species of arbuscular mycorhizal fungi with a concentration of 250-300 spores/10 g product. The product contains a mixture of species from the genera *Glomus*, *Gigaspora*, *Acaulospora* and *Scutellospora*. Fertilization using the common fertilizer in forest nurseries, NPK Blue Special 12:12:17 at the rate of 2g/polybag (2 kg soil) was done 1 week after transplanting for 5 consecutive months for standard fertilization regime. Each 1g NPK Blue Special fertilizer contains 120 mg of N, P, K and 2 mg of Mg respectively. The exponential rates were based on increasing amount of P. Exponential fertilization was done using the same fertilizer at different rates for F2 and F3 treatments for 20 weeks (5 months) whereby the weekly nutrient addition delivery schedule was based on the exponential function described by Ingestad & Lund (1986) and Timmer & Armstrong (1987):

$$\mathbf{N_T = N_S (e^{rt}-1)}$$

$\mathbf{N_T}$ is the selected amount of P to be added for 20 weeks, $\mathbf{N_S}$ is the initial amount of P in seedling (0.5% for *K. senegalensis* and 0.4% for *A. mangium*), t is the number of fertilization periods and r is the relative addition rate needed to increase $\mathbf{N_S}$ to a final level $\mathbf{N_T + N_S}$. The rates for exponential fertilization were 120 mg P kg $^{-1}$ soil for F2 and 240 mg P kg $^{-1}$ soil for F3 respectively. The fertilizer application rates for F1, F2 and F3 are shown in Figure 1. All pots were irrigated using an automated sprinkler system for 3 minutes twice daily.

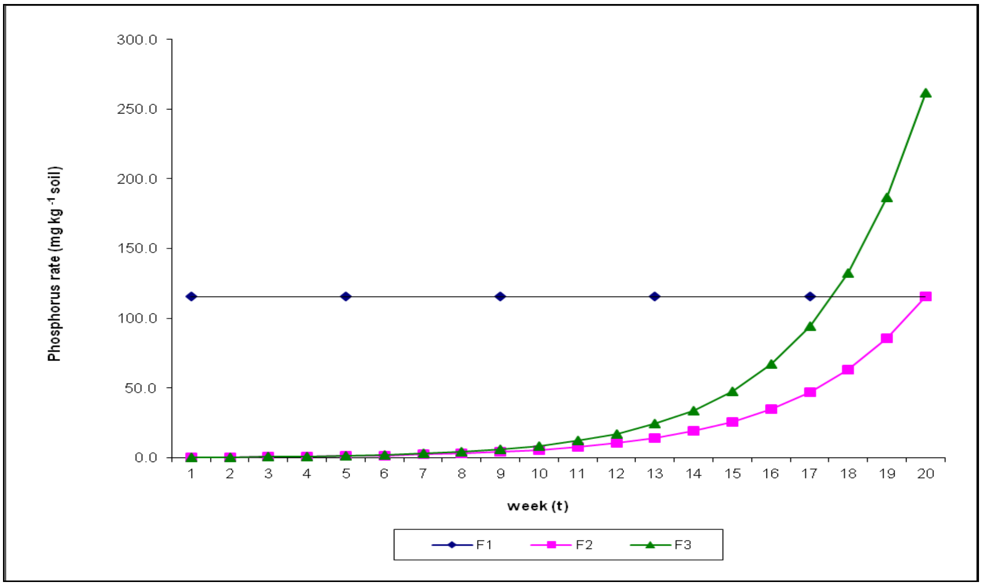

Figure 1: Weekly application of phosphorus fertilization rate for a 20 week duration
F1: Standard fertilization (120 mg P kg^{-1} soil)
F2: Exponential fertilization (120 mg P kg^{-1} soil)
F3: Exponential fertilization (240 mg P kg^{-1} soil)

Experimental design and analysis

Each treatment consists of 135 replications for *K. senegalensis* and 118 replications for *A. mangium*. The experiment was laid out in a Complete Randomized Design due to minimal environmental effects such as sunlight exposure, humidity and temperature. The effects of the treatments were evaluated for 5 months starting from October 2008 based on stem diameter (mm), seedling height (cm) and number of phyllodes. However, stem diameter data at the initial stage (October 2008) was not reported as the values were less than 0.5 mm for both species. The seedlings growth was monitored in the nursery prior to outplanting in field conditions. All variables were analyzed using one-way ANOVA. Data transformation was done for phyllodes to fit the assumption of a normalized distribution before analysis. Tukey Test was used for means comparison using Statistical Analysis System version 9.13. In this study, only physical growth of seedlings were reported as the seedlings were subjected to be utilized for outplanting trials in varying soil fertility gradients. Thus, destructive sampling for further laboratory analyses was avoided.

RESULTS

Mean stem diameter

It was apparent that standard fertilization with inoculation (F1M2) was able to significantly increase stem diameter of *A. mangium* seedlings (12 and 7%) in November and December respectively (Table 1). Control was same with F1M2 and F2M2 in January. Results of F1M2 were comparable with all other treatments in February except with F3M2. Generally, higher values were obtained for F1M2 compared to control for most of the months. Relatively lower values compared to control were recorded in F3M2 from November to February.

Initially, F1M2 and control was comparable and significantly superior compared to all other treatments for *K. senegalensis* seedlings (Table 2). By time, all treatments were same with control except F3M2 in February. Stem diameter of F3M2 was relatively lower compared to control during the whole duration of the experiment.

Mean height

The mean value for control (F1M1) in terms of height was significantly the highest among all treatments from October to February for *A. mangium* seedlings. Exponential fertilization without inoculation (F3M1) apparently had the lowest values for October, December and February.

In October, most treatments had the same effect on mean height except for F3M2 for *K. senegalensis*. However, values for December, January and February significantly increased for treatment with standard fertilization and inoculation (F1M2) compared to control almost 6, 29 and 10% respectively.

Table 1: Growth performance of *A. mangium* seedlings at 5 months old

Growth Parameter	Month	F1M1	F2M1	F3M1	F1M2	F2M2	F3M2
Mean stem diameter (mm)	Nov	1.88c † (0.03)	1.89c (0.02)	1.92bc (0.02)	2.11a (0.02)	2.00b (0.02)	1.84c (0.02)
	Dec	3.20b (0.05)	2.90c (0.04)	2.51d (0.03)	3.44a (0.03)	2.87c (0.04)	2.51d (0.03)
	Jan	3.73a (0.06)	3.12b (0.04)	2.79b (0.04)	3.92a (0.04)	4.11a (0.21)	2.82b (0.04)
	Feb	5.12ab (0.10)	5.06ab (0.08)	4.86ab (0.08)	5.85a (0.60)	4.90ab (0.08)	4.30b (0.06)
Mean height (cm)	Oct	6.82a (0.14)	5.75bc (0.12)	5.48c (0.10)	6.18b (0.11)	5.88bc (0.12)	5.84bc (0.13)
	Nov	15.37a (0.47)	12.91b (0.35)	12.03bc (0.38)	9.49d (0.17)	10.60d (0.22)	10.72cd (0.27)
	Dec	27.46a (0.71)	20.30b (0.62)	17.95c (0.63)	19.14bc (0.33)	19.48bc (0.43)	20.13bc (0.61)
	Jan	37.80a (1.05)	31.18b (1.02)	26.83c (0.98)	19.86d (0.33)	20.08d (0.43)	20.74d (0.62)
	Feb	46.67a (1.19)	38.89bc (1.24)	35.37c (1.13)	42.46ab (1.10)	40.60b (1.15)	40.73b (1.37)
No. of phyllodes	Oct	3b (0.01)	3b (0.01)	3b (0.01)	3b (0.01)	3b (0.01)	4a (0.01)
	Nov	7a (0)	5b (0)	5b (0.01)	7a (0.01)	5b (0.01)	5 b (0.01)
	Dec	12a (0)	10b (0)	9c (0)	11b (0)	10b (0)	9c (0)
	Jan	16a (0)	14b (0)	13c (0)	14b (0)	14b (0)	13c (0)
	Feb	17bc (0.01)	24bc (0.01)	23 c (0.01)	31a (0.01)	28b (1.15)	23c (0.01)

Mean values in rows followed by different letters are significantly different for treatments by Tukey ($p \leq 0.05$).
[†] Values in parentheses represent standard error.
(control, F1M1: Standard fertilization, - AM; F2M1: exponential fertilization [120 mg P kg^{-1}soil], - AM;
F3M1 : exponential fertilization [240 mg P kg^{-1} soil],- AM; F1M2: standard fertilization, + AM; F2M2:
exponential fertilization [120 mg P kg^{-1} soil], +AM; F3M2 : exponential fertilization [240 mg P kg^{-1} soil], + AM)

Number of phyllodes/leaves

Although F3M2 was significantly higher for number of phyllodes in October compared to control, control fared better in November to January, whereby the increase was 29 and 19% higher respectively. At the end of the experiment, F1M2 outdid control almost 2- folds.

Results for control were consistently higher or the same compared to other treatments for the months of November till January in *K. senegalensis* seedlings. At the end, F1M2 had the highest number of leaves but was the same as control, F2M1 and F2M2.

DISCUSSION

The combined effects of standard fertilization with inoculation gave an added advantage in improving the overall seedling growth of *A. mangium* (stem diameter, phyllodes number) and *K. senegalensis* seedlings (stem diameter, mean height and leaf number). Similar results were obtained in our previous study (Jeyanny et al. 2011) where significant physical growth was pertinent in *A. mangium* seedlings. Another study by Ghosh & Verma (2006) substantiated that VAM fungi inoculation significantly increased stem diameter, leaf area and chlorophyll content of *A. mangium* seedlings. However, the same treatment did not show substantial changes for mean height of *A. mangium* seedlings. Control outdid other treatments for mean height of *A. mangium* seedlings from October to February. In contrary, the mean height of *K. senegalensis* seedlings was the highest for standard fertilization and inoculation treatment from December to February. Investigation by Fisher & Cox (1980) demonstrated an increase of 26% in seedling height for *Quercus rubra* seedlings for inoculated seedlings compared with uninoculated seedlings.

Table 2: Growth performance of *K. senegalensis* seedlings at 5 months old.

Growth parameter	Month	F1M1	F2M1	F3M1	F1M2	F2M2	F3M2
Mean stem diameter (mm)	Nov	2.11a † (0.03)	1.89c (0.02)	1.91bc (0.02)	2.11a (0.02)	2.00b (0.02)	1.84c (0.02)
	Dec	3.22b (0.04)	2.90c (0.04)	2.50d (0.03)	3.44a (0.03)	2.88c (0.04)	2.52d (0.03)
	Jan	3.76a (0.05)	3.32b (0.05)	2.78c (0.04)	3.92a (0.04)	4.13a (0.21)	2.84c (0.04)
	Feb	5.15a (0.09)	5.05a (0.09)	5.14a (0.28)	5.23a (0.08)	4.90a (0.08)	4.30b (0.09)
Mean height (cm)	Oct	6.12a (0.12)	6.38a (0.12)	6.52a (0.11)	6.41a (0.12)	6.40a (0.11)	5.24b (0.10)
	Nov	10.70a (0.15)	8.37b (0.13)	8.16b (0.14)	10.74a (0.15)	8.37b (0.14)	6.83c (0.11)
	Dec	17.02b (0.28)	14.02c (0.23)	12.18d (0.18)	18.01a (0.25)	14.25c (0.24)	11.24d (0.16)
	Jan	17.48b (0.29)	17.04bc (0.32)	15.91c (0.26)	22.60a (0.34)	18.08b (0.36)	14.47d (0.24)
	Feb	25.83b (0.48)	23.38c (0.42)	22.65c (0.37)	28.48a (0.43)	25.70b (0.43)	20.52d (0.34)
No. of leaves	Oct	3ab (0.01)	3ab (0.01)	3ab (0.11)	3ab (0.12)	3ab (0.01)	4a (0.01)
	Nov	7a (0)	5b (0)	5b (0.01)	6a (0.15)	5b (0.01)	5b (0.01)
	Dec	12a (0)	10b (0)	9c (0)	11b (0)	10b (0)	9c (0)
	Jan	13a (0)	11b (0)	10c (0)	11b (0.34)	14a (0.01)	10c (0.24)
	Feb	27b (0.01)	24bc (0.01)	23c (0.01)	31b (0.43)	27b (0.01)	23c (0.34)

Mean values in rows followed by different letters are significantly different for treatments by Tukey ($p \leq 0.05$).
† Values in parentheses represents standard error
(control, F1M1: Standard fertilization, - AM; F2M1: exponential fertilization [120 mg P kg $^{-1}$soil], - AM;
F3M1 : exponential fertilization [240 mg P kg^{-1} soil],- AM; F1M2: standard fertilization, + AM; F2M2:
exponential fertilization [120 mg P kg^{-1} soil], +AM; F3M2 : exponential fertilization [240 mg P kg^{-1} soil], + AM)

Arbuscular mycorrhizas play an important role in P transfer (Mosse 1981) and growth promotion by AM was also observed at high P availability in soil (Moyersoen et al. 1998) in our study. In glasshouse experiments, N fertilization was known to either decrease or increase mycorrhizal infection (Abbott 1991). However, K fertilization was known to have minimal effects on infection rates (Alizadeh et al. 2010). The formation and the function of mycorrhiza are affected by plant species (Krishna & Dart 1984), soil composition, moisture, temperature, pH, CEC and anthropogenic abiotic and biotic stresses. Some AM fungi perform poorly in low ph soils, which are highly acidic due to excessive fertilization (Entry et al. 2002). Exponential fertilization which was administered in this study, especially F3M2 was detrimental for most of the variables tested in both species given the duration of the experiment. The effects of F3 rates on *A. mangium* and *K. senegalensis* seedlings would have been more conclusive if the experiment was extended but had to be shortened due to funding constraints. High soluble salts in the F3 treatments may have reduced plant growth by osmotic effect, damaging the young seedlings (Miller & Gardiner 1998) or may have deterred the effectiveness of AM mycorhiza (Sieverding 1991; Chen et al. 2006), affecting plant growth. Generally, EF was not suitable in this study but standard fertilization with inoculation (F1M2) was found to be significant for most of the variables tested in both species. Investigations on both species in this study were able to forecast whether EF treatments would be beneficial or not in enhancing nutrient capacity of seedlings for outplanting trials.

CONCLUSION

The combined effects of standard fertilization with AM inoculation positively increased seedling growth of *K. senegalensis* and *A. mangium*. Exponential fertilization administered at 240 mg P kg $^{-1}$ soil was detrimental for seedling growth of both species in the preliminary stage.

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