# GROWTH EFFECTS BY ARBUSCULAR MYCORRHIZA FUNGI ON OIL PALM (*Elaeis guineensis* Jacq.) SEEDLINGS

**SHAMALA SUNDRAM***

**ABSTRACT**

*The effects of arbuscular mycorrhiza fungi (AMF) infection on the growth of oil palm seedlings in nursery trials showed that the seedlings treated with* Glomus etunicatum *soil inocula resulted in 80.36% increased growth response. Oil palm seedlings treated with* G. etunicatum *in association with* Gigaspora rosea, Acaulospora morrowiae *and* Scutellaspora heterogama, *respectively, gave lower to negative growth responses. Oil palm seedlings that showed growth depression, however, had significantly higher root colonizations than the non-AMF plants.* G. etunicatum *thrives better as a single inoculant and successfully increases the vegetative growth of oil palm seedlings.*

**Keywords:** AMF, *Glomus etunicatum*, growth, oil palm.

**Date received**: 8 October 2009; **Sent for revision:** 27 October 2009; **Received in final form:** 27 January 2010; **Accepted:** 6 April 2010.

## INTRODUCTION

Malaysian palm oil industry contributes about 10% of the world's edible oils and fats. In 2008, Malaysia's area under oil palm (*Elaeis guineensis* Jacq.) cultivation represents about 1.84% of the world's total oilseed area (MPOC, 2008). The industry is also subjected to heavy usage of chemical fertilizers. Sustainable practices in the oil palm industry involve careful fertilizer management to reduce excess application over the required amount in the field. This study was undertaken to investigate whether arbuscular mycorrhiza fungi (AMF) could serve as a biological fertilizer for oil palm and eventually reduce the fertilizer cost.

* Malaysian Palm Oil Board,
P. O. Box 10620,
50720 Kuala Lumpur,
Malaysia.
E-mail: shamala@mpob.gov.my

AMF are obligate fungi which form a symbiotic relationship with their host plant, whereby the latter receives mineral nutrients supplied by the fungal partner, while the fungus in return obtains photosynthetically-derived carbon compounds (which the host has in abundance) as food and energy sources. Studies have shown that AMF-inoculated plants such as patchouli (Arpana *et al*., 2008) and soyabean (Meghvansi *et al*., 2008) had increased vegetative plant growth; some researchers used a single-species inoculum while others combined one AMF with another AMF or a non-AMF microbe as partners in a consortium. Species of *Glomus* are particularly prevalent in agricultural systems (Smith *et al*., 2003). In soils collected from several oil palm and cocoa plantations in Malaysia were also found to be mostly being the members of *Glomus* (Nadarajah, 1980). Several studies have shown that AMF fungi were especially good absorbers of phosphates from phosphorus-deficient soils. Plantation soils in Malaysia are generally acidic and deficient in some minerals, particularly phosphorus (Coulter, 1972). Based on these reports, this investigation

was initiated with *Glomus etunicatum*, a species that has also been reported as a promising plant growth promoter in many studies (Rubio *et al*., 2002; Arpana *et al*., 2008).

The objective of this study was to assess the response of oil palm seedlings to a soil inoculum consisting of single-species *G. etunicatum* compared to two species-consortium inocula of *G. etunicatum* with *Gigaspora rosea*, *Scutellspora heterogama* or *Acaulospora morrowiae*, respectively.

## MATERIALS AND METHODS

### Sources of Biological Materials

Spores of *G. etunicatum*, *Gigaspora rosea*, *Scutellaspora heterogama* and *Acaulospora morrowiae* delivered in vials of 40 spores per species in sterilized sand were purchased from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM), University of Virginia, USA. Oil palm seedlings (henceforth referred as 'plants') of *dura* x *pisifera* (DxP) at two and a half months of age were purchased from Sime Darby, Malaysia. The plants were raised in sandbeds in Universiti Putra Malaysia (UPM), Serdang, Selangor, for four weeks to stimulate root elongation, then transferred into garden pots for greenhouse acclimatization and used for nursery trials. The potting medium used was a horticultural soil formulation of 3:2:1 of peat, clay and sand. All soils used were non-sterilized to imitate field conditions. All nursery experiments were carried out under greenhouse conditions at UPM.

### Application of AMF Fungi

A total of 100 healthy plants, all four months old and of fairly equal size, were selected, divided into five groups or treatments, *i.e*. four experimental variants and one control. The first group of plants was applied with a single-species inoculum of *G. etunicatum* at 80 spores per plant (Treatment I, or TI). The other three variants consisted of two-species consortium inocula of *G. etunicatum* with *Gigaspora rosea* (TII), with *Scutellaspora heterogama* (TIII) and with *Acaulospora morrowiae* (TIV), respectively, again comprising a total of 80 spores per plant. These inocula were applied to 20 replicate plants for each of the five treatments, giving a total of 100 plants. The TI soil inoculum was made by mixing two vials of *G. etunicatum* (*i.e*. a ratio of 2:0) in 300 g of unsterilized soil in a separate container. For the mixed-species inocula, one vial of *G. etunicatum* was mixed with one vial of either *Gigaspora rosea* (TII), *Scutellaspora heterogama* (TIII) or *Acaulospora morrowiae* (TIV) before application in accordance to the respective treatments. Earthen garden pots (28.0 × 25.5 × 16.0 cm) were filled up to one-third with unsterilized soil. The soil inoculum was then placed as a layer over the soil in the pots and manually mixed very lightly to a depth of about 1 cm to create an AMF-inoculated rhizosphere. Next, the plants were removed from their pots and replanted into the new pots containing the AMF soil inocula. More soil was used to fill the pot to the top, then the soil was manually compacted. No AMF was applied to the control plants (C). All the plants were liberally watered daily in the morning throughout the 16 weeks of experimentation.

### Growth Parameters

Four parameters were used for growth assessment, namely, (i) leaf surface area, (ii) dry weights of shoots and roots, along with calculations of the mycorrhizal growth response (MGR) based on dry weights, (iii) mycorrhiza root colonization, and (vi) leaf nutrient contents of nitrogen (N), phosphorus (P) and potassium (K). Data on week 16 were used in this study for statistical analyses. All data on growth parameters were analysed using the analysis of variance (ANOVA), and Duncan's multiple range test (DMRT) for ranking of statistically significant data.

For the leaf surface area assessment, 10 plants per treatment were randomly selected. All leaves from each plant were passed through a portable leaf area meter (LI-COR; Li-3000A), and the readings recorded.

For the N, P and K leaf contents, five plants per treatment were randomly selected. Three mature leaf blades (leaf No. 3, 4 and 5) were excised from each plant, oven-dried to constant weight, then ground using a household electric grinder. Samples of approximately 0.25 g each were weighed for NPK analysis, using three replicate samples per treatment. The samples were sent to the Soil Analysis Laboratory, UPM, where the content of N was determined by the standard Kjeldahl method, while P and K contents were analysed following the standard dry digestion method (Kjeldahl, 1883).

Determination of plant dry weights were made on 10 randomly selected plants per treatment, not including those from which leaves had been cut for the NPK analysis. Each plant was carefully uprooted and cut into an upper part (which consisted of leaves and a short stem, henceforth referred as 'shoots') and a lower part, comprising the roots. Each group was labelled according to the experimental variants, then sun-dried followed by oven-drying at 80°C until constant weight. The dry weight or biomass data for shoots and roots were recorded.

The total plant dry weight was obtained from the arithmetical sum of the means of shoot and root weights, and these data were used in the calculation of the growth response of the plants to mycorrhizal colonization *(i.e.* MGR), using the following equation (Dickson *et al*., 1999):

$$\text{MGR (\%)} = \frac{\text{M - NM}}{\text{NM}} \times 100$$

where M is the total plant weight for AMF-inoculated plants, and NM is the total plant weight for non-AMF inoculated plant (measured in dry weight).

Mycorrhizal root colonization was assessed from five randomly selected replicate plants per treatment, not including any of the previously sampled plants. The plants were carefully uprooted and cut at the base. The roots were gently washed, blotted dry then oven-dried at 80°C to constant weight. Approximately 1 g biomass of fine tertiary root hairs was cut to 1 cm lengths ('segments') and stained using the method of Koske and Gemma (1989). Briefly, this involved clarifying the roots in aqueous KOH during which process the roots were heated at 90°C for 10 to 30 min, rinsed in several changes of water, bleached in alkaline $H_2O_2$, rinsed again and then soaked in 1% HCl for 1 to 24 hr. After root clarification, they were stained in 0.05% Trypan Blue for 10 to 30 min at 90°C then destained in acidic glycerol. AMF-root colonization was measured using the grid line intersect method of Giovannetti and Mosse (1980). A minimum of 100 intersects per treatment was used, and resampling was done three times. The segments were randomly dispersed in a petri dish with grid lines drawn on it, and examined over a white-light box to count the number of stained (presence of AMF) and non-stained (absence of AMF) segments. Details on how the data were scored are well described in Brundrett (2008). The percentage of roots with AMF inoculation was calculated from:

$$\text{Mycorrhizal root coloniza (\%)} = \frac{\text{Number of mycorrhizal segments (stained)}}{\text{Total number of sampled segments}} \times 100$$

## RESULTS

### Leaf Area Measurements

The highest leaf area reading was recorded on T1 plants at 506.73 $cm^2$, or a 63.73% increase compared to the control (non-AMF inoculated seedlings), while the lowest was from TII (consortium with *G. rosea*) at 247.22 $cm^2$, representing a negative value of -19.96% compared to the control, C (*Table 1*). The ranking of the treatments for this parameter in descending order was I > IV > III > C > II (*Table 1*). The percentage of leaf area increase or decrease in each treatment was calculated using the difference obtained between the value of the respective treatment and that of the control C.

### Leaf, Root and Total Plant Dry Weights

The highest and most significant value for shoot dry weight was obtained from plants of TI and TIV (consortium with *A. morrowiae*) at 6.21 g and 5.12 g, respectively, with no statistical difference between them at the 95% confidence level. This was followed by the plants of the non-AMF control at 3.48 g. Although plants of TII (consortium with *Gigaspora rosea*) and TIII (consortium with *S. heterogama*) had lower dry weights than the control, but were not significantly different from each other with a ranking from highest to lowest mean shoot dry weights at week 16 as follows: I > IV > C > III > II.

Plants of TI and TIV again gave the significantly highest values for root dry weights at 1.41 g and 1.39 g, respectively, while all other experimental variants showed no statistical difference from the control plants (0.74 g), giving the same ranking from highest to lowest dry weights as for the shoots as may be seen in *Figure 1*. When both plant parts were summed to give the total plant biomass, the ranking in order of decreasing total plant biomass was the same as for the root and shoot dry weights as shown in *Table 1*.

### Mycorrhizal Growth Response (MGR)

MGR was highest for TI at 80.36% compared to the non-AMF inoculated seedlings (control). This was followed by a small but positive response for TIV plants at 53.96%. For TII and TIII (in consortia with *G. rosea* and *S. heterogama*, respectively), negative MGRs was recorded at -22.45% and -21.0%, respectively, indicating growth depression. The order of percentage MGR ranked from highest to lowest was I > IV > III > II (*Table 1*).

### Root Colonization

AMF root colonization was highest for TI and TIII (which resulted in growth depression) at 51.08% and 52.18%, respectively compared to the non-AMF control plants (4.82%). The ranking of percentage root colonization in decreasing order was I > III > IV > II (*Table 1*).

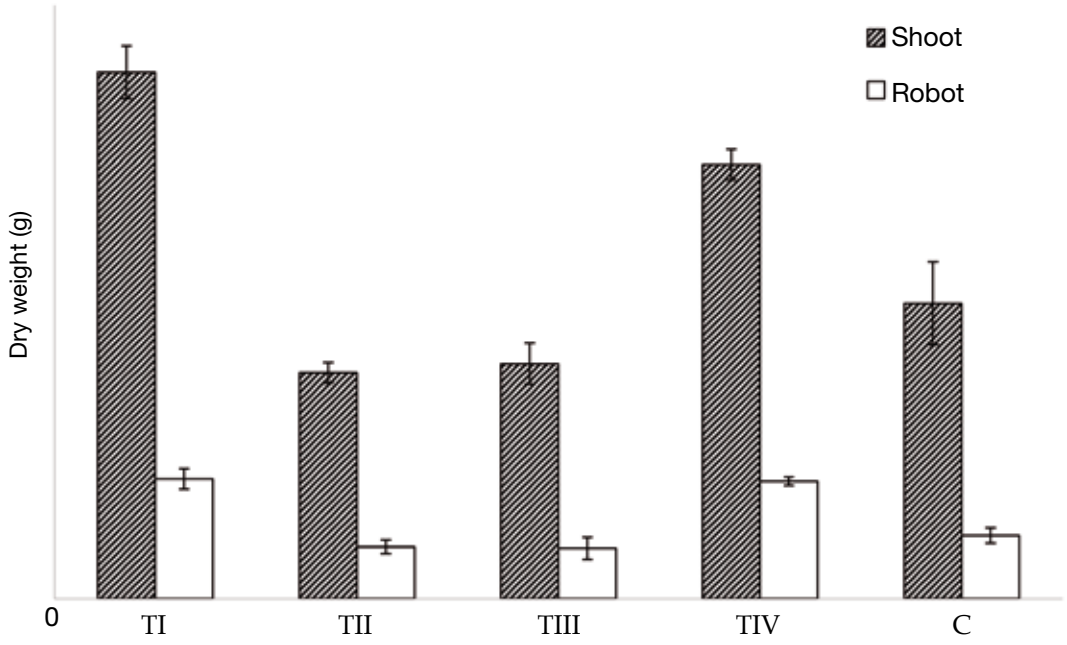
### Nitrogen, Phosphorus and Potassium Leaf Contents

There was no effect on N and P uptake as shown by leaf contents of these nutrients from all the experimental plants as no significant difference

**TABLE 1. MEAN OF LEAF AREA, TOTAL PLANT BIOMASS, MYCORRHIZAL GROWTH RESPONSE (MGR) AND ROOT COLONIZATION BY ARBUSCULAR MYCORRHIZA FUNGI (AMF) ACCORDING TO TREATMENT AFTER 16 WEEKS**

| Treatment | Leaf area ($cm^2$) (% increase) | Total dry biomass weight (g) | MGR (%) | AMF root colonization (%) |
|---|---|---|---|---|
| TI = 2:0 Ge | $506.73^{a} \pm 31.7$ (63.73) | $7.62^{a} \pm 0.22$ | 80.36 | $51.08^{a}$ |
| TII = 1:1 Ge + Gr | $247.22^{d} \pm 17.5$ (-19.96) | $3.28^{c} \pm 0.13$ | -22.45 | $32.49^{c}$ |
| TIII = 1:1 Ge + Sh | $345.82^{c} \pm 24.3$ (11.74) | $3.37^{c} \pm 0.19$ | -21.08 | $52.18^{a}$ |
| TIV = 1:1 Ge + Am | $455.45^{b} \pm 19.5$ (47.16) | $6.51^{a} \pm 0.12$ | 53.96 | $41.31^{b}$ |
| C = Non-AMF inoculated control | $309.48^{c} \pm 11.6$ (-) | $4.23^{b} \pm 0.29$ | - | $4.82^{d}$ |

Note: Values within a column followed by the same alphabet are not significantly different at $p \leq 0.05$ using Duncan's multiple range test (DMRT).
TI : Oil palm seedlings pre-inoculated with *Glomus etunicatum* (Ge).
TII: Oil palm seedlings pre-inoculated with Ge and *Gigaspora rosea* (Gr).
TIII: Oil palm seedlings pre-inoculated with Ge and *Scutellaspora heterogama* (Sh).
TIV: Oil palm seedlings pre-inoculated with Ge and *Acaulospora morrowiae* (Am).
C : Oil palm seedlings with no inoculation of AMF.
1:1 and 2:0 : AMF application ratios in oil palm seedlings according to weight of each inoculum.




Legend:
TI : Oil palm seedlings pre-inoculated with *Glomus etunicatum* (Ge).
TII : Oil palm seedlings pre-inoculated with Ge and *Gigaspora rosea* (Gr).
TIII : Oil palm seedlings pre-inoculated with Ge and *Scutellaspora heterogama* (Sh).
TIV : Oil palm seedlings pre-inoculated with Ge and *Acaulospora morrowiae* (Am).
C : Oil palm seedlings with no inoculation of AMF.

*Figure 1. Mean dry weights of shoots and roots of oil palm seedlings at 16 weeks after inoculation with arbuscular mycorrhiza fungi (AMF).*

**TABLE 2. NITROGEN, PHOSPHORUS AND POTASSIUM (NPK) CONTENTS IN LEAVES OF OIL PALM SEEDLINGS AT 16 WEEKS ACCORDING TO TREATMENT**

| Treatment | Nitrogen (%) | Phosphorus (%) | Potassium (%) |
|---|---|---|---|
| TI = 2:0 (Ge) | $2.84^{a} \pm 0.03$ | $0.37^{a} \pm 0.02$ | $2.76^{a} \pm 0.12$ |
| TII = 1:1 (Ge + Gr) | $2.80^{a} \pm 0.01$ | $0.36^{a} \pm 0.06$ | $2.39^{b} \pm 0.02$ |
| TIII = 1:1 (Ge + Sh) | $2.79^{a} \pm 0.03$ | $0.35^{a} \pm 0.05$ | $2.44^{b} \pm 0.05$ |
| TIV = 1:1 (Ge + Am) | $2.82^{a} \pm 0.02$ | $0.35^{a} \pm 0.04$ | $2.57^{ab} \pm 0.07$ |
| C = Non-AMF control | $2.78^{a} \pm 0.03$ | $0.34^{a} \pm 0.04$ | $2.35^{b} \pm 0.07$ |

Note: Values in a column followed by same alphabet are not significantly different at $p \leq 0.05$ using Duncan's multiple range test (DMRT).
TI : Oil palm seedlings pre-inoculated with *Glomus etunicatum* (Ge).
TII : Oil palm seedlings pre-inoculated with Ge and *Gigaspora rosea* (Gr).
TIII : Oil palm seedlings pre-inoculated with Ge and *Scutellaspora heterogama* (Sh).
TIV : Oil palm seedlings pre-inoculated with Ge and *Acaulospora morrowiae* (Am).
C : Oil palm seedlings with no inoculation of AMF.
1:1 and 2:0 : AMF application ratios in oil palm seedlings according to weight of each inoculum.

was observed amongst each other at $p>0.5$ (*Table 2*). However, leaf K content was highest and significantly different for TI at 2.76% and for TIV at 2.57% (I = IV > III > II > C). For all others treatments, leaf K content was not statistically different from the control.

## DISCUSSION AND CONCLUSION

The present nursery trial reported that the single-species inoculation of *G. etunicatum* (TI) gave a 80.36% increase in plant growth response, which is comparable to the results of field-replicated trials by Owusu-Bennoah and Mosse (1979) who reported increased plant growth of 33% to 77% in onion, lucerne and barley using only one AMF species as inoculum. A variety of growth parameters has been employed by researchers, but central to this issue are plant dry weight, root colonization and nutrient uptake as reflected by leaf nutrient content. TI plants in the present study gave the highest and most significant values for all the growth parameters assessed. The mean dry weight of TI plants was 7.62 g, with a mycorrhizal root colonization of 51.08% and these plants showed a significantly higher leaf content for potassium. Thus, *G. etunicatum* was successful in establishing a symbiotic relationship with the oil palm seedlings. The study proved that oil palm was indeed a suitable and compatible host for *G. etunicatum* as a single-species application.

Studies have shown that responses to AMF fungal species may vary with different host plant species (Gaur and Adholeya, 2002; Smith *et al*., 2004), and therefore several species may need to be screened for a compatible outcome with that plant host (Harinikumar and Bagyaraj, 1989). At times, native and selected strains of the same taxonomic species too can give different responses in the plants (Camprubi *et al*., 2008). Plant dry weight is a good indicator of the growth response to mycorrhizal inoculations. In this study, only TI and TIV (with *A. morrowiaea*) plants showed a positive growth response (at 80.36% and 53.69% respectively) whereas TII (with *G. rosea*) and TIII (with *S. heterogama*) resulted in growth depression of -22.45% and -21.08%, respectively. Growth depression can occur with some mycorrhizal colonizations (Smith, 1980), and this has also been demonstrated in some AMF-inoculated seedlings (Ong *et al*., 2002) and is an outcome of an increased carbon cost in the plant-mycorrhizal relationship given that certain conditions of growth are not limiting (Dickson *et al*., 1999). Mycorrhizal root colonization has been a hallmark of successful AMF-plant interaction. The present study showed that the highest root colonizations occurred in both TI (51.08%) and TIII (52.18%) plants. However, while TI showed excellent growth response, TIII plants gave a growth depression of -21.08%, resulting in smaller plants than those of the non-AMF inoculated control. Two other treatments, TII and TIV, showed root colonizations of 41.31% and 32.49%, respectively, as opposed to 4.82% in the non-AMF seedlings; however, TIV still gave a positive mycorrhizal growth response of 53.69%. Although TII and TIII gave higher root colonization than the control but the overall growth response was comparatively poorer. Ong *et al*. (2002) also reported a significantly higher mycorrhizal root colonization in AMF-inoculated *A. excelsa* seedlings was observed even though the plants were smaller than the non-AMF control.

This study perceived that the combined effect of two AMF species may have resulted in a very strong interspecies competition, thus causing a higher carbon drain from the host plant, as other factors relating to soil and greenhouse conditions were constant for all treatments. Some studies have

shown that productivity may increase with a mixture of species in the AMF inoculum because of the added beneficial effects of each single species in host plants such as soyabean (Meghavansi *et al*., 2008), buffel grass (Khan *et al*., 2007) and *Curculigo orchioides*, a medicinal herb (Sharma *et al*., 2008). Results from the present study showed that mychorrizal species with a compatible partnership in a consortium with *G. etunicatum* have yet to be found, and that each of the three species selected as a partner (*G. rosea*, *S. heterogama*, *A. morrowiae*) resulted in various degrees of an antagonistic effect rather than a synergistic reaction. In addition, the optimum ratio of combination of one species with another should also be investigated as it could probably lead to a more favourable response.

Most Malaysian soils under plantation crop cultivation are acidic, nutritionally poor and deficient in fungal flora (Ong *et al*., 2002). AMF-inoculated plants have been reported to show an increase in the uptake of P, especially from P-deficient acidic soils (Gerdemann, 1968; Safir *et al*., 1971; Tinker, 1984). This study found that only TI showed a positive response in nutrient uptake, and that was only for K. This may be attributed to the use of horticultural soil as the potting medium, which was probably not phosphorus-deficient.

The study found that *G. etunicatum* was able to successfully promote plant growth on its own, but when individually mixed with three other AMF species, resulted otherwise. The study concludes that none of the combinations exceeded the achievement of the singular application by *G. etunicatum* in the growth promotion of oil palm seedlings.

## ACKNOWLEDGEMENT

The author wish to thank the Director-General of MPOB for permission to publish this article. In addition, the author would like to thank Ms V Shanmugasundari and Hwang, L B, for assisting in the nursery studies; Universiti Putra Malaysia, and the Ministry of Science, Technology and Innovation (MOSTI) Malaysia, for providing the grants to make this study possible. The author would also like to thank Dr Norman Kamarudin and Dr Idris Abu Seman of MPOB, for their time and effort in editing this article.

## REFERENCES

ARPANA, J; BAGYARAJ, D J; RAO, E K S P; PARAMESWARAN, T N and ABDUL RAHIMAN, B (2008). Symbiotic response of patchouli [Pogostemon cablin (Blanco) Benth] to different arbuscular mycorrhizal fungi. *Adv. Environmental Biology, 2(1)*: 20-24.

BRUNDRETT, M (2008). Mycorrhizal Associations: The Web Resource. Section 10: Methods for identifying mycorrhizas. url: http://mycorrhizas.info/method.html#examine, accessed on 11 May 2009.

CAMPRUBÍ, A; ESTAÚN, V; NOGALES, A; GARCÍA-FIGUERES, F; PITET, M and CINTA, C (2008). Response of the grapevine rootstock Richter 110 to inoculation with native and selected arbuscular mycorrhizal fungi and growth performance in a replant vineyard. *Mycorrhiza, 18(4)*: 211-216.

COULTER, J K (1972). Soils in Malaysia: a review of investigations on their fertility and management. *Soil Fertility, 35*: 475-498.

DICKSON, S; SMITH, S E and SMITH, F A (1999). Characterisation of two arbuscular mycorrhizal fungi in symbiosis with *Allium porrum*: colonization, plant growth and phosphate uptake. *New Phytologist, 144*: 163-172.

GAUR, A and ADHOLEYA, A (2002). Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biology and Fertility of Soils, 35*: 214-218.

GERDEMANN, J W (1968). Vesicular-arbuscular mycorrhiza and plant growth. *Annual Review of Pythopathology, 6*: 397-418.

GIOVANNETTI, M and MOSSE B (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist, 84*: 489-500.

HARINIKUMAR, K M and BAGYARAJ D J (1989). Effect of cropping sequence, fertilizers and farmyard manure vesicular-arbuscular mycorrhizal fungi in different crops over three consecutive seasons. *Biology and Fertility of Soil, 7*: 173-175.

KHAN, I A; AHMAD, S; MIRZA, S N; NIZAMI, M; ATHAR, M and SHABBIR, S M (2007). Growth response of buffel grass (*Cenchrus ciliaris*) to phosphorus and mycorrhizal inoculation. *Agriculturae Conspectus Scientificus (ACS), 72(2)*: 129-131.

KJELDAHL, J Z (1883). A new method for the determination of nitrogen in organic bodies. *Analytical Chemistry, 22*: 366.

KOSKE, R E and GEMMA, J N (1989). A modified procedure for staining roots to detect vesicular arbuscular mycorrhizas. *Mycological Research, 92*: 486-505.

MPOC (MALAYSIAN PALM OIL COUNCIL) (2008). Fact sheet - Malaysian palm oil. url: http://www.malaysiapalmoil.org/pdf/20080908-factsheet.pdf, accessed May 2009.

MEGHVANSI, M; PRASAD, K; HARWANI, K and MAHNA, S K (2008). Response of soybean cultivars towards inoculation with 3 arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. *European Journal of Soil Biology, 44(3)*: 316-323.

NADARAJAH, P (1980). Species of Endogonaceae and mycorrhizal asociation of *Elaeis guineensis* and *Theobroma cacao*. *Tropical Mycorrhiza Research, 27*: 232-237.

ONG, K H; AWANG, K; HASHIM, A and MAJID, N M (2002). Effects of fertilizers and vesicular-arbuscular mycorrhizas on the growth and photosynthesis of *Azadirachta excelsa* (Jack) Jacobs seedlings. *Forest Ecology and Management, 158(1-3)*: 51-58.

OWUSU-BENNOAH, E and MOSSE, B (1979). The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals IV. Field inoculation responses in barley, lucerne and onion. *New Phytologist, 83*: 671-679.

RUBIO, R; BORIE, F; SCHALCHLI, C; CASTILLO, C and AZCON, R (2002). Plant growth responses in natural acidic soil as effected by arbuscular mycorrhizal inoculation and phosphorus sources. *J. Plant Nutrition, 25(7)*: 1380-1405.

SAFIR, G R; BOYER, J S and GERDEMANN, J W (1971). Mycorrhizal enhancement of water transport in soybean. *Science, 72*: 581-583.

SHARMA, D; KAPOOR R and BHATNAGAR, R (2008). Arbuscular mycorrhizal (AM) technology for the conservation of *Curculigo orchioides* Gaertn: an endangered medicinal herb. *World Journal of Microbiology and Biotechnology, 24(3)*: 395-400.

SMITH, S E (1980). Mycorrhizas of autotrophic higher plants. *Biological Reviews, 55*: 475-510.

SMITH, S E; SMITH, F A and JAKOBSEN, I (2003). Mycorrhizal fungi can dominate phosphate supply to plant irrespective of growth responses. *Plant Physiology, 133*: 16-20.

SMITH, S E; SMITH, F A and JAKOBSEN, I (2004). Functional diversity in arbuscular mycorrhizal fungi (AMF) symbiosis: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist, 162(2)*: 511-524.

TINKER, P B (1984). The role of microorganism in mediating and facilitating the uptake plant nutrients from the soil. *Plant Soil, 76*: 77-91.