

## Early Vegetative Growth of Themba Oil Palm (*Elaeis guineensis* Jacq.) Seedlings under Graded AMF Formulation Rates

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### ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are widely used as biological inputs to improve nutrient acquisition and plant growth. However, their effectiveness may vary depending on inoculum rates, host genotype, and nursery conditions. This study evaluated the early vegetative response of Themba oil palm seedlings to graded rates of a commercial arbuscular mycorrhizal fungi (AMF) formulation. Design/ methodology/ approach: A nursery experiment used a Completely Randomized Design with five replications and six formulation rates (0, 5, 12.5, 15, 17.5, and 20 g seedling<sup>-1</sup>). Plant height, leaf length, and leaf number were recorded weekly for four weeks after inoculation. Findings: AMF formulation rate did not significantly affect plant height ( $p = 0.276$ ), leaf length ( $p = 0.129$ ), or leaf number ( $p = 0.670$ ) at week four. The 20 g treatment had the highest mean, but the differences were not statistically significant. Polynomial regression was descriptive ( $R^2 = 0.54\text{--}0.88$ ) and did not establish an optimum rate. Research limitations/implications: The four-week duration, the absence of root colonization and tissue nutrient measurements, and the lack of a carrier/product-only control limit the attribution of effects specifically to AMF. Originality/value: The study provides preliminary rate-response information for the Themba variety and identifies methodological priorities for longer AMF nursery trials.

### ARTICLE INFO

Received : July 1, 2026  
 Revised : August 22, 2026  
 Accepted : September 25, 2026  
 Published : September 27, 2026

### KEYWORDS

Arbuscular mycorrhizal fungi, biofertilizer, nursery stage, oil palm, themba variety

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### DOI

[10.25077/jrp.7.2.84-90.2026](https://doi.org/10.25077/jrp.7.2.84-90.2026)

### INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an indispensable global agricultural commodity, with Indonesia accounting for more than half of global palm oil production. The palm oil sector underpins vital food, oleochemical, and bioenergy industries. However, the long-term productivity of plantations is closely linked to the quality of planting material established during the nursery phase. Nursery

production can be constrained by nutrient limitations and suboptimal growing conditions, which may reduce root development and vegetative vigor. Sustainable biological inputs are therefore being investigated as complementary approaches to conventional nursery management.

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with plant roots and can extend the effective volume of soil exploration

through extraradical hyphae. This mycorrhizal pathway is particularly relevant to phosphorus (P), a relatively immobile nutrient in soil. Reviews and meta-analyses indicate that AMF can improve plant P acquisition and, in many systems, plant biomass. However, the magnitude and direction of the response vary with AMF identity, host plant, nutrient supply, soil conditions, and inoculation characteristics (Etesami et al., 2021; Marro et al., 2022; Qiu et al., 2022; Simanjuntak et al., 2013; Wu et al., 2024).

For oil palm, responses to AMF inoculation have also varied among varieties and nursery conditions (Mohd Noor et al., 2022; Rini et al., 2022; Noviana et al., 2021; Perdana et al., 2015; Sodikin et al., 2022). However, evidence for graded AMF rates in the Themba variety remains limited. Commercial inoculants may contain multiple AMF taxa and non-fungal carrier or nutrient components, so dose comparisons based on product mass should be interpreted as responses to the complete formulation rather than to viable AMF propagules alone (Basiru & Hijri, 2022; Duell et al., 2022). The present study, therefore, evaluated the early vegetative response of Themba oil palm seedlings to graded rates of a commercial AMF formulation under nursery conditions, with an emphasis on plant height, leaf length, and leaf number during the first four weeks after inoculation.

The objective was to characterize the short-term numerical and statistical responses to the tested formulation rates rather than to establish a definitive long-term optimum. The study also provides a preliminary basis for designing longer-term nursery experiments that include AMF root colonization measurements and nutrient uptake assessments.

## MATERIALS AND METHODS

### Experimental Site and Plant Materials

The experiment was conducted from September to November 2024 in Langkan Village,

Banyuasin Regency, South Sumatra Province, Indonesia. The study was conducted in an open-air nursery under natural tropical field conditions. Uniform one-month-old oil palm (*Elaeis guineensis* Jacq.) seedlings of the Themba variety were selected based on morphological uniformity. Seedlings were potted in standard nursery polybags containing a growing medium of topsoil and compost mixed at a 1:1 (v/v) ratio. Shredded oil palm empty fruit bunches (EFB) were placed on the soil surface as organic mulch to conserve soil moisture and suppress weed emergence. Routine nursery maintenance, including uniform daily irrigation, sanitation, and pest management, was applied equally across all units. AMF inoculation was performed at potting/transplanting, and the four-week observation period began immediately after inoculation. The September–November period, therefore, refers to the broader nursery activity period; the response analysis reported in this manuscript is restricted to the first four weeks after AMF application.

### AMF Inoculum, Formulation, and Treatment Application

Phosphorus fertilization was supplied using triple superphosphate (TSP; 46% P<sub>2</sub>O<sub>5</sub>) at a rate of 5 g seedling<sup>-1</sup>. TSP was applied once at the beginning of the experiment, immediately after AMF inoculation, by evenly broadcasting the fertilizer around the base of each oil palm seedling. The same TSP application was applied uniformly to all experimental units. The AMF inoculum was applied directly beneath the seedling root mass during potting to optimize physical contact between fungal propagules and emerging roots. The experiment evaluated six AMF application rates:

- A0: 0 g seedling<sup>-1</sup> (Control)
- A1: 5 g seedling<sup>-1</sup>
- A2: 12.5 g seedling<sup>-1</sup>
- A3: 15 g seedling<sup>-1</sup>
- A4: 17.5 g seedling<sup>-1</sup>
- A5: 20 g seedling<sup>-1</sup>

## Experimental Design and Growth Observations

The trial was arranged in a Completely Randomized Design (CRD) with single-factor AMF dosage replicated five times, generating 30 experimental units (one seedling per unit).

Vegetative growth parameters were recorded weekly over a four-week post-inoculation duration:

1. Plant height (cm): Measured from the substrate level to the tip of the highest erect spear leaf.
2. Leaf length (cm): Measured from the petiole base to the distal leaflet tip of the newest fully expanded leaf.
3. Number of leaves (leaves seedling<sup>-1</sup>): Counted as the cumulative number of fully opened, functionally green leaves.

Growth increments over four weeks were calculated as:

Growth increment=final measurement–initial measurement

## Statistical Analysis

Assumptions of normality and homogeneity of variance were examined using the Shapiro–Wilk test and Levene’s test, respectively. Data were subjected to one-way analysis of variance (ANOVA) at  $\alpha = 0.05$ . Treatment means were evaluated, and post-hoc Duncan’s Multiple Range Test (DMRT) was planned only when the omnibus F-test was significant. Polynomial regression was used for descriptive analysis of the relationship between AMF product rate and treatment means; the regression results were not interpreted as evidence of statistically significant treatment effects when the corresponding ANOVA was non-significant.

## RESULTS AND DISCUSSION

### Plant Height

ANOVA indicated that differing AMF formulation rates did not exert a statistically significant influence on seedling height during any observation interval over the four weeks ( $p > 0.05$ ;

Table 1). At week four, the p-value was 0.276, indicating that the observed numerical differences among treatment means were not statistically significant.

Table 1. Analysis of variance for plant height of Themba oil palm seedlings across observation periods.

| Observation period | F-value | p-value | Significance    |
|--------------------|---------|---------|-----------------|
| Week 1             | 0.787   | 0.569   | Not significant |
| Week 2             | 0.482   | 0.786   | Not significant |
| Week 3             | 0.860   | 0.522   | Not significant |
| Week 4             | 1.356   | 0.276   | Not significant |

Although no statistical divergence was detected, numerical differences were documented. At week four, seedlings treated with 20 g AMF formulation had the tallest average height ( $22.20 \pm 0.37$  cm), followed by 17.5 g ( $21.60 \pm 0.60$  cm) and 5 g ( $21.40 \pm 1.21$  cm), whereas the uninoculated control reached  $21.00 \pm 0.89$  cm and the 12.5 g treatment produced the lowest mean ( $19.40 \pm 0.60$  cm). In terms of absolute four-week increment, the 17.5 g and 20 g treatments generated increases of 6.00 cm and 5.20 cm, respectively, compared with 4.40 cm in the control. These numerical differences should not be interpreted as treatment effects because the ANOVA was non-significant.

The absence of statistically significant differences in seedling height may reflect the short evaluation period and the time required for AMF establishment. AMF responses depend on colonization, host compatibility, inoculum characteristics, and environmental conditions (Marro et al., 2022; Qin et al., 2022; Wang, M. et al., 2023; Wu et al., 2024). Meta-analytic evidence also indicates that AMF effects vary with nutrient supply and inoculum type (Wu et al., 2024). Thus, the numerical differences observed among the 17.5–20 g treatments provide descriptive information for future testing. However, they do not demonstrate that these rates increased plant height under the present four-week conditions.

Leaf Length

Leaf length responses showed no statistically significant differences among treatments over the four weeks (Table 2;  $p > 0.05$ ). The week-four ANOVA gave  $p = 0.129$ , so the numerical differences in leaf length should be interpreted descriptively rather than as evidence of a significant AMF-rate effect.

Table 2. Analysis of variance for leaf length of Themba oil palm seedlings across observation periods

| Observation period | F-value | p-value | Significance    |
|--------------------|---------|---------|-----------------|
| Week 1             | 0.721   | 0.614   | Not significant |
| Week 2             | 0.721   | 0.614   | Not significant |
| Week 3             | 1.326   | 0.287   | Not significant |
| Week 4             | 1.918   | 0.129   | Not significant |

Numerically, seedlings inoculated with the 20 g AMF formulation produced the longest average leaf length at week four ( $19.40 \pm 0.51$  cm), followed by 15 g ( $18.60 \pm 0.51$  cm) and 17.5 g ( $17.80 \pm 0.97$  cm), whereas the control measured  $17.00 \pm 1.26$  cm. The highest cumulative leaf elongation increment occurred in the 15 g (7.00 cm), 20 g (6.60 cm), and 17.5 g (6.50 cm) treatments, compared with 5.00 cm in the control. These numerical patterns were not statistically significant. AMF can contribute to P acquisition through extraradical hyphae, but the magnitude of the response depends on host–fungus compatibility, nutrient status, inoculum characteristics, and environmental conditions (Etesami et al., 2021; Jiang et al., 2021; Madrid-Delgado et al., 2021; Wang, G. et al., 2023; Wu et al., 2022; Wu et al., 2024). Therefore, the present data do not establish enhanced P mobilization as the mechanism underlying the numerical leaf-length differences because P uptake and root colonization were not measured.

Number of Leaves

Leaf production was not significantly altered by AMF formulation rate across the four weeks (Table 3;  $p > 0.05$ ). The week-four ANOVA gave  $p = 0.670$ ,

indicating that the numerical differences among treatments were not statistically significant.

Table 3. Analysis of variance for leaf number of Themba oil palm seedlings across observation periods.

| Observation period | F-value | p-value | Significance    |
|--------------------|---------|---------|-----------------|
| Week 1             | 1.412   | 0.256   | Not significant |
| Week 2             | 1.620   | 0.193   | Not significant |
| Week 3             | 1.214   | 0.332   | Not significant |
| Week 4             | 0.642   | 0.670   | Not significant |

Seedlings treated with 20 g and 17.5 g AMF formulation had numerically higher leaf counts at week four, ending with  $4.80 \pm 0.37$  and  $4.60 \pm 0.40$  leaves per seedling<sup>-1</sup>, respectively, compared with  $4.20 \pm 0.20$  in the control. However, the cumulative leaf-number increments were relatively close among treatments, and the ANOVA remained non-significant. Leaf emergence in young perennial monocots is influenced by developmental stage, genotype, carbohydrate status, and environmental conditions; consequently, a four-week observation period may capture only an early phase of treatment response. The present results, therefore, support continued evaluation rather than a definitive dosage recommendation.

Dose-Response Regression Modeling and Synthesized Discussion

Polynomial regression analysis characterized the dose-dependent response across all evaluated vegetative traits.

Table 4. Polynomial regression equations for the relationships between AMF formulation rate and vegetative growth parameters.

| Growth parameter | Regression equation                 | R <sup>2</sup> |
|------------------|-------------------------------------|----------------|
| Plant height     | $Y = 21.4968 - 0.3302X + 0.0179X^2$ | 0.54           |
| Leaf length      | $Y = 17.2279 - 0.3135X + 0.0210X^2$ | 0.60           |
| Leaf number      | $Y = 4.2553 - 0.0538X + 0.0040X^2$  | 0.88           |

Polynomial regression produced  $R^2$  values of 0.54 for plant height, 0.60 for leaf length, and 0.88 for leaf number. The relatively high  $R^2$  for leaf number reflects the pattern of the treatment means across the tested rates. However, it should not be interpreted as evidence of a significant dose effect, as the corresponding ANOVA was non-significant ( $p = 0.670$  at week four). Similarly, the quadratic equations describe the observed numerical pattern and should not be used to identify a statistically validated optimum dose from this four-week experiment. Response magnitude can vary with AMF species or community, host genotype, nutrient availability, and experimental conditions (Carrara & Heller, 2022; Horsch et al., 2023; Wang, G. et al., 2023).

The short four-week observation period is an important limitation when interpreting these findings. Experimental duration, AMF colonization, nutrient availability, and inoculum characteristics can modify the magnitude of plant responses (Basiru & Hijri, 2022; Qin et al., 2022; Wu et al., 2024). In addition, the present commercial formulation contained organic nutrient components, and no carrier-only or product-only control was included. Consequently, the study cannot separate the contribution of viable AMF propagules from other components of the product. Future experiments should extend the observation period, quantify root colonization, measure tissue nutrient concentrations, and include a suitable carrier/product control to isolate the specific contribution of AMF.

## CONCLUSIONS

Application of the tested commercial AMF formulation rates did not cause statistically significant differences in plant height, leaf length, or leaf number of Themba oil palm seedlings during the first four weeks after inoculation. At week four, the 20 g seedling<sup>-1</sup> treatment showed the highest numerical means for plant height (22.20 cm), leaf length (19.40 cm), and leaf number (4.80 leaves seedling<sup>-1</sup>). However, these numerical differences were not statistically significant. The polynomial regressions were descriptive of treatment-mean

patterns and should not be interpreted as evidence of a statistically validated optimum rate.

The findings indicate that the four-week period provides preliminary information on early vegetative responses but is insufficient to establish a definitive AMF dosage recommendation for the Themba variety. Interpretation is also limited by the use of a commercial formulation that contains organic nutrient components, without a carrier/product-only control. Future studies should evaluate the seedlings over a longer nursery period, quantify root colonization, measure tissue nutrient uptake, and include a suitable carrier/product control to distinguish AMF-specific effects from formulation effects.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the PNB program of Politeknik Negeri Sriwijaya for the fiscal year 2025, under contract number 07012/PL6.2.1/LT/2025 dated June 26, 2025.

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