Elaeis guineensis phenotypic traits and non-enzymatic antioxidant responses to the combination of biofertilizer and chemical fertilizer in infertile soil

Aaronn Avit Ajeng [a], Noor Sharina Mohd Rosli [a], Pei Xin Chen [a],
Rosazlin Abdullah [a, b, **], Jamilah Syafawati Yaacob [a, b], Tau Chuan Ling [a],
Kuan Shiong Khoo [c, d, *]

[a] *Institute of Biological Sciences, Universiti Malaya, 50603, Kuala Lumpur, Malaysia*
[b] *Centre for Research in Biotechnology for Agriculture (CEBAR), Institute of Biological Sciences, Universiti Malaya, 50603, Kuala Lumpur, Malaysia*
[c] *Department of Chemical Engineering and Materials Science, Yuan Ze University, Taoyuan, Taiwan*
[d] *Centre for Herbal Pharmacology and Environmental Sustainability, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, 603103, Tamil Nadu, India*

ARTICLE INFO

ABSTRACT

A 7-month glasshouse study was conducted to assess the growth responses, nutrient status, and non-enzymatic antioxidant properties of *E. guineensis* seedlings grown on infertile Ultisol which were subjected to different combinations of chemical fertilizers (CF) and commercial biofertilizer (IBG) as follows: [T1] 100% CF [T2] 70% CF + 30% IBG biofertilizer [T3] 50% CF + 50% IBG biofertilizer [T4] 70% CF only and [T5] Absolute Control. A combination of CF70 and IBG30 led to 15.8% increase in the growth of seedlings as compared to CF100, presenting significantly higher fresh shoot and root weights as well as an ideal root-to-shoot ratio. Absolute control seedlings on the other hand, showed less desirable phenotypical traits across all the observed parameters. Significantly higher levels of relative chlorophyll were recorded for the seedlings treated with CF70 + IBG30, which positively correlated with the chlorophyll *a/*b ratio. Moreover, the biofertilizer and chemical fertilization allowed increased uptake of nutrients where higher uptake of B and P was positively correlated ($p < 0.05$) with enhanced frond production, while larger roots mass was associated with primary growth traits. The positive impacts of the combined IBG biofertilizer and chemical fertilizer application were likely attributed to enhanced accumulation of non-enzymatic antioxidants to counteract the effects of soil infertility, with seedlings in CF70 + IBG30 mostly recorded the highest phenolic, anthocyanin, flavonoid, photosynthetic pigments, DPPH radical activity and proline levels.

Abbreviations

2,2-Diphenyl-1-picrylhydrazyl   DPPH
Ascorbic Acid   AsA

* Corresponding author. Department of Chemical Engineering and Materials Science, Yuan Ze University, Taoyuan, Taiwan.
** Corresponding author. Institute of Biological Sciences, Universiti Malaya, 50603, Kuala Lumpur, Malaysia.
E-mail addresses: rosazlin@um.edu.my (R. Abdullah), kuanshiong.khoo@saturn.yzu.edu.tw, kuanshiong.khoo@hotmail.com (K.S. Khoo).

Cation Exchange Capacity   CEC
Chemical Fertilizer   CF
Nitrogen   N
Phosphorus   P
Potassium   K
Total Anthocyanin Content   TAC
Total Flavonoid Content   TFC
Total Phenolic Content   TPC

1. Introduction

Worldwide demand for edible oil is causing a major increase in the oil palm agricultural acreage for industrial oil palm (*E. guineensis*) cultivation particularly in the Southeast Asia regions such as Malaysia in which converted lands are highly weathered, acidic or over-fertilized (Ghazali et al., 2016). Soil acidification can be caused by a variety of factors, including natural processes, industrial contaminants, and intensive agricultural activities within the areas. Acidity influences the soil by modifying the belowground geochemical and biological interactions (Souri, 2017), resulting in increased leaching of vital plant nutrients and the availability of harmful substances (Souri, 2016). Although acidic soils such as oxisol and ultisol are generally infertile, such tropical soils exhibit some of the highest productivity in the world once chemical limitations are addressed through adequate routine application of lime and fertilizers (Fatai et al., 2017, Fageria and Baligar, 2008).

However, in a long-extended cultivation period, such a routine may not be environmentally friendly. Implementing more proper and sustainable management practices for infertile tropical soils has become crucial for maintaining the optimal oil palm nutrition while achieving high yields. One of many strategies in ensuring a sustainable oil palm plantation practice includes the utilization of degraded land for the new plantation since the cost for land reclamation for the new plantation will be significantly reduced compared to deforestation (Purwadi et al., 2023). Moreover, practices of utilizing biofertilizers as a complementary supplement to chemical fertilizer is also considered sustainable and eco-friendly alternative as they can improve soil fertility and promote plant growth and quality (Serri et al., 2021). These living microbes enrich soil through a variety of direct and indirect methods, enhancing soil fertility and supporting the conditions necessary for plant growth (Ajeng et al., 2024, Ajeng et al., 2020a). In contrast to chemical fertilizers, which pose risks to the environment and human health (Pandey et al., 2024), biofertilizers offer a more environmentally friendly and economically viable solution to meet the growing demands of food production.

More recently, industries and research institutions are developing novel formulation techniques to improve the shelf life and stability of biofertilizers. Encapsulation, immobilization, and carrier-based formulations are being explored to ensure the prolonged survival and viability of beneficial microorganisms during storage and application (Vejan et al., 2021; Ajeng et al., 2020b). There is also a growing emphasis on tailoring biofertilizers or plants-associated microbiome to specific crop or climate requirements thanks to the modern biotechnology tools such as the synthetic biology and genetic engineering (Ke et al., 2021). Recent advancements in genome engineering, *meta*-omic technologies, computational tools, and genome-wide functional genomics can help us create microorganisms for biocontrol, biofertilization, and biostimulation, as well as increased agricultural production and yield. Customized microbial consortia and improvised formulations are being designed to exhibit superior nutrient uptake capabilities and provide enhanced plant growth-promoting (Naiji and Souri, 2018; Sharma et al., 2023). Such technology is considered novel and important since there is no 'one size fits all' type of biofertilizer which is why continuous research and development (R&D) needs to focus on tailoring microbial consortia to the unique requirements of different crops and agroecosystems, ensuring sustainable agricultural practices and maximizing productivity while minimizing environmental impact.

IBG Biofertilizer is among the specialized formulation developed specifically for oil palm plantations, which fully utilizes the power of beneficial microorganisms to enhance nutrient availability, improve soil health, and promote the growth and productivity of oil palm trees (Lim and Matu, 2015). One unique selling point of IBG biofertilizer is in its proven ability to induce inflorescence rate of female to male ratio, leading to an increased fruit bunch quantity, high in organic matter as substrate for the bacteria and plants nutrients. Although the effects of biofertilizers on *E. guineensis* productivity have been extensively studied in recent decades, it is important to note that different combinations and types of microorganisms were formulated into the biofertilizer, which could result in varying efficacies. Therefore, it is essential to study different biofertilizer combination and formulation such as IBG Biofertilizer for a more inclusive approach towards enhancing soil health and crop productivity across diverse agricultural contexts.

Soil infertility is one of the biotic stressors frequently faced by the oil palm plantation which could affect the developmental, structural, physiological and biochemical processes in the crops. During stress, high-energy electrons are transferred to molecular oxygen (O_2) to form reactive oxygen species (ROS), which are toxic molecules that target high-molecular mass molecules, such as membrane lipids or mitochondrial DNA, by forming lipid or nucleotide peroxides, particularly at the thymine level, and causing damage to cellular macromolecules, including DNA (Ahmad et al., 2010; Govaichelvan et al., 2024). Enzymatic and nonenzymatic antioxidative systems, such as glutathione reductase (GR), catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), ascorbic acid (AsA), tocopherol, glutathione, and phenolic substances, among others, combat the damaging and toxic effects of these reactive oxygen species (ROS) ensuring plants resilience.

Biofertilizer together with soil microbiome, particularly bacteria and fungus, could play a crucial role in boosting plants development during stress condition by strategizing different mechanisms such as colonizing rhizospheres and roots and producing beneficial metabolites and substances through enzymatic and non-enzymatic processes. Among early reports which studied the implications of

biofertilizer on the stress alleviation, Khanna et al. (2019) discovered that the biofertilizer increased the cellular ascorbic acid (AsA) levels in tomatoes (*Lycopersicum esculentum)* which led to an improved protection against pathogen (*Meloidogyne incognita*). Similar mechanisms in inducing other non-enzymatic antioxidants could be hypothesized upon supplementation of biofertilizer to assist the oil palm, to overcome soil infertility condition. In another comprehensive studies of stressors on oil palm, Cha-um et al. (2012) investigated the effects of water stress and nutrient stress on *E. guineensis* physiological parameters which includes several non-enzymatic antioxidant properties such as chlorophyll *a*, *b* and SPAD-meter relative chlorophyll reading. Furthermore, Harkousse et al. (2021) studied the non-enzymatic antioxidant characteristics of date palm, an intraspecies to oil palm. The authors revealed that the use of biofertilizers lowered the content of antioxidants such as the activities of S-transferase (GST) and carotenoid in date palm tissues.

Hence, the study aims to fill information gaps about the effects of IBG Biofertilizer supplementation on the non-enzymatic antioxidant properties of *E. guineensis*, particularly in terms of alleviating soil infertility. We will specifically look the growth productivity, nutrient status of the seedlings and the levels of chlorophyll *a* and chlorophyll *b* and carotenoid, total phenolic content (TPC), total flavonoids content (TFC), total anthocyanin content (TAC), proline content, DPPH, FRAP, and ascorbic acid (AsA) in *E. guineensis* after various treatment regimens, including the use of IBG Biofertilizer. We hypothesize that using IBG biofertilizer would improve overall growth productivity, alleviate nutritional constraints caused by infertile soil, and induce the content of non-enzymatic antioxidants, making the plants more resilience. Therefore, we aim to investigate the effects of combined IBG biofertilizer and chemical fertilization routines on the phenotypic, nutritional status, and non-enzymatic antioxidant properties of *E. guineensis* cultivated on infertile soil under glasshouse conditions.

2. Materials and methods

2.1. Experimental set-up

The glasshouse experiment was carried out at Rimba Ilmu, Universiti Malaya, Kuala Lumpur (3.13105, 101.65820). Ultisol (*Bungor* series) was selected for the study as it was characterized as highly weathered and acidic infertile soil (Ajeng et al., 2020a,b; Sabir et al., 2015). The clay fraction of the soil is mostly dominated by kaolinite, hematite, goethite and gibbsite; and the CEC and basic cations are low. Soil reaction was acidic in nature with soil pH slightly below 5, but the exchangeable Al was more than 1 cmolc/kg soil. 15 kg of Ultisol was precisely weighed and added into each polybag (34 × 45 cm) and incubated for a week before the transplant.

Oil palm commercial seeds (Deli Dura × AVROS Pisifera) were obtained from the Oil Palm Breeding Unit, Sime Darby Plantation Research Sdn. Bhd., Malaysia. IBG Biofertilizer (oil palm) (NPK 5:7:8 + Trace Elements (TE)) was obtained from IBG Manufacturing Sdn. Bhd. and NPK 14:13:9:2.5 + TE was purchased from single distributor throughout the trial to ensure the fertilizer quality remained unchanged. Complete IBG biofertilizer (oil palms) characterizations and properties were supplemented ini Supplementary Table 1. The experiments were conducted in the Complete Block Design (CBD) with four replicates for each treatment in a single trial for 7 months from August 15, 2022 to February 26, 2023. The experiment consisted of five treatments: [T1] 100% chemical fertilizer (CF), [T2] 70% CF + 30% IBG biofertilizer, and [T3] 50% CF + 50% IBG biofertilizer, [T4] 70% CF only and [T5] Absolute Control. The rates of the treatments are listed in Table 1.

2.2. Measurement of oil palm seedlings growth

The growth parameters of the oil palms were observed and taken every two weeks during morning at 8.00 a.m. The height of the fronds (leaflets plus rachis) was measured using measuring tape from the lowest rudimentary to the tip of the rachis. The number of fronds was taken and recorded. The girth size was measured using a digital Vernier caliper at 5 cm from the planting medium. The chlorophyll was measured using a chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan) of leaf blades from the third frond with a visually green color at the midrib to maximize the calibration. The readings were taken at three random spots and the

Table 1
Fertilizer application rates.

Months	Frequency	T1	T2		T3		T4	T5
		100% NPK Fertilizer (g)	70% NPK Fertilizer (g)	30% IBG (mL)	50% NPK Fertilizer (g)	50% IBG (mL)	70% NPK Fertilizer (g)	
Treatments		**CF100**	**CF70 + IBG30**		**CF50 + IBG50**		**CF70**	**C**
1	1st	5	3.5	2.5	2.5	4.5	3.5	0
	2nd	5	3.5	–	2.5	–	3.5	
2	1st	10	7	–	5	–	7	
	2nd	15	10.5	2.5	7.5	4.5	10.5	
3	1st	20	14	–	10	–	14	
4	1st	25 + 10 g ks	17.5 + 7 ks	3	12.5 + 5 g ks	4.5	17.5 + 7 ks	
5	1st	25	17.5	–	12.5	–	17.5	
6	1st	25	17.5	0	12.5	–	17.5	
7	1st	25	17.5	–	12.5	–	17.5	
Total		155.0 g NPK	108.5 g NPK	8 ml	77.5 g NPK	13.5 ml	108.5 g NPK	0
		10 g ks	7 g ks		5 g ks		7 g ks	

*Nutrient Content Ratio NPK 14:13:9:2.5. Ks: Kieserite. The percentage of IBG Biofertilizer added (30% and 100%) followed the recommended rate (100%) by IBG Biofertilizer Application Method (~25 ml/palm for 1–2-year-old seedlings every six month).

amounts were averaged throughout the study period. The SPAD-502 was calibrated after and before another reading on different seedlings.

2.3. Physicochemical analyses of soil and plant samples

The seedlings were harvested after 7 months of planting. Sampling was conducted in 8.00 a.m. to help ensure plants tissues were in the same physiological state. They were carefully removed from the soil and the roots were cleansed of soil particles. The seedlings were cut at the soil level and separated from the roots. The fresh weight (FW) of all the seedlings and roots were taken on the same day followed by dry weight (DW) by drying in an oven at 71–75 °C until constant weight was achieved. The aboveground shoots and roots were ground separately using a grinding machine (<2 mm) separately for macronutrients analysis. The soil used after the treatment was thoroughly mixed and air-dried, sieved using a 2 mm mesh sieve, and measured for macronutrients content (Ajeng et al., 2021).

The pH, total nitrogen (TN) (%), total phosphorus (TP)(PPM), available phosphorus (AP) (PPM), exchangeable cations (potassium (K)(meq/100q), were then measured for these soil samples. Additionally, the plant samples were examined independently for TN, TP, K, Mg, and Ca. The dry combustion technique was used to determine the total N in soil and plant samples using an elemental analyzer (Matejovic, 1993). The vanadate-molybdate technique (Tandon et al., 1968) was used with a spectrophotometer at 425 nm to quantify total phosphorus. Cations (K, Ca, and Mg) were extracted using 1 M ammonium acetate (NH_4OAc) at pH 7, followed by inductively coupled plasma (PerkinElmer, USA) (David, 1960). The pH was accessed using a glass electrode pH meter with 1:2.5 soil to water suspensions (Eutech Instruments, Thermo Fisher Scientific, Woodlands, Singapore). All soil and plants analyses were conducted in accordance with the M.S 678 (soil) standard method outline by the Standard and Industrial Research Institute of Malaysia (Malaysian Standard, MS, 1980) and units presented as percentage (%) and cmol(+)/kg for Exch. K.

2.4. Determination of non-enzymatic antioxidants

2.4.1. Quantification of carotenoid, chlorophyll a, and chlorophyll b pigments

The leaves in Frond 1 (2–4 weeks after opening) were used for phytochemical analysis. To measure the carotenoid, chlorophyll *a*, and chlorophyll *b* contents, 0.5 g of freeze-dried biological plant samples was ground with 15 mL methanol using a mortar and pestle. The resulting extract was centrifuged at 12,000 rpm for 10 min, and the supernatant was filtered using Whatman No.1 filter paper before analysis. The absorbance of the extracts was recorded at 665.2 nm, 652.4 nm, and 470 nm using a microplate spectrophotometer (Multiskan GO, Thermo Scientific, USA). The, chlorophyll *a* (Chl$_a$) (Eq. (1)), chlorophyll *b* (Chl$_b$) (Eq. (2)) and carotenoid (TC)(mg/g FW) (Eq. (3)) contents were determined using the formulas described by Lichtenthaler and Buschmann (2001):

$$Ch_a \, (mg/L) = 16.72 \, A_{665.2} - 9.16 \, A_{652.4} \tag{1}$$

$$Ch_b \, (mg/L) = 34.09 \, A_{652.4} - 15.28 \, A_{665.2} \tag{2}$$

$$TC \, (mg/L) = \frac{1000 \, A_{470} - \left(1.63 \, C_a - 104.96 \, C_b\right)}{221} \tag{3}$$

2.4.2. Measurement of total anthocyanin, phenolic, and flavonoid contents

The quantification of total anthocyanin, phenolic, and flavonoid contents followed the protocols previously published (Rusli et al., 2022; Yusof et al., 2018). The freeze-dried plant samples were ground with 15 mL methanol and incubated for 48 h before further processed. The dried extract was dissolved in absolute methanol at a stock concentration of 10 mg/mL for subsequent quantification of total flavonoid (TFC) and total phenolic (TPC) contents, as well as antioxidant assays. The pH differential method was employed for the quantification of monomeric anthocyanin content (cyanidin-3-glucoside). The sample extracts' pH was adjusted to pH 1 and 4.5, and the absorbance at 510 nm and 700 nm was measured using an Epoch Microplate spectrophotometer. The monomeric anthocyanin pigment concentrations were calculated using the following **Equation (4)**:

$$Anthocyanin \; pigment \; content^{(mg/L)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \tag{4}$$

Where $A = (Abs_{510} - Abs_{700}) \, pH1 - (Abs_{510} - Abs_{700}) \, pH4.5$, MW (Cyanidin $-$ 3 $-$ glucoside) = 449, *DF = dilution factor* and $\varepsilon = 26,900$

The aluminum chloride colorimetric method was utilized for the quantification of TFC. The absorbance at 415 nm was measured using an Epoch Microplate spectrophotometer in triplicates. For the quantification of TPC, the Folin-Ciocalteu reagent (FCR) method was employed. The absorbance of the extract at 765 nm was measured using an Epoch Microplate spectrophotometer.

2.4.3. Determination of proline content and DPPH radical scavenging activity

Proline levels were determined following the methods described by Bates et al. (1973) with minor modifications. Fresh leaf samples (0.15 g) were ground with 3 mL of 3% sulfosalicylic acid using a mortar and pestle. Then, 0.6 mL of the extract was mixed with 1.2 mL of glacial acetic acid and 1.2 mL of acid ninhydrin. The mixture was shaken vigorously and placed in a water bath at 75 °C for 1 h. Afterward, the mixture was cooled on ice to stop the reaction, and 2 mL of toluene was added to extract the proline content from the chromophore layer. The absorbance of the chromophore layer at 520 nm was measured using a microplate spectrophotometer (Multiskan GO, Thermo Scientific, USA). The proline content of the samples was calculated by comparing with a standard and ex-

pressed as μ mol per gram of fresh weight (μ mol/g FW) of the samples. The DPPH radical scavenging activity was measured following protocol described by Rusli et al. (2022); Yusof et al. (2018).

2.4.4. Determination of ascorbic acid content

Ascorbic acid content was determined using the 2, 6-dichlorophenolindophenol visual titration method based on the procedure by Offia-Olua and Ekwunife (2015) with minor modifications. A solution of indophenol was made by mixing 0.08 g of DCPIP with 50 mL of water and 0.042 g of sodium bicarbonate. After filtering, the volume was adjusted to 200 mL. This solution was standardized against ascorbic acid by titration. The ascorbic acid solution was prepared by dissolving 0.05 g of ascorbic acid in 100 mL of water. In a flask, 5 mL of this solution was mixed with 20 mL of water and 10 mL of extraction solution and titrated until a faint pink color persisted. The concentration was determined in mg of ascorbic acid per mL of DCPIP. For the blank, distilled water was used instead of ascorbic acid. To measure the ascorbic acid in samples, 0.1 g of sample was mixed with extraction solution and water, stirred, and titrated with DCPIP until a faint pink color appeared. The ascorbic acid content was calculated using **Equation (5):**

$$\text{Ascorbic Acid Content} \left(\frac{\text{mg ascorbic acid}}{\text{g}} \right) \text{FW of Sample} = X - B \times \left(\frac{F}{E} \right) \times \left(\frac{V}{Y} \right) \tag{5}$$

Where, X = Average DCPIP volume used for sample titration (mL) B = Average DCPIP volume used for blank titration (mL) F = Titre of dye (mg ascorbic acid equivalent to 1 mL DCPIP) E = Weight of sample (g) V = Volume of the initial sample solution (mL) Y = Volume of the sample aliquot titrated (mL)

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was carried out to evaluate the effects of the treatments on growth, soil properties, and plant nutrient uptake using SPSS (version 26.0; IBM, Chicago, Illinois, USA). The simple correlation analysis was carried out using SAS 9.2 to analyze the correlation between the soil properties and plant nutrient uptake with biomass. The principal component analysis (PCA) and Pearson's and Spearman's correlation matrix were also carried out using the same software. Pearson's correlation coefficients were used to identify the relationship between soil and crop variables. Tukey Post Hoc analysis was used to determine the significant difference between the treatments in plants growth responses, meanwhile Duncan MRT was used to determine the difference between the means of the non-enzymatic antioxidant properties as it allows identification of specific treatment groups that differ significantly and pinpoint the most effective treatment influencing the non-antioxidant levels. Graph was plotted and visualized using SigmaPlot for Windows Version 10.0 (Germany).

3. Results and discussion

3.1. Growth and biomass properties of E. guineensis seedlings

Table 2 shows the average heights of seedlings and girth diameter, shoots and root properties of *E. guineensis* seedlings after 7-month glasshouse study. The phenotypes and characteristics of the biomass and roots were visualized in Fig. 1. The results indicated that CF70 + IBG30, exhibited the highest average height of 116.75 cm. When compared to the standard fertilization (CF100, seedlings in CF70 + IBG30 had a significant growth. There was a 15.8% increase in height in 30% IBG when compared to the standard 100% fertilizer treatment. The treatment CF50 + IBG50, showed an average height of 95.25 cm, showing a significant decrease of 5.6% when compared to CF100. Although lower, this result still suggests a positive impact of the biofertilizer component on plant growth.

In contrast, CF70, exhibited a lower average height of 82.13 cm. although suffice, the growth of seedlings in addition of 30% IBG biofertilizer had a profound and significant impact on the seedling's growth. On the other hand, the control treatment, displayed the lowest average height of 64.25 cm, as expected which was the most pronounced decrease showing a statistically significant decline of 36.3% when compared to the heights of seedlings in the standard fertilization plot. It is undeniable that the presence of beneficial microbes in biofertilizers can enhance plant nutrient uptake, stimulate root development, and improve overall plant health (Keni et al., 2023). These microbes establish symbiotic relationships with plants, facilitating nutrient absorption and providing growth-promoting substances. Additionally, they contribute to the establishment of a diverse microbial community in the soil, enhancing nutrient cycling and overall soil fertility (Kumar et al., 2022). In a finding from Keni et al. (2023), the authors found that a combination of chem-

Table 2
The shoots and root properties of *E. guineensis* seedlings after 7-month glasshouse study.

Treatments	Fresh Biomass				Dry Biomass		
	Height (cm)	Girth (mm)	FS (g)	FR (g)	DS (g)	DR (g)	R:S
CF100	100.86 ± 10.22b	3.77 ± 0.28a	114.39 ± 6.34b	66.45 ± 8.91a	43.47 ± 3.00 ab	**14.75 ± 0.24a**	0.35 ± 0.02 ab
CF70 + IBG30	**116.75 ± 20.32a**	**3.88 ± 0.25a**	**174.66 ± 9.41a**	**70.93 ± 2.72a**	**49.61 ± 6.96a**	13.40 ± 0.59b	**0.29 ± 0. 04a**
CF50 + IBG50	95.25 ± 25.12b	3.79 ± 0.16a	106.29 ± 4.77b	69.17 ± 2.10a	36.90 ± 4.63b	13.16 ± 0.30b	0.37 ± 0.06 ab
CF70	82.13 ± 23.30c	3.14 ± 0.18b	79.52 ± 4.22BCE	43.80 ± 2.06b	34.64 ± 3.94BCE	13.99 ± 0.50 ab	0.42 ± 0.04 BCE
C	64.25 ± 10.30d	2.37 ± 0.37c	59.01 ± 2.06c	14.54 ± 3.49c	24.95 ± 4.60c	12.05 ± 0.61c	0.50 ± 0.11c

*Fs: Fresh Shoot, FR: Fresh Roots, DS: Dried Shoot, DR: Dried Roots, R:B: Root to Shoot Ratio.
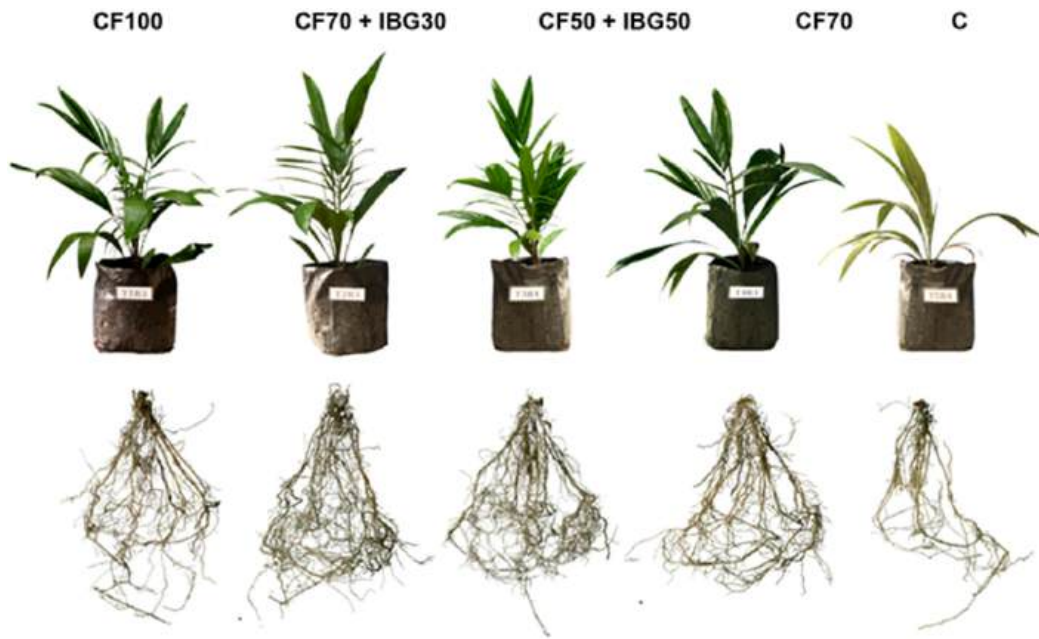
Fig. 1. Phenotypic trait responses of different fertilization on *E. guineensis* seedlings aboveground leaves and roots.

ical fertilizers with organic fertilizer made from a consortium of effective microbes resulted in taller seedlings compared to treatments using chemical fertilizers alone. This finding supports our results, as biofertilizer with reducing dosage of chemical fertilizer exhibited the tallest average height among other treatments. Additional factors such as the specific strains of beneficial microbes, the concentration of biofertilizer, and the compatibility with chemical fertilizers can all influence the outcomes (Mitter et al., 2021).

CF70 + IBG30 also had the significantly higher fresh shoots (174.66 ± 9.41 g) and fresh root weight (70.93 ± 2.72 g), compared to the other treatments. Control showed the lowest values for fresh shoots weight (79.52 ± 4.22 g) and fresh root weight (43.80 ± 2.06 g). In terms of dry shoots (DB), CF70 + IBG30 (49.61 ± 6.96 g) and CF50 + IBG50 (36.90 ± 4.63 g) displayed higher values compared to CF100 (43.47 ± 3.00 g) and CF70 (34.64 ± 3.94 g), suggesting greater shoots production in those treatments. C treatment exhibited the lowest DS value (24.95 ± 4.60). For dry root (DR), CF100 (14.75 ± 0.24 g) and CF70 (13.99 ± 0.50 g) had higher values compared to CF70 + IBG30 (13.40 ± 0.59 g) and CF50 + IBG50 (13.16 ± 0.30 g).

Our investigation indicated significant differences in root-to-shoot (R:S) ratios between treatments, with the C treatment having the greatest ratio (0.50 ± 0.11 g) and the CF70 + IBG30 having the lowest (0.29 ± 0.04 g). A higher R:S ratio in the C treatment indicates a preferred allocation of resources to root growth, indicating an investment approach that might benefit efficient nutrient uptake and structural integrity under stress circumstances (Lynch, 1995). In contrast, the lower R:S ratio seen in CF70 + IBG30 indicates a prioritization of shoot production, which presumably promotes rapid above-ground development but may jeopardize below-ground resource acquisition and stress resilience (Brouwer, 1962). These apparent patterns highlight the need for balanced resource allocation in regulating plant growth responses and stress tolerance systems (Lambers et al., 2008).

3.2. Relative chlorophyll properties of E. guineensis seedlings

The relative chlorophyll index readings obtained from the SPAD meter for the different treatments are shown in Fig. 2. The chlorophyll index serves as a critical measure of photosynthetic capacity and overall health in oil palm seedlings. Higher chlorophyll index readings are typically associated with improved photosynthetic efficiency and better growth performance. From Fig. 1, it is clearly that C treatment has the least visually green color which indicated that the plant had lower photosynthetic efficiency and translated into stunted growth as compared to other treatment regimens with chemical fertilizers and IBG biofertilizer. CF70 + IBG30 consistently exhibited higher chlorophyll index readings compared to the other treatments. This indicates that the 70% chemical fertilizer with 30% IBG biofertilizer addition positively influenced the chlorophyll content and photosynthetic activity of the oil palm seedlings.

Fig. 3 illustrates the number of fronds of the seedlings as a response to the fertilization regimes. CF70 + IBG30 treatment resulted in the highest number of healthy fronds followed by the CF100 plots with C treated seedlings with the lowest number of fronds observed over the period of the glasshouse studies. We also used principal components analysis (PCA) Fig. 4 to study the clusters of observed variables studied in this work based on their similarity where PCA does not discard any samples or characteristics. Instead, it reduces the overwhelming number of dimensions by constructing principal components (PCs). There was a high positive loading in which components such as heights, root-to-shoot ratio, and girth which indicated that the corresponding components strongly contribute to the principal component. Same can be observed for the principal components which contained fresh biomass weight and number of fronds, dry biomass weight and fresh root weight, and dry root weight and relative chlorophyll index, respectively.
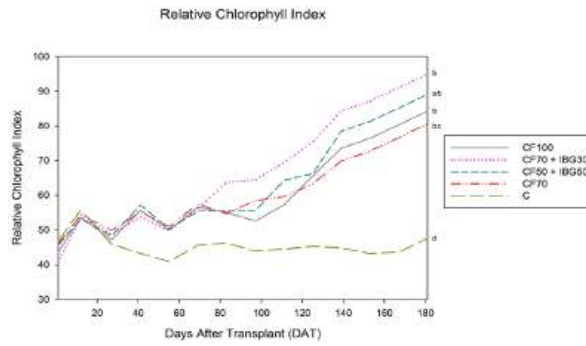
Fig. 2. The relative chlorophyll index of *E. guineensis* seedlings foliar as influenced by different treatment regimens during the 7 months trials. Different letters between treatments signified significant difference at $p < 0.05$ according to Tukey post-hoc test.
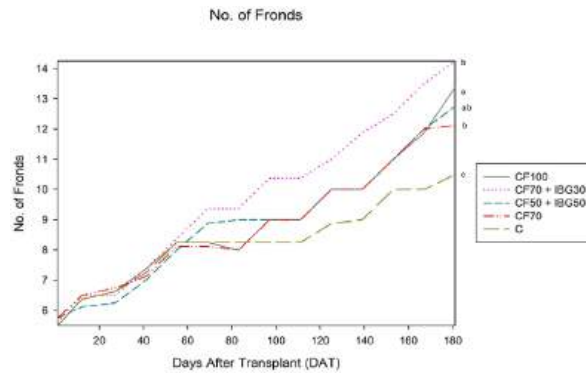

Fig. 3. The number of *E. guineensis* seedlings fronds as influenced by different treatment regimens during the 7 months trials. Different letters between treatments signified significant difference at $p < 0.05$ according to Tukey post-hoc test.
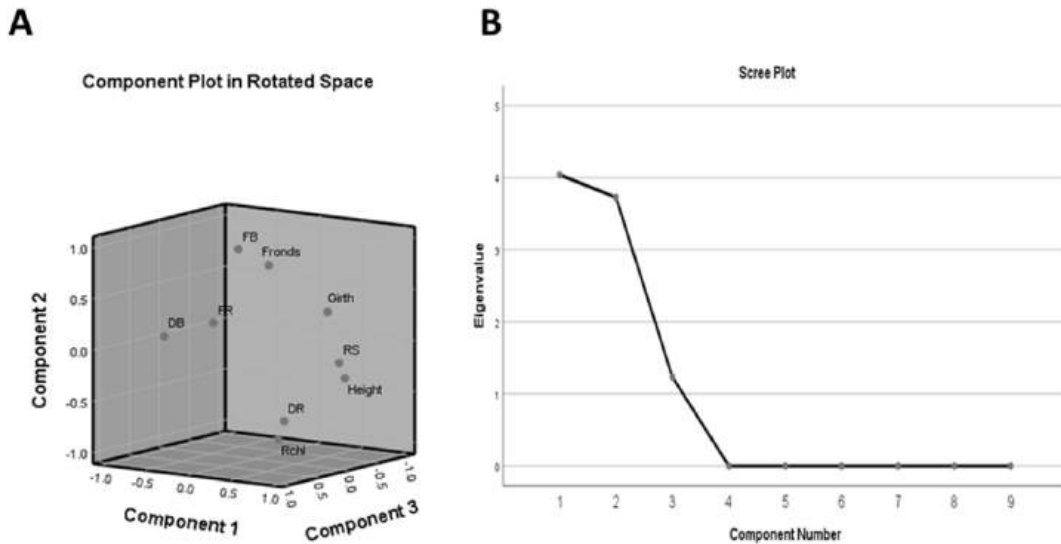

Fig. 4. (A) Principal component analysis of *E. guineensis* growth parameters and (B) postharvest biomass properties and the scree plot explained variance of each of the principal components which illustrated the criteria used for selecting the number of principal components studied.

3.3. Seedlings and soil nutrition and uptake analyses

Figs. 5 and 6, and **7** showed the soil, plant macronutrients content and their uptake, respectively. For the soil macronutrients contents, CF70 + IBG30 had significantly higher total nitrogen (0.066%), available phosphorus (0.92%) and Exch. K (0.21 cmol (+)/kg), respectively. Most Ultisols also have low exchangeable cations of K and even if complete recycling of Empty fruit bunch (EFB) and palm oil mill effluent (POME) produced from the Fresh Fruit Bunches (FFB) yield is to be carried out, the nutrients required by the palms for high yield levels are still higher than the nutrient supply from the oil palm agro-ecosystem. Therefore,
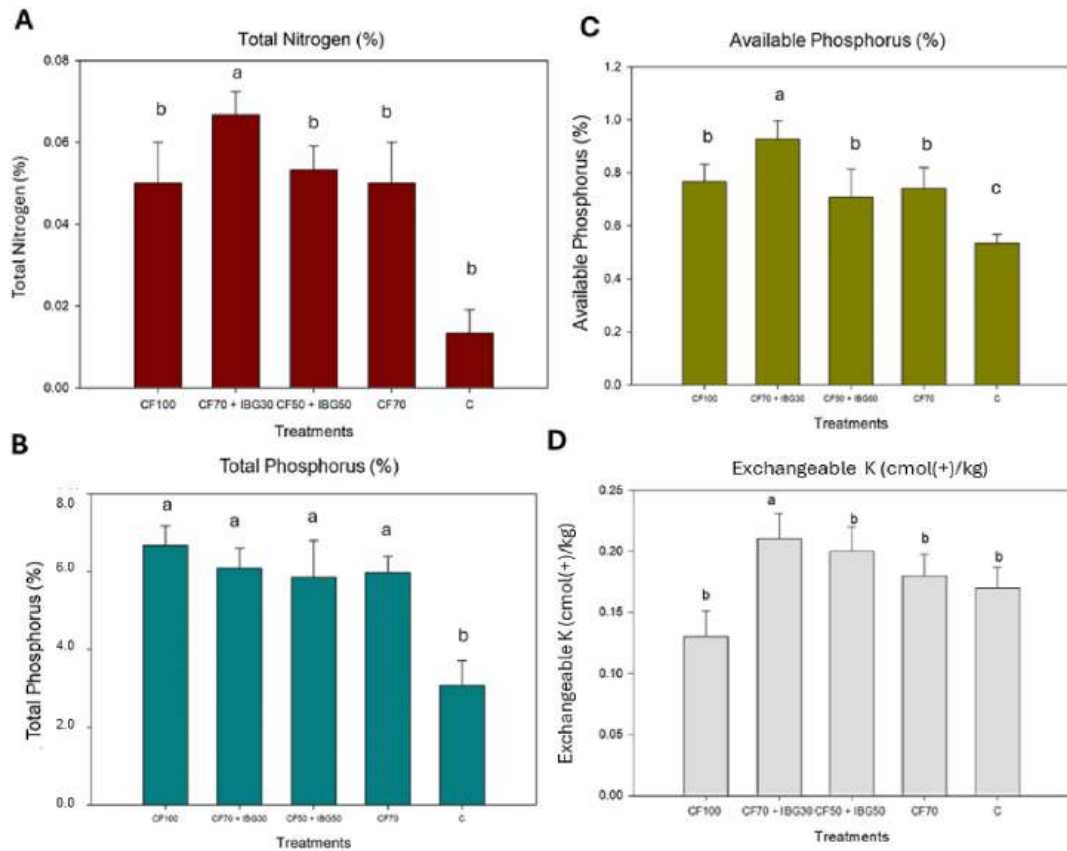
Fig. 5. Soil macronutrients (A) total nitrogen (%), (B) total phosphorus (%), (C) available phosphorus (%) and (D) exchangeable potassium (cmol(+)/kg) contents after the harvest. Different letters across the treatments represent significant differences at $p < 0.05$ according to Tukey Post-Hoc test.

they need to be augmented by other means to maintain the yield levels (Ng et al., 2012). The potential increase of Exch. K in CF70 + IBG30 could be due to the synergistic application of both inorganic and IBG biofertilizer.

The seedlings without any amendment remained the lowest and it was evident through the seedlings growth responses which was due to nutrient deficiency, leading to the poor plant's growth. Meanwhile, CF100 had the highest total phosphorus in soil after the treatment (6.173%) however the difference was not significant compared to CF70 + IBG30 (6.087%). In terms of the plant's macronutrients content and their respective uptake by the seedlings, overall CF70 + IBG30 treatment had highest for macronutrients contents the biomass of the seedlings, however, only P, K, Ca were the highest being uptake by the biomass in this particular treatment (Fig. 7). Although the IBG biofertilizer has a lower NPK ratio (5:7:8 + Trace Elements) compared to the chemical fertilizer (14:13:9:2.5 + TE), its inclusion in the treatment contributes additional nutrients to the soil. The biofertilizer provides a supplementary source of N, P, and potassium (K), albeit in smaller quantities than the chemical fertilizer. When combined with the chemical fertilizer, these added nutrients together with beneficial bacteria contained in the biofertilizer helped create a more balanced nutrient profile in the soil.

The IBG Biofertilizer, as detailed in the company's catalogue contains phosphate-solubilizing bacteria that play a crucial role in nutrient management. These bacteria dissolve rock phosphate by releasing organic acids, allowing for a significant reduction in the use of phosphate-based or chemical fertilizers. According to the catalogue, the application of IBG biofertilizer enables a 50% reduction in chemical fertilizers, as the bacteria release bound phosphorus, calcium phosphate, iron phosphate, and aluminum phosphate, thus reestablishing the biological chain and restoring soil productivity. Our investigation corroborated these conclusions. The findings, notably the total nitrogen and total phosphorus content shown in Fig. 5, showed that a 50% reduction in chemical fertilizer (CF50) paired with 50% IBG biofertilizer results in nutrient levels comparable to the 100% chemical fertilizer (CF100) treatment. This means that crops' nutritional requirements may be satisfied with less reliance on chemical fertilizers, with at least 50% substitution of IBG biofertilizer. The treatment combining 70% chemical fertilizer and 30% IBG biofertilizer (CF70+IBG30) showed even more desirable outcomes. This treatment resulted in significantly higher levels of total nitrogen, total phosphorus, and exchangeable potassium compared to the CF100 treatment (100% chemical fertilizer). When comparing the CF70 treatment (70% chemical fertilizer without any biofertilizer) to the CF50 + IBG50 treatment (50% chemical fertilizer and 50% IBG biofertilizer), the total nitrogen and phosphorus levels in the CF50+IBG50 seedlings were similar to those in the CF100 and CF70 treatments. This suggests that adding 50% IBG biofertilizer contributed less to the overall soil nutrients, particularly total nitrogen and phosphorus.

However, using 30% IBG biofertilizer with 70% chemical fertilizer effectively improved soil macronutrients. While theoretically, 50% IBG biofertilizer could enhance microbial activity to restore the soil's fertility, the low macronutrient content observed may be
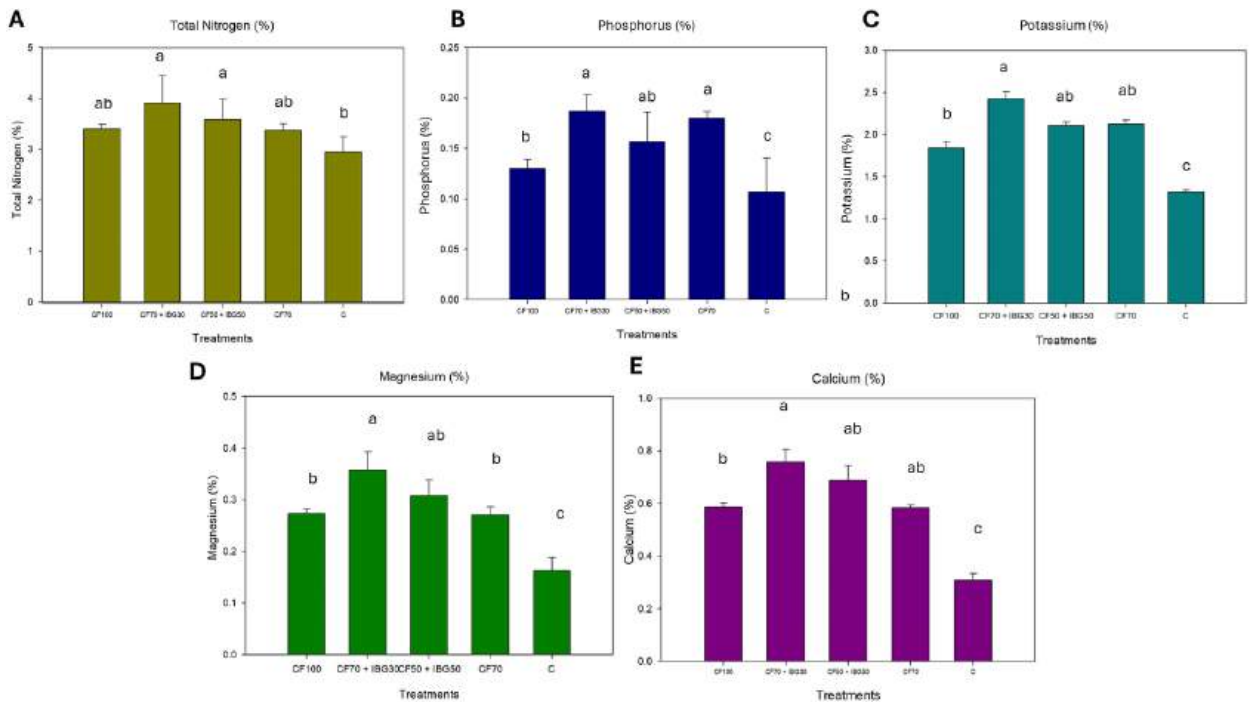
Fig. 6. Plants macronutrients (A) total nitrogen, (B) phosphorus (C) potassium (D) magnesium and (E) calcium across different treatments. Different letters across the treatments represent significant differences at p < 0.05 according to Tukey Post-Hoc test.

Fig. 7. Uptake of (A) nitrogen, (B) phosphorus, (C) potassium, (D) magnesium (E) calcium (F) boron across different treatments. Different letters across the treatments represent significant differences at p < 0.05 according to Tukey Post-Hoc test.
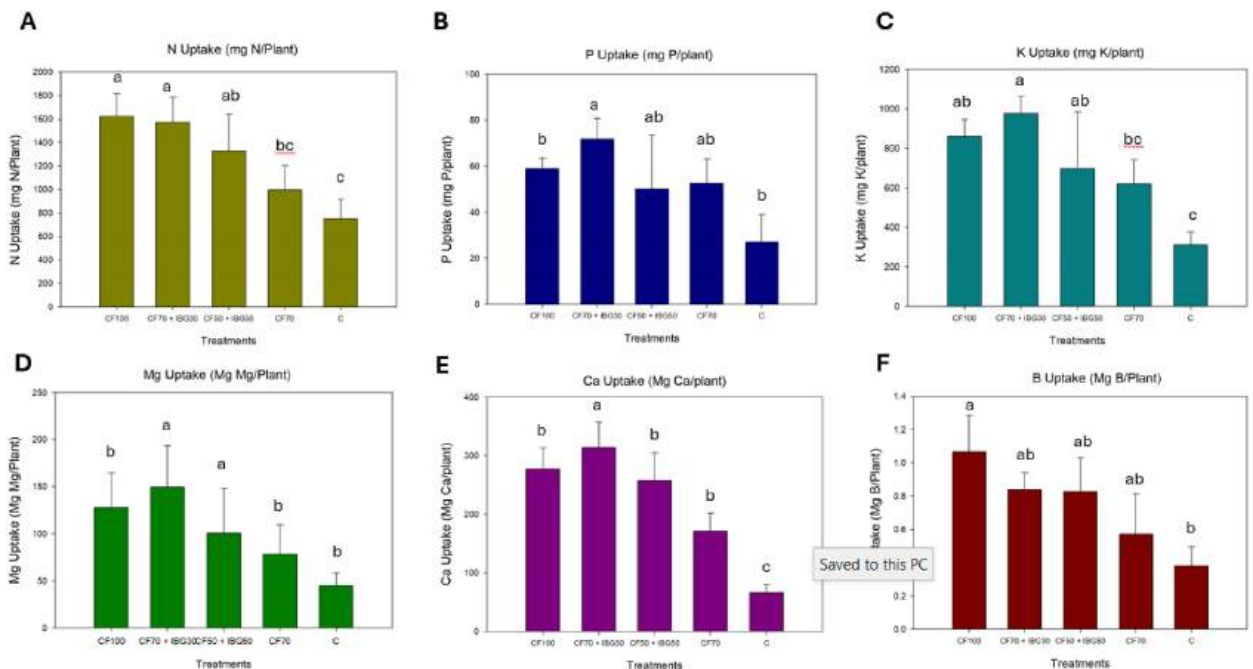
due to the low initial nutrient levels in the ultisol. In such conditions, a large portion of the 50% NPK applied would be directly taken up by plants, leaving fewer residual nutrients. This reduction in residual nutrients leads to lower organic carbon levels, which are vital for supporting belowground microorganisms. The nutrient dynamics were balanced with NPK fertilizer application slightly higher than 50% combined with IBG biofertilizer, leading to better soil fertility outcomes. The combined effects of the direct nutrient supply

from the commercial biofertilizer and the enhanced nutrient cycling facilitated by its microbial components, facilitated by the chemical fertilizers were key factors in the observed improvements in the growth and physiological properties of *E. guineensis* seedlings.

One limitation of the current study is the absence of an inactivated biofertilizer control. The inclusion of such a control would have allowed for better isolation of the effects of functional microbes present in the biofertilizer. Without this control, it is challenging to distinguish between the effects of the biofertilizer's microbial activity and other potential variables. To address this limitation, future experiments should incorporate an inactivated biofertilizer control. This control can be prepared by autoclaving or otherwise sterilizing the biofertilizer to ensure that all microbial activity is halted. By comparing the results from plants treated with active and inactivated biofertilizer, researchers can more accurately determine the specific contributions of the functional microbes to plant growth and health. Additionally, including a broader range of controls and replicates would further enhance the robustness and reliability of the findings. By implementing these improvements, future studies can provide a more comprehensive understanding of the role of biofertilizers and the specific mechanisms by which functional microbes influence plant development.

Table 3 and Table 4 represent the Pearson and Spearman correlation on the growth parameters of seedlings and biomass properties and nutrient uptake respectively. Pearson and Spearman correlation coefficients are two commonly used statistical techniques for determining the link between variables. The Pearson correlation coefficient measures the linear link between variables, whereas the Spearman correlation coefficient analyzes the monotonic relationship (Hauke and Kossowski, 2011). For the Pearson's correlation, there was a strong positive correlation at $P < 0.05$ (2-tailed) between the height of the seedlings to the girth diameter, number of fronds, weight of the fresh roots (FR) and dry weight of the biomass (DB) which suggest that the increase in the plant growth parameters would positively influence the increase of the biomass properties of the seedlings. However, one of the cons of using Pearson's correlation to study the relationship between the growth parameters and uptake of nutrients by the seedlings is that this correlation assumes linearity which in some growth conditions and stages of the seedlings might not be the suitable given there could be other factors affecting the variables. To evaluate such relationship which is robust to outliers, we utilize Spearman's correlation in an attempt to describe the middle or center point of a distribution which in the presence of outlier or extreme values observed, the median will be preferred over the mean.

In the correlation, we observed that the number of fronds of the oil palm seedlings is positively correlated with the uptake of B and P at $p < 0.05$ (2-tailed) (Table 4). From the glasshouse trial, seedlings amended with CF100 had the statistically highest B uptake, and CF70 + IBG30 with P, K, Mg, and Ca uptake, respectively. B and P play a critical part in various crucial processes in oil palms, including cell division, root growth, cell wall production, sugar transport, and calcium absorption (Putra and Purwanto, 2015). Boron (B) insufficiency is a common concern in oil palm plantations. The symptoms can be seen in leaf deformities such as hook leaf, crinkle

Table 3
Pearson's correlation between growth parameters and biomass properties of *Elaeis guineensis*.

	Height	Girth	Rchl	Fronds	FB	FR	DB	DR	RS
Height	1								
Girth	0.927[a]	1							
Rchl	0.922[a]	0.892[a]	1						
Fronds	0.892[a]	0.827[a]	0.804[a]	1					
FB	0.838[a]	0.705[a]	0.727[a]	0.809[a]	1				
FR	0.889[a]	0.901[a]	0.886[a]	0.740[a]	0.732[a]	1			
DB	0.830[a]	0.746[a]	0.776[a]	0.819[a]	0.799[a]	0.733[a]	1		
DR	**0.481**[b]	**0.558**[b]	0.602[a]	**0.480**[b]	0.224	**0.537**[b]	**0.445**[b]	1	
RS	−0.774[a]	−0.650[a]	−0.739[a]	−0.725[a]	−0.742[a]	−0.729[a]	−0.930[a]	−0.306	1

RChl – Relative chlorophyll index, FB- Fresh Biomass, FR – Fresh Roots, DB – Dry Biomass, DR- Dry Roots and RS – Root:Shoot ratio.
[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).

Table 4
Spearman's correlation between growth parameters and nutrient uptake properties of *E. guineensis*.

	Height	Girth	RCHL	Frond	Nupt	Pupt	Kupt	Mgupt	Caupt	Bupt
Height	1									
Girth	0.842[a]	1								
RCHL	0.875[a]	0.748[a]	1							
Frond	0.852[a]	0.703[a]	0.768[a]	1						
Nupt	0.658[a]	0.684[a]	0.583[a]	0.653[a]	1					
Pupt	0.725[a]	0.645[a]	0.605[a]	**0.551**[b]	0.647[a]	1				
Kupt	0.795[a]	0.725[a]	0.629[a]	0.630[a]	0.767[a]	0.958[a]	1			
Mgupt	0.841[a]	0.773[a]	0.641[a]	0.653[a]	0.777[a]	0.928[a]	0.976[a]	1		
Caupt	0.804[a]	0.788[a]	0.729[a]	0.761[a]	0.940[a]	0.719[a]	0.839[a]	0.838[a]	1	
Bupt	0.631[a]	0.688[a]	**0.550**[b]	**0.554**[b]	0.815[a]	0.629[a]	0.711[a]	0.728[a]	0.731[a]	1

RChl – Relative chlorophyll index, FB- Fresh Biomass, FR – Fresh Roots, DB – Dry Biomass, DR- Dry Roots and RS – Root:Shoot ratio.
[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).

leaf, transitory leaf, fishbone leaf, and stump leaf shape in which could have contributed to the reduction of number of healthy fronds in plots unamended with any fertilization or in stressed and acidic soil in general. These leaf malformation symptoms are caused by interference with leaf lamina development, resulting in reduced photosynthetic activity (Broeshart et al., 1957), and our correlation also indicated that B uptake (Table 4) was positively correlated with the relative chlorophyll content of the seedlings. From here we could deduce that decreased B uptake in oil palm seedlings under unamended infertile Ultisol have reduced fronds and malformed leaves which lowered the photosynthetic activity.

It is worth noting that the uptake of B in IBG biofertilizer amended seedlings was higher than the CF70 and Control. IBG biofertilizer could enhance Boron (B) availability through microbial activity that solubilizes insoluble forms into plant-accessible compounds. Beneficial microorganisms produce enzymes and organic acids that break down complex B compounds. Additionally, some microbes within the IBG biofertilizer could chelate B ions, keeping them soluble for uptake. Improved soil structure and root growth facilitated by biofertilizers also aid B uptake by seedlings (Shireen et al., 2018). Overall, biofertilizers contribute to a healthier soil environment and more efficient nutrient utilization, promoting better Boron availability and plant growth.

3.4. Non-enzymatic antioxidant properties of E. guineensis seedlings

3.4.1. Chlorophyll a/b ratio and carotenoid contents

Fig. 8 (a) and Fig. 8 (b) illustrate the responses of *E. guineensis* leaves on chlorophyll *a*/b and carotenoid. The CF100 treatment mitigates infertility issues by delivering adequate nutrients, leading to a substantial chlorophyll *a*/b ratio and increased carotenoid content. However, supplementation of CF70 + IBG 30% has the significantly highest chlorophyll *a*/b observed in both leaves with 13.18% (1.07 ± 0.01) higher chlorophyll *a*/b than CF100 although CF100 had a significantly higher leaves carotenoid content (19.03 ± 0.02 mg/g FW), 24.28% higher than that of CF70 + IBG30 amendment (14.41 ± 0.03 5 FW). Similarly, the CF50 + IBG50 treatment reduces stress through nutrient supplementation, resulting in better chlorophyll *a*/b ratio in leaves compared to CF100 and CF70 which further affirming the positive influence of IBG biofertilizer addition to ameliorate the side effects of soil infertility on oil palm seedlings by improving the antioxidant defense These advantages are offset by a 7.48% (0.99 ± 0.00) lower chlorophyll *a*/b ratio and a 31.02% (13.13 ± 0.03 mg/g FW) drop in carotenoid concentration in leaves when compared to high-
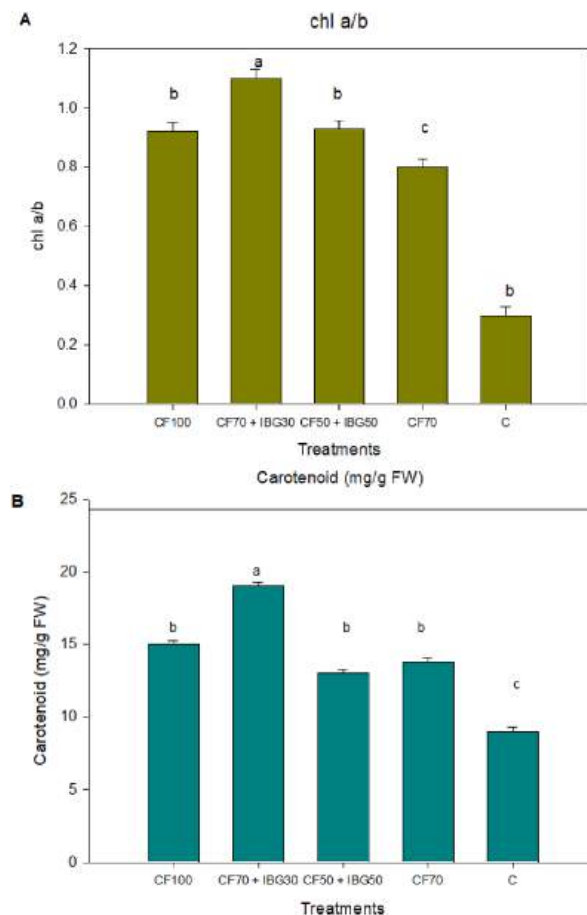

Fig. 8. Pigment properties of *E. guineensis* (A) Chlorophyll *a*/b of leaves and roots and (B) carotenoid contents in leaves. Different letters across the treatments represent significant differences between means observed at p < 0.05 according to Duncan's Multiple Range Test (MRT).

est ratio observed in seedlings supplemented with CF70 + IBG30 (1.07 ± 0.01) and CF100 (19.03 ± 0.01 mg/g FW), respectively.

Specifically, the addition of 30% IBG biofertilizer resulted in a 30.22% increase in carotenoid content above CF70 alone. Carotenoids act as potent antioxidants, scavenging reactive oxygen species (ROS) and protecting plant cells from oxidative damage (Yusof et al., 2023; Li et al. 2011). Thus, the higher levels of carotenoids in seedlings treated with biofertilizers suggest an enhanced capacity to mitigate oxidative stress and maintain cellular homeostasis under adverse environmental conditions (Ng et al., 2024; Aung et al., 2022, Cha-um et al., 2012; Keller, 2005). Soil infertility and acidity can hinder photosynthesis by inhibiting enzymes like rubisco, leading to excess energy. To prevent damage, leaves normally reduce chlorophyll and activate thermal dissipation and antioxidant mechanisms. Lower chlorophyll in C treated oil palm seedlings is a consequence, not a cause, of reduced photosynthesis caused by the inhibition of photosynthetic enzymes caused by the infertility. Similar results were found by Shah et al. (2017), showing a sharp decrease in chlorophyll a/b ratio in nutrient-deficient *E. guineensis* plots, exacerbated by water stress.

3.4.2. Total phenolic content, total anthocyanin content, and total flavonoid content

Fig. 9 shows the total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC) for leaves and roots, respectively. In the case of leaves, the treatment CF70 + IBG30 had a significantly higher TPC than CF100 by around 9.18% (2.25 ± 0.02 GAE/g FW). The CF100 and CF70 + IBG30 treatments also had greater TPC in roots, with percentage differences of roughly 24.66% and 23.63%, respectively. The CF70 supplemented with IBG30 treatment has the highest TFC and a percentage dif-
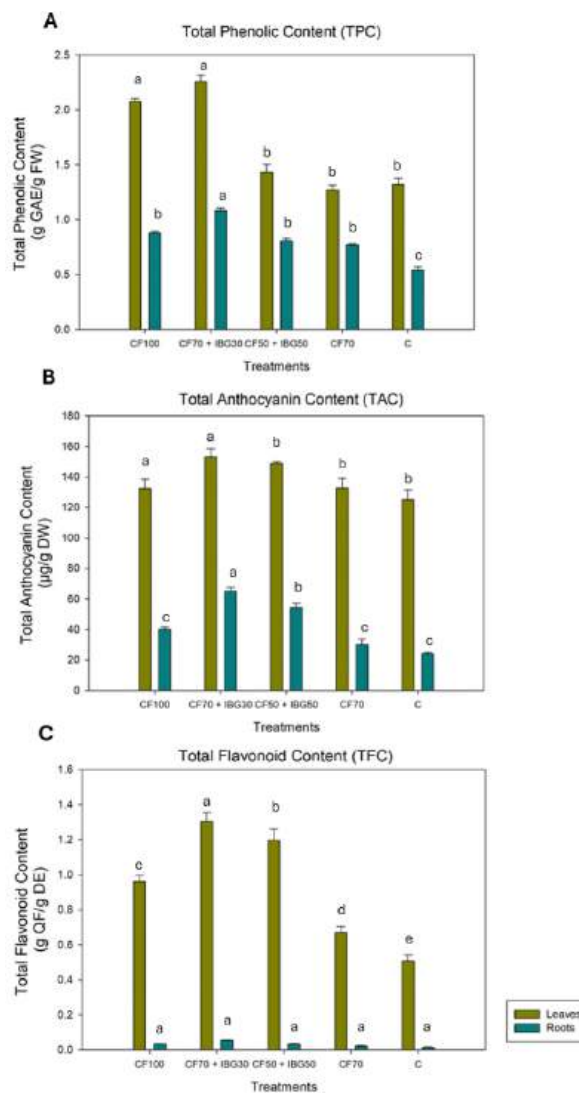

Fig. 9. (A) Total phenolic content (TPC) and (B) total anthocyanin content (TAC) (C) total flavonoid content (TFC) in leaves and roots. Different letters across the treatments represent significant differences between means observed at p < 0.05 according to Duncan's Multiple Range Test (MRT).

ference of around 30.40% when compared to C treatment in leaves. Similarly, TAC was higher in seedlings amended with CF70 + IBG30.

Phenolics, flavonoids, and anthocyanins are active chemicals that play critical functions in plant survival. Anthocyanins contribute to UV protection and stress tolerance, whereas phenols and flavonoids protect against oxidative stress and infections. The increased synthesis of these metabolites in response to different treatments demonstrates plants' flexibility to the nutrient deficiency and the techniques they use to preserve their health and function during the abiotic stress. The addition of biofertilizer to the seedlings might have mediated the stress signaling programs in the oil palm seedlings, prompting phenolics, flavonoid, and anthocyanin synthesis as a defensive strategy (Al-Mohammad et al., 2021). Plants frequently increase the synthesis of secondary metabolites such as phenolics, flavonoids, and anthocyanins in response to possible stressors as a defence mechanism (Nabavi et al., 2020).

There could be variability in the level non-antioxidants observed in this study, in which Mihai et al. (2022) previously noted that the synthesis of TPC and TFC in the oil palms is highly dependent on the disease progression, in our case, the infertility level of the soil. Interestingly, the authors observed that longer exposure to biotic stressors such as bud rot diseases in *E. guineensis* has led to a decreased level in TPC and other antioxidants capacities of the plants owing to the high number of pathogens. Similarly, decreased anthocyanin levels in oil palms was observed during the exposure to AI stress (Hidayah et al., 2020; Hasan and Abd Manan, 2020 suggested that the TPC and TFC of the *E. guineensis* increased when higher N fertilizers was applied however, these effects were varied when different progenies of *E. guineensis* were used.

Our findings can be further corroborated by Samsoon et al. (2022) and Haque et al. (2023) where the application of nano- and biofertilizer via soil and foliar improved the non-enzymatic antioxidants and photosynthetic components of the cowpea and tomato crops, respectively. This could be further explained via the potential intervening role of biofertilizer through the increased content of proteins such as the tyrosine, where Atif et al. (2023) proposed the role of the protein such as tyrosine in increasing the levels of non-enzymatic antioxidants in the maize grown under cadmium contamination. Such claims that the biofertilizer could induce the production of proteins such as the tyrosine, methionine, and asparagine was proven by Wichrowska and Szczepanek, 2020, where the composition of the amino acids in the potato tubers was positively influenced by the biofertilizer addition. The improved metabolic state frequently results in increased synthesis of secondary metabolites, and this is seen in combination of biofertilizer and chemical fertilizer seedlings where the TPC, TAC and TAC were higher. Similar postulates could be applied whereby longer exposure to such condition without any fertilizers application (such as C treatment) would result in *E. guineensis* unable to produce higher protective compounds and ultimately leading to its demise due and vulnerability towards soil infertility.

3.4.3. FRAP, ascorbic acid (AsA), DPPH and proline

Fig. 10 shows DPPH and b) Proline activity C) Ascorbic acid (AsA) and D) FRAP in leaves and roots of *E. guineensis,* respectively. Our observations on the radical scavenging activity (Fig. 10(a) revealed that seedlings under CF70 + IBG30 had the highest activity among other treatments (5.86 mg/g). Meanwhile, the radical scavenging activity of seedlings roots was the highest under the CF70 (6.07 mg/g) followed by the absolute control treatment (5.46 mg/g). Under prolonged exposure to stress, plants roots may suffer from a decrease in the DPPH free radical scavenging activity (Kaur et al., 2017). Meanwhile, the proline activity (Fig. 10(b)) in seedlings under the treatment CF70 + IBG30 was significantly highest, which could be an indication the seedlings are imparting the infertility stress by maintaining cell turgor or osmotic balance, preventing electrolyte and nutrients leakage, and bringing the free radicals to a balanced state (Hayat et al., 2012). Similar observation was made by Kaur et al. (2017) where based on seedling development, seedlings that performed better under stress exhibited higher levels of proline and DPPH radical scavenging activity.

Proline (Pro) is an essential and adaptive amino acid that can play an important role not only in plant development but also in stress responses (Szepesi and Szőllősi, 2018). It was suggested that higher proline accumulation in the vegetative parts of plant supported our hypothesis that IBG biofertilizer could impart antioxidative defense mechanism in the plants, leading resilience against infertility stress and overall tolerance conferment to the plants. Similar investigation was reported by Li et al. (2019) during cold stress where higher proline was associated with improved resilience towards the stress. Fig. 10(c) shows the AsA levels in *E. guineensis* across different fertilization routines. Both AsA levels in leaves and roots of the seedlings were enhanced in CF70 + IBG30 treatment (0.54 mg g/fm and 0.55 mg g/fm respectively) than CF100, CF70 and C however these differences were not statistically significant according to Duncan's Multiple Range Test (MRT) at $p < 0.05$ for both leaves and roots samples. Ascorbic acid (AsA) is a universal non-enzymatic antioxidant with significant potential for not only scavenging ROS but also influencing a variety of key processes in plants under both stress and non-stress situations (Akram et al., 2017). AsA is essential for plant growth because it aids in cell division, osmotic adjustment, hormone manufacture, and acts as an enzyme cofactor (Celi et al., 2023). Fig. 10(d) shows the ferric reducing antioxidant power (FRAP) level for the seedlings as amended by the treatments. No significant difference was observed between the highest FRAP level in leaves of seedlings in CF50 + IBG50 when compared to all other treatments. Additionally, the FRAP root level for all treatments were statistically insignificant.

4. Conclusions

This study assessed the role of IBG biofertilizer combined with reduced chemical fertilization on the growth responses, nutrients status and non-enzymatic antioxidant properties of *E. guineensis* grown under infertile *Bungor* series (ultisol). The glasshouse studied revealed a 30% application of IBG Biofertilizer is proven to be effective together under reduced fertilizer with the treatment resulted in comparable traits and non-enzymatic antioxidants properties to standard fertilization regimen (CF100). The seedlings properties in C treatment having no amendment, were far below the means observed in other treatments, where the DPPH radical scavenging activity was the highest which could explain in less accumulation of antioxidant compounds extracted from the seedlings grown under
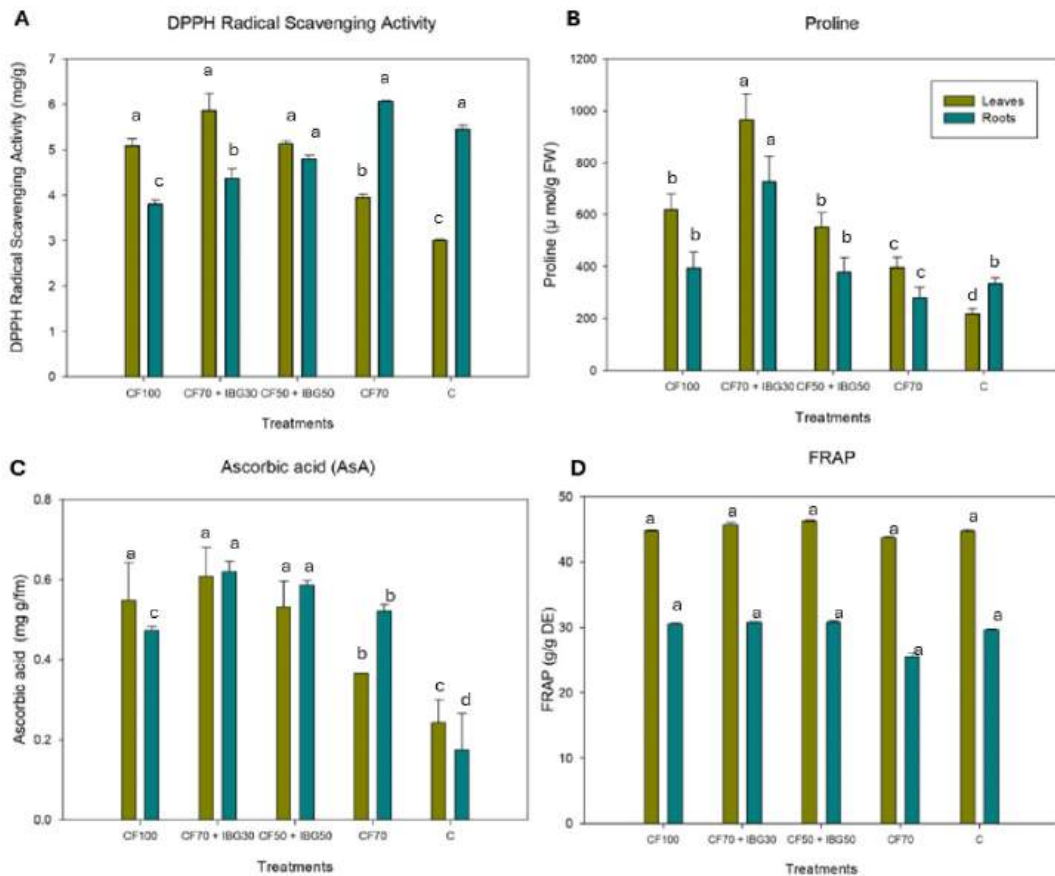
Fig. 10. (A) DPPH and (B) Proline activity (C) Ascorbic acid (AsA) and (D) FRAP in leaves and roots of *E. guineensis*. Different letters across the treatments represent significant differences between means observed at $p < 0.05$ according to Duncan's Multiple Range Test (MRT).

no amendment (stressed soil). Ideally, CF70 + IBG30 could restore the soil fertility status and improve plants growth activity under infertile without the need of full fertilization regime which is significantly important in ushering the oil palm plantation sector to a more sustainable and greener practice using biofertilizer. This study however by no means necessarily could contribute to the complete fertilization framework regulation overhaul in the particular sector studied but it would provide sufficient scientific evidence of the beneficial biofertilizer in improving the growth and enhance non-enzymatic antioxidants properties of oil palm seedlings under infertile Ultisol.

CRediT authorship contribution statement

Aaronn Avit Ajeng: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Noor Sharina Mohd Rosli:** Writing – review & editing, Validation, Investigation. **Pei Xin Chen:** Writing – review & editing, Validation, Investigation. **Rosazlin Abdullah:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jamilah Syafawati Yaacob:** Writing – review & editing, Validation, Investigation. **Tau Chuan Ling:** Writing – review & editing, Validation, Investigation. **Kuan Shiong Khoo:** Writing – review & editing, Validation, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2024.103321.

References

Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G., Sharma, S., 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Crit. Rev. Biotechnol. 30 (3), 161–175. https://doi.org/10.3109/07388550903524243.

Ajeng, A.A., Abdullah, R., Kadir, W.R.A., Suki, N.I.A., Sa'adah, N., 2021. Growth of maize and residual nutrients in soil treated with palm Kernel Shell biochar. Ama, Agric. Mech. Asia, Afr. Lat. Am. 52 (1), 2979–2994.

Ajeng, A.A., Abdullah, R., Ling, T.C., Ismail, S., Lau, B.F., Ong, H.C., Chang, J.S., 2020a. Bioformulation of biochar as a potential inoculant carrier for sustainable agriculture. Environ. Technol. Innov. 20, 101168. https://doi.org/10.1016/j.eti.2020.101168.

Ajeng, A.A., Abdullah, R., Malek, M.A., Chew, K.W., Ho, Y.C., Ling, T.C., Show, P.L., 2020b. The effects of biofertilizers on growth, soil fertility, and nutrients uptake of oil palm (*Elaeis guineensis*) under greenhouse conditions. Processes 8 (12), 1681. https://doi.org/10.3390/pr8121681.

Ajeng, A.A., Vejan, P., Abdullah, R., 2024. The role of microbes in biofortification for sustainable nutrient management and agricultural crop productivity: current trends and imminent challenges. In: Microbial Technology for Agro-Ecosystems. Academic Press, pp. 293–306. https://doi.org/10.1016/B978-0-443-18446-8.00009-7.

Akram, N.A., Shafiq, F., Ashraf, M., 2017. Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Front. Plant Sci. 6, 613. https://doi.org/10.3389/fpls.2017.00613.

Al-Mohammad, M.H., Sachet, T.F., Al-dulaimi, Z.S., 2021. Effect of phenylalanine, jasmonic acid and biofertilizer on growth, yield and anthocyanin pigments of roselle calyces. IOP Conf. Ser. Earth Environ. Sci. 910 (1), 012077. https://doi.org/10.1088/1755-1315/910/1/012077.

Atif, M., Perveen, S., Parveen, A., Zafar, S., Batool, F., Saeed, F., 2023. Intervening role of tyrosine in cadmium Detoxification, balancing of mineral ions homeostasis, antioxidants, and secondary metabolites in maize. J. Soil Sci. Plant Nutr. 23 (4), 5975–5992. https://doi.org/10.1007/s42729-023-01455-5.

Aung, T.T., Shi, F., Zhai, Y., Xue, J., Wang, S., Ren, X., Zhang, X., 2022. Acidic and alkaline conditions affect the growth of tree peony plants via altering photosynthetic characteristics, limiting nutrient assimilation, and impairing ROS balance. Int. J. Mol. Sci. 23 (9), 5094. https://doi.org/10.3390/ijms23095094.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. Plant Soil 39, 205–207.

Broeshart, H., Ferwerda, J.D., Kovachich, W.G., 1957. Mineral deficiency symptoms of the oil palm. Plant Soil 8, 289–300. https://doi.org/10.1007/BF01666319.

Brouwer, R., 1962. Nutrition influences the distribution of dry matter in the plant. Neth. J. Agric. Sci. 10 (5), 399–408. https://doi.org/10.18174/njas.v10i5.17581.

Celi, G.E.A., Gratão, P.L., Lanza, M.G.D.B., dos Reis, A.R., 2023. Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. Plant Physiol. Biochem. 107970. https://doi.org/10.1016/j.plaphy.2023.107970.

Cha-um, S., Takabe, T., Kirdmanee, C., 2012. Physio-biochemical responses of oil palm (*Elaeis guineensis* Jacq.) seedlings to mannitol-and polyethylene glycol-induced iso-osmotic stresses. Plant Prod. Sci. 15 (2), 65–72. https://doi.org/10.1626/pps.15.65.

David, D.J., 1960. The determination of exchangeable sodium, potassium, calcium and magnesium in soils by atomic-absorption spectrophotometry. Analyst 85, 495–503. https://doi.org/10.1039/an9608500495.

Fageria, N.K., Baligar, V.C., 2008. Ameliorating soil acidity of tropical Oxisols by liming for sustainable crop production. Adv. Agron. 99, 345–399. https://doi.org/10.1016/S0065-2113(08)00407-0.

Fatai, A.A., Shamshuddin, J., Fauziah, C.I., Radziah, O., Bohluli, M., 2017. Formation and characteristics of an Ultisol in Peninsular Malaysia utilized for oil palm production. Solid Earth Discuss 2017, 1–21. https://doi.org/10.5194/se-2017-60.

Ghazali, A., Asmah, S., Syafiq, M., Yahya, M.S., Aziz, N., Tan, L.P., et al., 2016. Effects of monoculture and polyculture farming in oil palm smallholdings on terrestrial arthropod diversity. J. Asia. Pac. Entomol. 19 (2), 415–421. https://doi.org/10.1016/j.aspen.2016.04.016.

Govaichelvan, K.N., Hamid, N., Kantono, K., Simarani, K., Yaacob, J.S., 2024. Unleashing the power of nature: Investigating the effects of storage on plant-based pigments and Bioactivities in tropical Ficus spp. extracts. J. Appl. Res. Med. Aromat. Plants 100528. https://doi.org/10.1016/j.jarmap.2024.100528.

Haque, M.M., Khatun, M., Mosharaf, M.K., Rahman, A., Haque, M.A., Nahar, K., 2023. Biofilm producing probiotic bacteria enhance productivity and bioactive compounds in tomato. Biocatal. Agric. Biotechnol. 50, 102673. https://doi.org/10.1016/j.bcab.2023.102673.

Harkousse, O., Slimani, A., Jadrane, I., Aitboulahsen, M., Mazri, M.A., Zouahri, A., Al Feddy, M.N., 2021. Role of local biofertilizer in enhancing the oxidative stress defence systems of date palm seedling (*Phoenix dactylifera*) against abiotic stress. Appl. Environ. Soil Sci. 2021, 1–13. https://doi.org/10.1155/2021/6628544.

Hasan, H., Abd Manan, F., 2020. Total phenolic and flavonoid content of *Elaeis guineensis* leaves treated with different amount of nitrogen-potassium fertilizer. Int. J. Life Sci. Biotechnol. 3 (2), 156–163. https://doi.org/10.38001/ijlsb.699527.

Hauke, J., Kossowski, T., 2011. Comparison of values of Pearson's and Spearman's correlation coefficients on the same sets of data. Quaest. Geogr. 30 (2), 87–93. https://doi.org/10.2478/v10117-011-0021-1.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A., 2012. Role of proline under changing environments: a review. Plant Signal. Behav. 7 (11), 1456–1466. https://doi.org/10.4161/psb.21949.

Hidayah, A.N., Yahya, S., Sopandie, D., 2020. The tolerance of oil palm (*Elaeis guineensis*) seedlings to Al stress is enhanced by citric acid and natural peat water. Biodiversitas J. Biol. Divers. 21 (10). https://doi.org/10.13057/biodiv/d211051.

Kaur, D., Grewal, S.K., Kaur, J., Singh, S., 2017. Free radical scavenging activities can mitigate the effect of water stress in chickpea. Crop Pasture Sci. 68 (6), 544–554. https://doi.org/10.1071/CP17022.

Ke, J., Wang, B., Yoshikuni, Y., 2021. Microbiome engineering: synthetic biology of plant-associated microbiomes in sustainable agriculture. Trends Biotechnol. 39 (3), 244–261. https://doi.org/10.1016/j.tibtech.2020.07.008.

Keller, M., 2005. Deficit irrigation and vine mineral nutrition. Am. J. Enol. Vitic. 56 (3), 267–283. https://doi.org/10.5344/ajev.2005.56.3.267.

Keni, M.F., Zainuddin, N., Masri, M.M.M., 2023. Improving the growth performance of oil palm seedlings by mixtures of organic and chemical Fertilisers. J. Oil Palm Res. 35 (1), 147–156. https://doi.org/10.21894/jopr.2022.0033.

Khanna, K., Jamwal, V.L., Kohli, S.K., Gandhi, S.G., Ohri, P., Bhardwaj, R., Ahmad, P., 2019. Role of plant growth promoting bacteria (PGPRs) as biocontrol agents of *Meloidogyne incognita* through improved plant defense of Lycopersicon esculentum. Plant Soil 436 (1–2), 325–345. https://doi.org/10.1007/s11104-019-03932-2.

Kumar, S., Sindhu, S.S., Kumar, R., 2022. Biofertilizers: an ecofriendly technology for nutrient recycling and environmental sustainability. Curr. Res. Microb. Sci. 3, 100094. https://doi.org/10.1016/j.crmicr.2021.100094.

Lambers, H., Chapin, F.S., Pons, T.L., 2008. Plant Physiological Ecology. Springer Science & Business Media. https://doi.org/10.1007/978-0-387-78341-3.

Li, J., Yang, Y., Iqbal, A., Qadir, R., Shi, P., Wang, Y., Wu, G., 2019. Correlation analysis of cold-related gene expression with physiological and biochemical indicators under cold stress in oil palm. PLoS One 14 (11), e0225768. https://doi.org/10.1371/journal.pone.0225768.

Chlorophylls and carotenoids: measurement and characterization by UV–VIS spectroscopy, 2001. Current Protocols in Food Analytical Chemistry. John Wiley and Sons, New York, NY, p. F4.3.1-F4.3.8. https://doi.org/10.1002/0471142913.faf0403s01.

Lim, S.F., Matu, S.U., 2015. Utilization of agro-wastes to produce biofertilizer. Int. J. Energy Environ. Eng. 6, 31–35. https://doi.org/10.1007/s40095-014-0147-8.

Lynch, J., 1995. Root architecture and plant productivity. Plant Physiol 109 (1), 7–13. https://doi.org/10.1104/pp.109.1.7.

Malaysian Standard, [MS], 1980. MS 678–Recommended Methods for Soil Chemical Analysis: Part I–V. Shah Alam. Standards and Industrial Research Institute of Malaysia.

Biocatalysis and Agricultural Biotechnology 60 (2024) 103321

Matejovic, I., 1993. Determination of carbon, hydrogen, and nitrogen in soils by automated elemental analysis (dry combustion method). Commun. Soil Sci. Plant Anal. 24, 2213–2222. https://doi.org/10.1080/00103629309368950.

Mihai, R.A., Guacollantes, G.M.C., Villacrés Mesias, S.A., Florescu, L.I., Catana, R.D., 2022. Variation of the antioxidative defense in *Elaeis guineensis* Jacq. Facing bud rot disease in the Coastal Area of Ecuador. Molecules 27 (21), 7314. https://doi.org/10.3390/molecules27217314.

Mitter, E.K., Tosi, M., Obregón, D., Dunfield, K.E., Germida, J.J., 2021. Rethinking crop nutrition in times of modern microbiology: innovative biofertilizer technologies. Front. Sustain. 5, 606815. https://doi.org/10.3389/fsufs.2021.606815.

Nabavi, S.M., Šamec, D., Tomczyk, M., Milella, L., Russo, D., Habtemariam, S., Shirooie, S., 2020. Flavonoid biosynthetic pathways in plants: versatile targets for metabolic engineering. Biotechnol. Adv. 38, 107316. https://doi.org/10.1016/j.biotechadv.2018.11.005.

Naiji, M., Souri, M.K., 2018. Nutritional value and mineral concentrations of sweet basil under organic compared to chemical fertilization. Acta Sci. Polon. Hortorum Cultus 17 (2). https://doi.org/10.24326/asphc.2018.2.14.

Ng, P.H.C., Gan, H.H., Goh, K.J., 2012. Soil nutrient changes in Ultisols under oil palm in Johor, Malaysia. J. Oil Palm, Env and Health (JOPEH) 2. https://doi.org/10.5366/jope.2011.10.

Ng, Z.Y., Ajeng, A.A., Cheah, W.Y., Ng, E.P., Abdullah, R., Ling, T.C., 2024. Towards circular economy: potential of microalgae-bacterial-based biofertilizer on plants. J. Environ. Manage. 349, 119445. https://doi.org/10.1016/j.jenvman.2023.119445.

Offia-Olua, B.I., Ekwunife, O.A., 2015. Production and evaluation of the physico-chemical and sensory qualities of mixed fruit leather and cakes produced from apple (*Musa Pumila*), banana (*Musa Sapientum*), pineapple (*Ananas Comosus*). Niger. Food J. 33 (1), 22–28. https://doi.org/10.1016/j.nifoj.2015.04.004.

Pandey, N., Vaishnav, R., Rajavat, A.S., Singh, A.N., Kumar, S., Tripathi, R.M., et al., 2024. Exploring the potential of Bacillus for crop productivity and sustainable solution for combating rice false smut disease. Front. Microbiol. 15, 1405090. https://doi.org/10.3389/fmicb.2024.1405090.

Purwadi, R., Adisasmito, S., Pramudita, D., Indarto, A., 2023. Strategies for Restoration and utilization of degraded lands for sustainable oil palm plantation and Industry. Agroecological Approaches for Sustainable Soil Management 373–408. https://doi.org/10.1002/9781119911999.ch17.

Putra, E.T.S., Purwanto, B.H., 2015. Physiological responses of oil palm seedlings to the drought stress using boron and silicon applications. J. Agron. 14 (2), 49–61. https://doi.org/10.3923/ja.2015.49.61.

Rusli, L.S., Abdullah, R., Yaacob, J.S., Osman, N., 2022. Organic amendments effects on nutrient uptake, secondary metabolites, and antioxidant properties of *melastoma malabathricum* l. Plants 11 (2), 153. https://doi.org/10.3390/plants11020153.

Sabir, M., Hanafi, M.M., Hakeem, K.R., 2015. Sulfur nutrition of oil palm for enhancing oil yield in Tropics. In: Hakeem, K. (Ed.), Crop Production and Global Environmental Issues. Springer, Cham. https://doi.org/10.1007/978-3-319-23162-4_15.

Samsoon, S., Azam, M., Khan, A., Ashraf, M., Bhatti, H.N., Alshawwa, S.Z., Iqbal, M., 2022. Green-synthesized MnO2 nanofertilizer impact on growth, photosynthetic pigment, and non-enzymatic antioxidant of *Vigna unguiculata* cultivar. Biomass Convers. Biorefinery 1–10. https://doi.org/10.1007/s13399-022-03686-5.

Serri, F., Souri, M.K., Rezapanah, M., 2021. Growth, biochemical quality and antioxidant capacity of coriander leaves under organic and inorganic fertilization programs. Chem. Biol. Technol. Agric. 8 (1), 1–8. https://doi.org/10.1186/s40538-021-00232-9.

Shah, S.H., Houborg, R., McCabe, M.F., 2017. Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). Agronomy 7 (3), 61. https://doi.org/10.3390/agronomy7030061.

Shireen, F., Nawaz, M.A., Chen, C., Zhang, Q., Zheng, Z., Sohail, H., et al., 2018. Boron: functions and approaches to enhance its availability in plants for sustainable agriculture. nt. J. Mol. Sci 19 (7), 1856. https://doi.org/10.3390/ijms19071856.

Sharma, B., Tiwari, S., Kumawat, K.C., Cardinale, M., 2023. Nano-biofertilizers as bio-emerging strategies for sustainable agriculture development: Potentiality and their limitations. Sci. Total Environ. 860, 160476. https://doi.org/10.1016/j.scitotenv.2022.160476.

Souri, M.K., 2016. Plants adaptation to control nitrification process in tropical region; case study with *Acrocomia totai* and *Brachiaria humidicola* plants. Open Agriculture 1 (1), 144–150. https://doi.org/10.1515/opag-2016-0019.

Souri, M.K., 2017. Inhibition of nitrification process by seed oil from *Acrocomia totai* palm tree. J. Plant Nutr. 40 (7), 1035–1044. https://doi.org/10.1080/01904167.2016.1263325.

Szepesi, Á., Szőllősi, R., 2018. Mechanism of proline biosynthesis and role of proline metabolism enzymes under environmental stress in plants. In: Plant Metabolites and Regulation under Environmental Stress. Academic Press, pp. 337–353. https://doi.org/10.1016/B978-0-12-812689-9.00017-0.

Tandon, H.L.S., Cescas, M.P., Tyner, E.H., 1968. An acid-free vanadatemolybdate reagent for the determination of total phosphorus in soils. Soil Sci. Soc. Am. J. 32, 48–51. https://doi.org/10.2136/sssaj1968.03615995003200010012x.

Vejan, P., Khadiran, T., Abdullah, R., Ahmad, N., 2021. Controlled release fertilizer: a review on developments, applications and potential in agriculture. J. Control. Release. 339, 321–334. https://doi.org/10.1016/j.jconrel.2021.10.003.

Wichrowska, D., Szczepanek, M., 2020. Possibility of limiting mineral fertilization in potato cultivation by using bio-fertilizer and its influence on protein content in potato tubers. Agriculture 10 (10), 442. https://doi.org/10.3390/agriculture10100442.

Yusof, Z., Mahmood, N.Z., Othman, R., Yaacob, J.S., 2023. Effect of fertilization on expression of bioactive carotenoid and chlorophyll pigments in *Clinacanthus nutans* Lindau for potential Use as functional natural Colourants. Sains Malays. 52 (4), 1121–1132. https://doi.org/10.17576/jsm-2023-5204-07.

Yusof, Z., Ramasamy, S., Mahmood, N.Z., Yaacob, J.S., 2018. Vermicompost supplementation improves the stability of bioactive anthocyanin and phenolic compounds in *Clinacanthus nutans* Lindau. Molecules 23 (6), 1345. https://doi.org/10.3390/molecules23061345.