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The Effects of Biofertilizers on Growth, Soil Fertility, and Nutrients Uptake of Oil Palm (Elaeis Guineensis) under Greenhouse Conditions

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Abstract: The full dependency on chemical fertilizers in oil palm plantation poses an enormous threat to the ecosystem through the degradation of soil and water quality through leaching to the groundwater and contaminating the river. A greenhouse study was conducted to test the effect of combinations of biofertilizers with chemical fertilizer focusing on the soil fertility, nutrient uptake, and the growth performance of oil palms seedlings. Soils used were histosol, spodosol, oxisol, and ultisol. The three treatments were T1: 100% chemical fertilizer (NPK 12:12:17), T2: 70% chemical fertilizer + 30% biofertilizer A (CF + BFA), and T3: 70% + 30% biofertilizer B (CF + BFB). T2 and T3, respectively increased the growth of oil palm seedlings and soil nutrient status but seedlings in oxisol and ultisol under T3 had the highest in almost all parameters due to the abundance of more efficient PGPR. The height of seedlings in ultisol under T3 was 22% and 17% more than T2 and T1 respectively, with enhanced girth size, chlorophyll content, with improved nutrient uptake by the seedlings. Histosol across all treatments has a high macronutrient content suggesting that the rate of chemical fertilizer application should be revised when planting using the particular soil. With the reduction of chemical fertilizer by 25%, the combined treatment with biofertilizers could enhance the growth of the oil palm seedlings and soil nutrient properties regardless of the soil orders.

Keywords: plant growth promoting rhizobacteria; oil palm seedlings nursery; biofertilizers; chemical fertilizer

1. Introduction

The agriculture sector is considered as one of the economy pillars in many developing nations [1]. However, continuous use of agrochemicals such as chemical fertilizers and pesticides in this sector is detrimental to human health such as infant methemoglobinemia [2] and which also cause ecological imbalance [3,4]. The use of chemical fertilizer will also cause air and ground water pollution resulting

from eutrophication. This practice also negatively affects the roots of the crops, making them unable to acquire nutrients [5,6]. Therefore, there is a need to replace this conventional agricultural practice by implying a safer alternative to promote the growth of the plants, without affecting the agroecosystem. The effort to reduce the dependence on the chemical fertilizers has been made through the establishment of biological based organic fertilizers (also known as biofertilizer) as an alternative [7]. Biofertilizers are made up from soil bacteria that are beneficial to the plants and it is known as an integrated nutrients system where nutrients required by the plants are provided by the activity of the below-ground microorganisms. This practice of using beneficial microbes in agriculture has started about 60 years ago [8].

The introduction of beneficial microbes in inorganic fertilizers have received a considerable amount of attention in the last decades as the microbes are effective in promoting plant growth by secreting phytohormones and metabolites [9]. The application of bioinoculants containing N-fixing bacteria and P-solubilizing bacteria have proven to improve leaf chlorophyll, plant nutrient uptake, and yield of rice in which the use of N and P fertilizer was able to be minimized by 50% [10]. These beneficial bacteria are termed as the Plant Growth Promoting Rhizobacteria (PGPR) which comprise of nitrogen—fixers, phosphorus (P), and potassium (K) solubilizers, and often combined as consortium with the some beneficial fungi in the production of biofertilizers [7]. The PGPR mechanism of action can be divided into direct and indirect mechanism, with direct mechanism including biofertilization, root stimulation, rhizoremediation, and plant stress control. The indirect mechanism includes the biological control against diseases which includes antibiosis, induction of systemic resistance, and competition for nutrient and niches [11]. Genera belonging to *Rhizobium* spp., *Azospirillum* spp., and *Bacillus* spp. are the symbiotic nitrogen fixing bacteria which efficiently fix the nitrogen in the nodules and roots of the plants, hence reducing the dependence on nitrogenous fertilizers [12,13]. Meanwhile, the production of organic acid such as the gluconic acid, oxalic acid, malic acid, formic acid, 2-ketogluconic acid, propionic acid, lactic acid, D-malic acid, and citric acid by PGPR belonging to the genera *Pseudomonas* spp. [14,15], *Acinetobacter* spp. [16], *Bacillus* spp. [17], *Klebsiella pneumonia* [18], and *Burkholderia fungorum* [19] aid in the P solubilization and making the nutrient accessible to the plants.

Malaysia is currently the world's second largest palm oil producer and the increase in the plantation area from 1.5 million hectares in 1985 to 5.39 million hectares in 2014 has resulted in extensive use of chemical fertilizers [9,20]. Malaysia has tropical soils, most of them are considered as problematic soils such as peat, sandy acid sulfate, and highly weathered soils such as ultisols and oixsols [21]. The acidity of the soils is due to the natural ecosystem through the weathering process, pyrite oxidation in acid sulfate soil, and organic matter deposition and accumulation of forming peat; where these processes are often enhanced by human activities through intensive land-based crop and animal production [22]. The aim of this study is to observe the effects of combined application of chemical fertilizer with the biofertilizer application on oil palm (*Elaeis guineensis*) seedlings using different soil orders under greenhouse conditions as we are looking into reducing the rates of chemical fertilizer application and investigate the effects of common problematic soils in Malaysia on the application of biofertilizers for oil palm seedlings plantation. Different biofertilizers with various compositions of beneficial microbes was used together with the chemical fertilizer and evaluated based on plant growth attributes including the uptake of nutrients by these seedlings.

2. Materials and Methods

2.1. Study Area and Experimental Design

The experiment was carried out at Rimba Ilmu, University of Malaya, Kuala Lumpur. Four soil orders were used in this experiment; histosol, spodosol, oxisol, and ultisol. The chemical properties of the soils used in this study were listed in Table 1. Three quarters of each polybags (n = 12) (20 kg for histosol and spodosol, 15 kg for oxisol and ultisol) was filled with each respective soil and 4 g of Christmas Island Phosphate Rock (CIRP) (15% Total P and available P was 58.86) was applied and mixed into each polybag (34 × 45 cm) and incubated for a week before the transplant.

The temperature ambience was 28–33 °C. The experiments were conducted in the Complete Block Design (CBD) with four replicates for each treatment in a single trial. Liquid biofertilizer A (BFA) (effective microorganisms: 1×10^7 CFU/mL) and biofertilizer B (BFB) (effective microorganisms: 1×10^6 CFU/mL) were purchased from local Malaysian manufacturers. BFA consists of *Bacillus* spp. such as *Bacillus cereus* JCM 2152, *Bacillus amyloliquefaciens* strain MPA 1034 and *Bacillus tequilensis* strain 10b *Lactobacillus* spp.; *Azospirillum* spp. and *Rhizobium* spp. Meanwhile, BFB consists of a very diverse group of microbes: Actinomycetes such as *Kocuria rhizophila*, *Arthrobacter methylotrophus*, *Bacillus* spp. such as *B. pumilus*, *B. subtilis* (subspecies Spizizenii), *B. vallismortis*, *B. Thurengiensis*, *B. mycoides*, *B. mucilaginosus*, *Brevibacillus reuszeri*, *Paenibacillus polymax*, and *Paenibacillus azoreducens*. *Azospirillum brasilense* and fungus such as *Aspergillus niger* and *Aspergillus awamori*; yeast such as *Saccharomyces cerevisiae* Hansen were also the beneficial microbes contained in the biofertilizer. The micro and macro nutrient with the organic matter of the biofertilizers were listed in Table 2. NPK blue with the formulation ratio of (12 N:12 P_2O_5:17 K_2O: 2 MgO + TE) was used as the chemical fertilizer. The experiment consists of three treatments: [T1] 100% of CF, [T2] 70% CF + 30% BFA, and [T3] 70% CF + 30% BFB. The amount and dose of fertilizers applied was listed in Table 3. Treatments were done for four rounds (every 30 days).

Table 1. Chemical properties of histosol, spodosol, ultisol, and oxisol.

Soil Properties	Histosol	Spodosol	Ultisol	Oxisol
pH	3.23	5.49	3.83	4.33
Total N (%)	0.61	0.34	0.10	0.12
Availlable P (mg/kg)	75.81	36.66	25.99	32.78
Exchangeable K (mg/kg)	455.2	487.93	358.33	471.1

Table 2. The micro and macro nutrient, and the organic matter of the biofertilizer A and biofertilizer B.

Micro and Macro Nutrients	Biofertilizer A	Biofertilizer B
N	7%	5–6%
P	6%	8–9%
K	9%	10–11%
Ca	2%	-
Mg	1%	0.5–1.0%
Su	1%	-
Bo	0.5%	0.9–1.1%
Fe	50 ppm	282 ppm
Cu	15 ppm	18.4 ppm
Mn	10 ppm	35.8 ppm
Zn	15 ppm	51.4 ppm
Mo	12 ppm	-
Organic matter	Aloe vera Seaweed extract Fulvic acid Amino acid Protein	Aloe vera Seaweed extract Humic acid Amino acid Fish emulsify

Table 3. Chemical fertilizer and biofertilizer application. The biofertilizer was diluted with 200 mL of distilled water before applied to a single seedling.

Month	Control Plot Dosage per Palm (g seedlings^{-1}) 100% Chemical Fertilizer	Treatment Plot (NPK 12-12-17-2 + TE) 75% Chemical Fertilizer	Biofertilizer (mL)
1	15	10	2
2	20	15	2
3	25	20	3
4	30	25	3

2.2. Analysis of Soil Chemical Properties

The soil used was thoroughly mixed and air dried, sieved using a 2 mm mesh sieve and measured for macronutrients (NPK) content. Soil chemical properties such as 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). Total N was analyzed using CNS analyzer (LECO TruMac® CNS, St. Joseph, MN, USA) [23] and K was determined using the leaching method with 1 M ammonium acetate buffered at pH 7 [24]. P was analyzed using the method described by Bray and Kurtz [25].

2.3. Measurement of Oil Palm Seedlings Growth

During the treatment period, the growth parameters of the oil palms were observed and taken every two weeks. 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 colour at the midrib to maximize the calibration [26]. The readings were taken at three random spots and the amount was averaged throughout the study period. The SPAD-502 was calibrated after and before another reading on different seedlings.

2.4. Plants Analysis

The planting material used in this study was D × P Yangambi (ML 161). The seedlings were harvested after four months of planting. 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 dry mass of both aboveground biomass and root were determined by drying in an oven at 71–75 °C until constant weight was achieved. The aboveground biomass and roots were ground separately using a grinding machine (<2 mm) separately for macronutrients (NPK) analysis. To determine the residual of plant nutrients, the amount of N was determined using a CNS analyzer (LECO TruMac® CNS, St. Joseph, MN, USA) [24] while P was analyzed using the wet digestion method [27] and exchangeable K using displacement of cations with 1.0 N of NH4OAc (pH 7.0) and displacement of absorbed NH_4^+ with 0.1 N K_2SO_4 [28].

2.5. Data Analysis

Tukey's HSD (honest significant difference) test was used to determine significant differences (p-value ≤ 0.05) between different types of soil orders and two-way ANOVA was used to determine significant differences (p-value ≤ 0.05) between the treatments. The data are statistically analyzed using the IBM SPSS Statistics for Windows, version 21.0.

3. Results

3.1. Growth Performance of Fronds

Figure 1 shows the height of the oil palm seedlings at DAT (days after transplant) 131. There is a significant difference between T1 and T3 plots. In general, all seedlings across every soil type show a better growth under the T3. Under the same biofertilizer treatment, seedlings planted in oxisol depicted the highest growth. The application of BFB has a positive effect on the growth of seedlings in oxisol with a total increase of 22% and 17% from T2 and T1, respectively. Most seedlings in the T3 plot are higher and have more developed fronds with leaves as compared to the other treatments. In terms of the number of fronds, no significant difference was observed between the treatments and the type of soils used. However, seedlings in oxisol under the T2 have more frond counts compared to T1 and T3 with an increase of 5% and 12%, respectively. Meanwhile, seedlings in T3 planted using spodosol and ultisol depicted the highest number of fronds where seedlings in T1 show the lowest number of fronds.

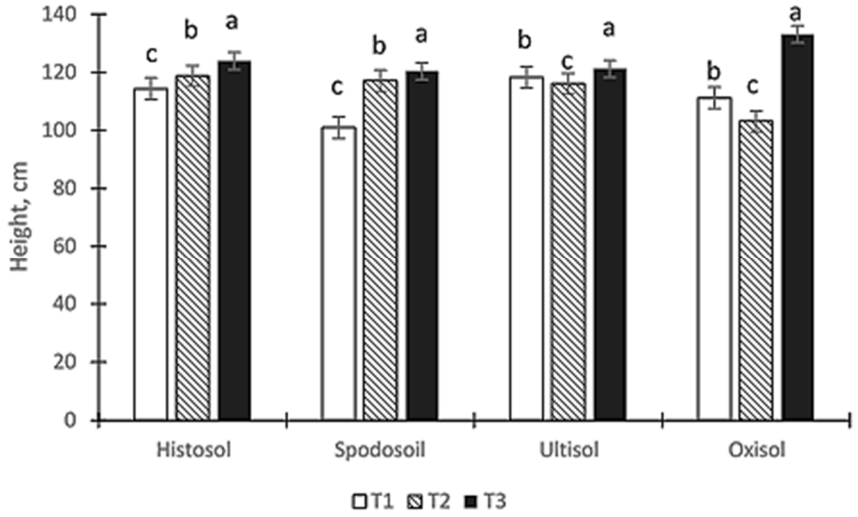

Figure 1. Highest frond height of oil palm seedlings at the end of treatment (DAT 131). Vertical bar represents the standard deviation. Different letters represent significant differences in Tukey's HSD comparison. Means sharing the same letter across treatments do not differ significantly at p-value ≤ 0.05.

3.2. Stem Girth Size

At the time of harvest, all seedlings in histosol, ultisol, and oxisol under T3 plots have the largest girth size (38.38, 38.87, and 38.25 mm, respectively) (Figure 2). The girth size of the seedlings was at least 26–30% larger than seedlings in T1. The stem girth size of seedlings in spodosol under the combined fertilization with BFA was the highest across all soils and treatments. Seedlings in spodosol also have the largest girth size under T1 while the girth size of seedlings in histosol, oxisol, and ultisol was less than 30 mm at 131 DAT. The other soils show an increase in girth size reading under T2 and no girth size below 30 mm was recorded.

3.3. Aboveground Biomass (ABG) and Root Dry Mass Ratio

Table 4 shows the aboveground biomass and root and their dry ratio. The highest ABG dry mass was obtained from oil palms seedlings treated under T3 under oxisol and ultisol while the least dry mass was from seedlings planted under 100% CF. This corresponded with oil palm seedlings height in which ultisol has the highest height compared to other soils. There was a significant difference between T1 and T3 for ABG, however there was no significant difference in ABG dry mass in between the soils used. Heavier root weights can be observed in seedlings under oxisol as compared to other soil types especially in the treatment with BFB. However, there was no significant difference between the treatments in root dry mass, and the root:Aboveground ratio. From this study, the combined fertilization with biofertilizers also showed a positive effect on the proliferation and development of the roots.

3.4. Chlorophyll Content

Figure 3 shows the chlorophyll content of the oil palm seedlings throughout the study period measured by the SPAD meter. The chlorophyll content of the seedlings in spodosol under T1 plots were decreasing starting DAT 41 until the harvesting day and remained the lowest. The combined treatment of chemical fertilizer with the biofertilizers show a positive response on the chlorophyll content of the seedlings. Our results indicate that biofertilizers can substitute chemical fertilizers to sustain N needs by the seedlings to enhance the chlorophyll content even at a reduced rate of fertilizers. There were significant differences among T1 and T3 in all the experimented seedlings across all soil types at p-value ≤ 0.05. Unlike T1, an increasing trend was seen in all seedlings under T2 and T3. Under the combined fertilization with BFA, all seedlings show an increment in the chlorophyll content except for chlorophyll in seedlings planted using oxisol which decreased at 67 DAT but increased again

after 131 DAT. Seedlings in histosol depicted the highest chlorophyll reading throughout the last two months of treatment period. The chlorophyll content of seedlings in T3 planted using histosol declined after 30 DAT but increased after 41 DAT and show a slight change from 67 and 131 DAT. Seedlings in ultisol under the same treatment reached the highest peak at 41 DAT with the chlorophyll content reading of 63.18 but decreased to 62.50 at 131 DAT. A steady increase in the chlorophyll content was seen in seedlings under oxisol but it remained the lowest reading throughout the last three months during the treatment period. The addition of biofertilizers seems also to have a positive impact on the chlorophyll reading of the seedlings.

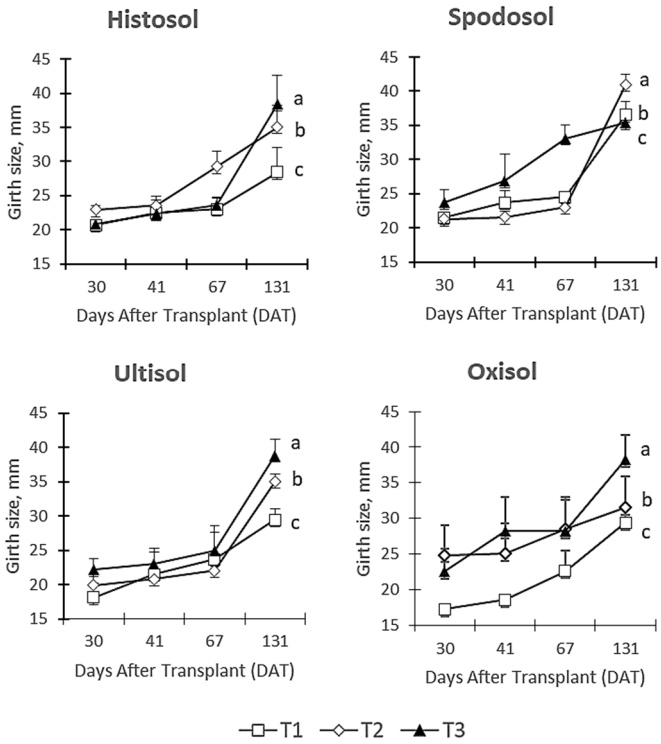

Figure 2. Girth size of the seedlings throughout the treatment period. Vertical bar represents the standard deviation. Different letters represent significant differences in Tukey's HSD comparison. Means sharing the same letter across treatments do not differ significantly at p-value ≤ 0.05.

Table 4. Aboveground biomass (ABG) and root dry weight with ABG:root. Different letters represent significant differences in Tukey's HSD comparison. Means sharing the same letter across treatments do not differ significantly at p-value ≤ 0.05.

Soil	Treatment	ABG	Root	Root:ABG
Histosol	T1	57.97 ± 9.92b	16.22 ± 4.46a	0.28 ± 0.03a
	T2	62.07 ± 3.47a	16.10 ± 3.31a	0.26 ± 0.04ab
	T3	59.50 ± 17.47b	15.09 ± 3.61a	0.26 ± 0.04b
Spodosol	T1	49.62 ± 14.32b	14.25 ± 4.21a	0.29 ± 0.02a
	T2	63.48 ± 7.08ab	16.52 ± 0.92a	0.26 ± 0.02ab
	T3	64.53 ± 4.99a	15.84 ± 1.17a	0.25 ± 0.02b
Ultisol	T1	53.61 ± 3.80b	11.70 ± 0.68a	0.22 ± 0.01ab
	T2	66.34 ± 2.50ab	15.20 ± 1.26a	0.23 ± 0.02a
	T3	70.39 ± 7.98a	13.92 ± 1.60a	0.20 ± 0.00b
Oxisol	T1	65.97 ± 4.61b	15.55 ± 2.95a	0.24 ± 0.06ab
	T2	58.70 ± 11.13ab	14.30 ± 1.03a	0.25 ± 0.02a
	T3	78.21 ± 14.91a	16.44 ± 0.95a	0.22 ± 0.00b

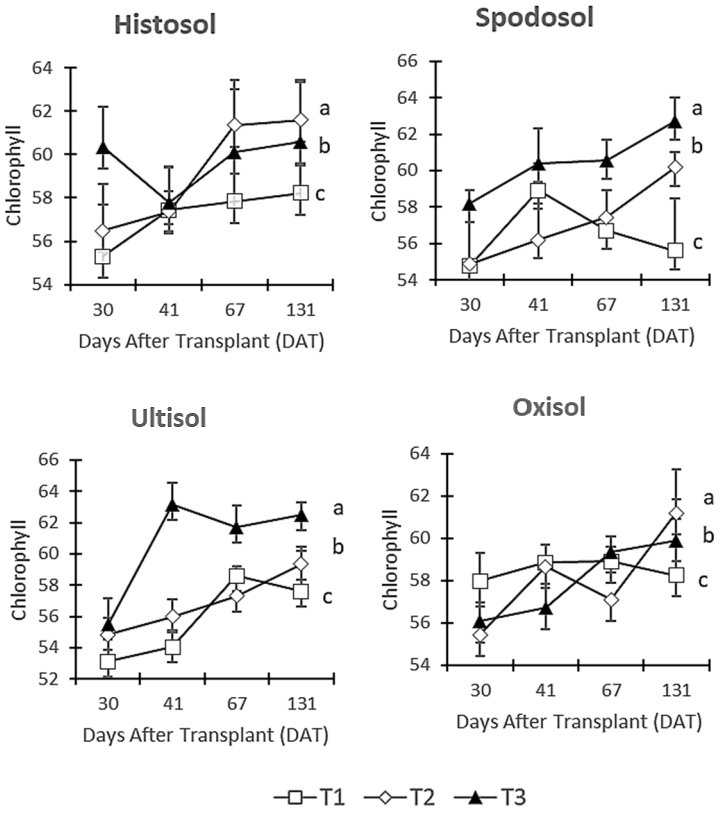

Figure 3. Chlorophyll index of the seedlings throughout the treatment period. Vertical bar represents the standard deviation. Different letters represent significant differences in Tukey's HSD comparison. Means sharing the same letter across treatments do not differ significantly at *p*-value ($p \leq 0.05$).

3.5. Soil Macronutrient Status

The nitrogen content in histosol was significantly higher across all treatments especially in the T3 plots as compared to spodosol, oxisol, and ultisol at the time of harvest *p*-value ($p \leq 0.05$) (Figure 4). This indicates that a lower N fertilizer rate is substantial for oil palms seedlings grown in the nursery prior to transplanting especially in tropical peat soil or the histosol. In the T3 treatment, the P content in the soil was higher than T2 followed by the T1 treatment especially in the histosol. However, no significant differences were found in all treatments and soils for P, as well as K soil content.

3.6. NPK Uptake by the Oil Palm Seedlings

The nutrient uptake by the oil palm seedlings is shown in Figure 5. The majority of the seedlings depicted an improved nutrient uptake in treatments with biofertilizers especially BFB under oxisol and ultisol. Overall, seedlings planted in ultisol under T3 have the highest NPK uptake, which is 20%, 38%, and 14% more than histosol, spodosol, and oxisol, respectively. However, there was no significant difference between the treatment at *p*-value ($p \leq 0.05$) but a significant difference was observed between histosol and spodosol, spodosol and ultisol in the uptake of N by the oil palm seedlings. Our results also indicate that there is a correlation between the high N uptake with an enhanced chlorophyll reading. However, there was no significant difference between the treatment at *p*-value ($p \leq 0.05$) but a significant difference was observed between histosol and spodosol, spodosol and ultisol in the uptake of N by the oil palm seedlings. No significant difference was found in all soil orders for the uptake of K at *p*-value ($p \leq 0.05$). Improved nutrient uptake is often correlated with root growth [20], however in our study the majority of seedlings depicted better root growth in histosol and spodosol as compared to the seedlings in oxisol (Figure 6) but the NPK uptake was higher in seedlings planted in oxisol and ultisol especially in T3.

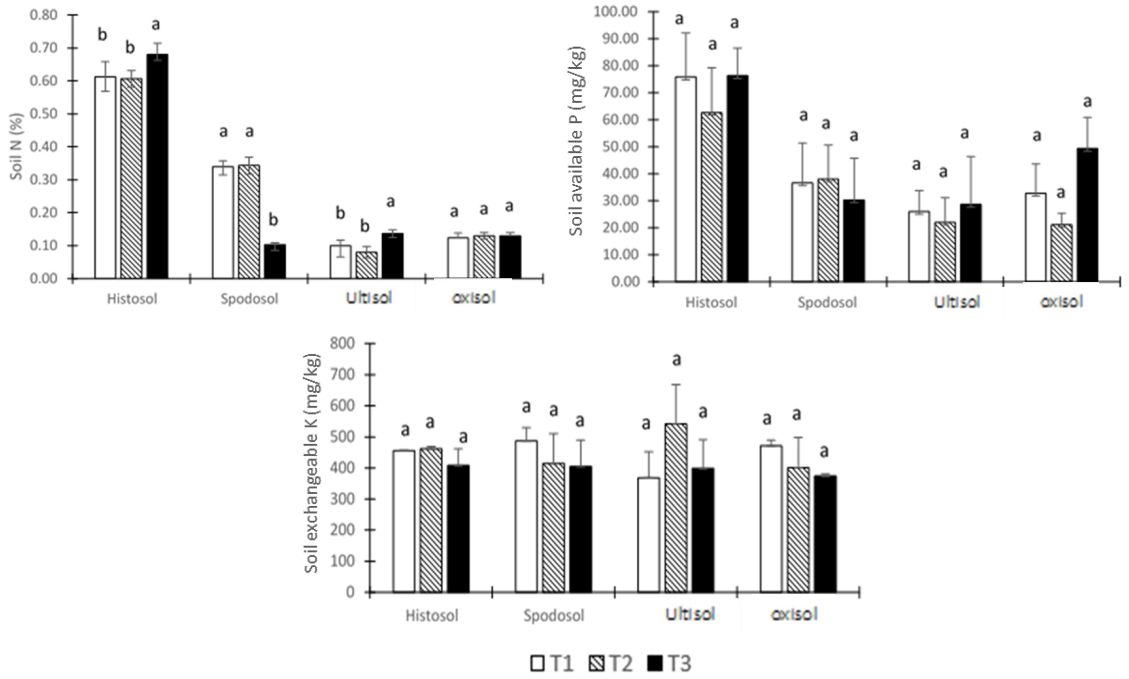

Figure 4. Soil macronutrient content (NPK) after the harvest. Vertical bar represents the standard deviation. Different letters represent significant differences in Tukey's HSD comparison. Means sharing the same letter across treatments do not differ significantly at *p*-value ($p \leq 0.05$).

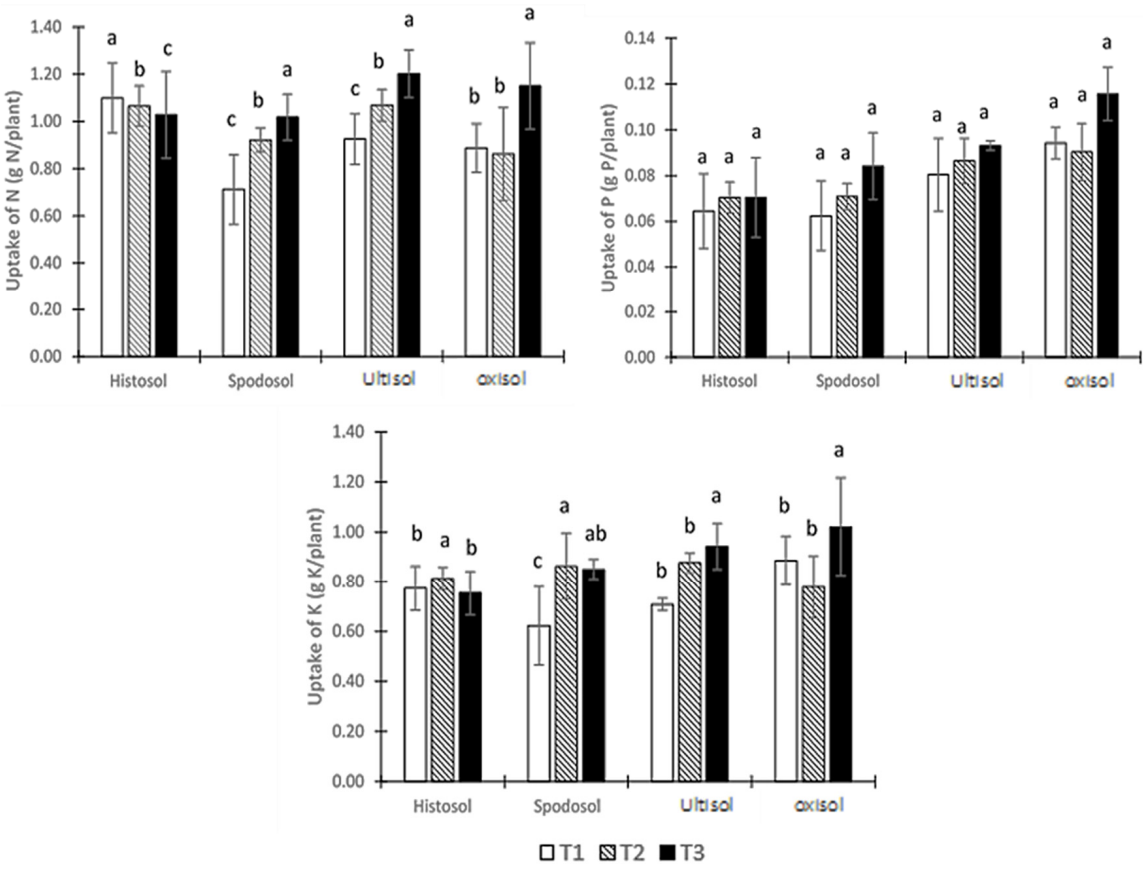

Figure 5. NPK uptake by the seedlings at time of harvest. Vertical bar represents the standard deviation. Different letters represent significant differences in Tukey's HSD comparison. Means sharing the same letter across treatments do not differ significantly at *p*-value ($p \leq 0.05$).

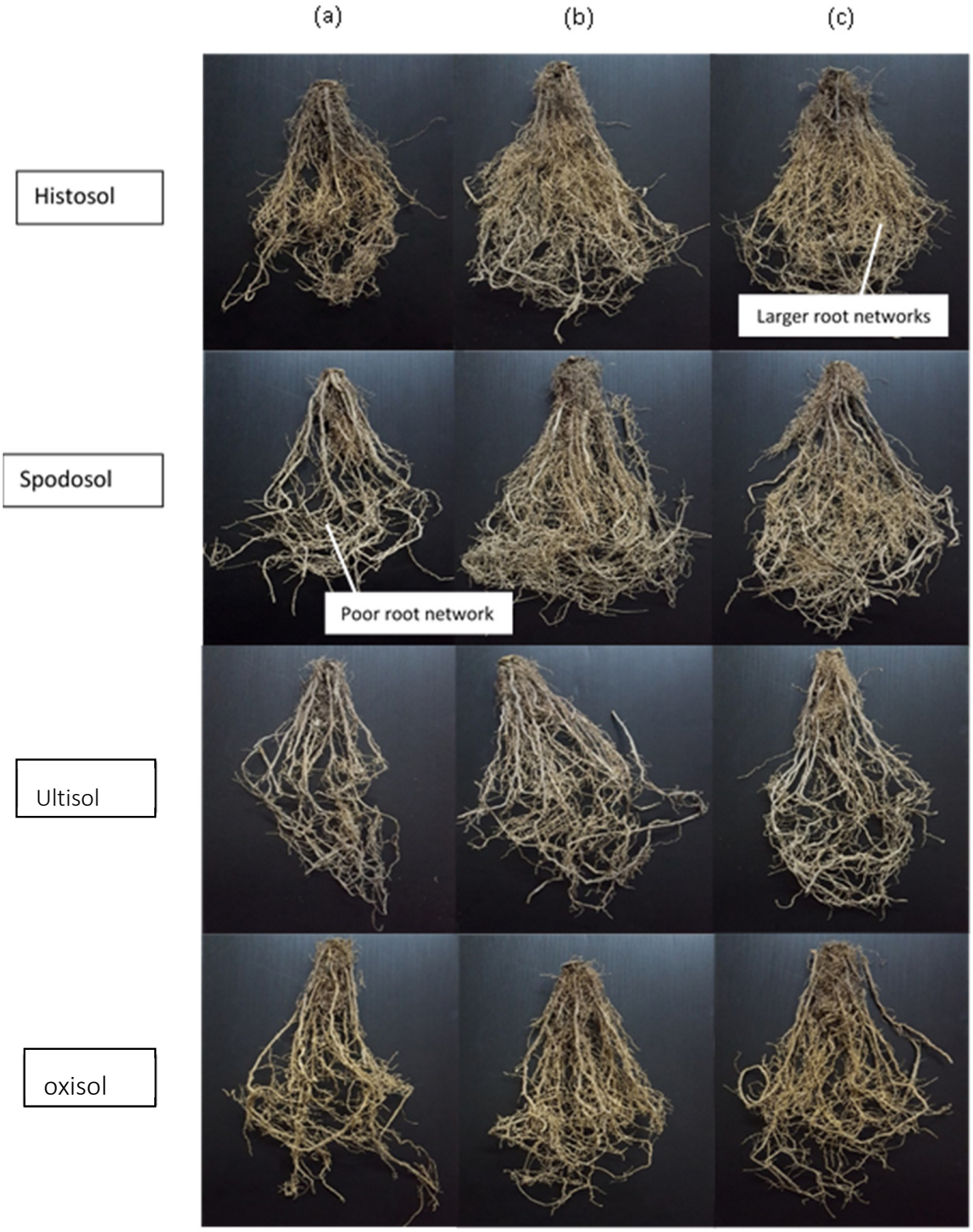

Figure 6. Roots of oil palm seedlings at the end of treatment. (**a**)T1, (**b**) T2, and (**c**) T3. The roots of oil palm seedlings treated with T3 were more in number, longer with more root hairs followed by seedlings in T2 then 100% T1 plots.

4. Discussion

4.1. Growth Performance of Fronds

FAO in 2011 states that about 175.5 million tons of chemical fertilizer is used in agriculture to achieve an optimum crop yield [29]. The enormous amount of chemical fertilizers deposited into soil causes a severe pollution of the river and groundwater which poses serious environmental

issues and public health. Thus, the initiative of using organic fertilizers such as bioinoculant has gained immense interest over the years. Although the production of phytohormones, suppression of phytopathogens, activation of phosphate solubilization, and promotion of plant nutrients uptake are the common mechanisms of PGPR in promoting the plant growth but the exact mechanisms are not clearly known [30]. PGPR also influence aerial growth of the inoculated crops which can be observed in increasing plant height, shoot, weight and stem width, as well as increasing the number of leaves per plant [31]. A similar finding was observed by Adiprasetyo et al. [32] where the multi-microbial biofertilizer was able to increase the height, chlorophyll, and the number of leaves of oil palm plants as compared to the sole treatment with chemical fertilizer. Zainuddin et al. [9] also reported the increase of vegetative growth of oil palm seedlings in the treatment containing biofertilizer made with *Bacillus* spp., *Providencia* sp., *Phyllobacterium* sp., *Sphingobacterium* sp., and few fungi species at a lowered rate of CF. In the present study, the bioinoculant is effective in conferring beneficial growth traits to the seedlings under greenhouse condition. Regarding the plant growth promotion mechanisms, *Bacillus* strains in BFA may promote the growth of the oil palm seedlings via the inhibition of pathogens. *B. amyloliquefaciens* is efficient in producing secondary metabolites such as lipopeptides, surfactins, bacillomycin D, and fengycins, which are secondary metabolites mainly with inhibiting pathogens activity [30,33,34]. *B. amyloliquefaciens* produce a mixture of organic acids such as lactic, isovaleric, isobutyric, and acetic acid that were identified as phosphate solubilizers [35]. A study by Yanti et al. [36] reported that the *B. cereus* strain JCM 2152 has the potential to promote the growth of the tomato plant and provide resistance towards *Ralstonia solanacearum* which usually causes bacterial wilt in tomato and other tropical crops. These *Bacillus* strains are ideal to be incorporated into biofertilizer formulation as they will be able to promote the plant growth.

4.2. Stem Girth Size

Abidemi et al. [37] reported that the phosphorus fertilization by the PGPR in most seedlings resulted in a higher number of leaves and stem girth size of oil palm seedlings. In the present study, the PGPR strains used in both biofertilizers A and B were excellent in enhancing the vegetative growth of the seedlings. Although seedlings in spodosol show poor frond growth, it is compensated with the large stem girth size.

4.3. Aboveground Biomass (ABG) and Root Dry Mass Ratio

During the treatment period, the growth of the oil palm seedlings in the greenhouse occurred predominantly aboveground especially the rachis and leaves as similar was observed by Zakry et al. [38]. Batool and Iqbal [39] reported that an increment of 10−95% in root length and shoot length was observed in seedlings treated by phosphate solubilizing bacteria (PSB) inoculum as compared to the control treatment. The *Azospirillum* strains used by Amir et al. [40] was also proven to increase the root growth of oil palm plantlets at the nursery stage. A diverse group of PGPR including few PSB and *Azosprillum brasilense* in BFB might have contributed to the growth and the elongation of the roots alongside the aboveground biomass of oil palm seedlings. The increase in aboveground and root biomass could also be related to the response to PGPR in the production of phytohormones such as auxin, gibberellin, and cytokinin which stimulated the root growth [12,39] and in turn increasing the essential nutrient uptake for an enhanced growth. Seedlings treated under both biofertilizers have a positive impact on the root development and proliferation. The root dry mass was not significantly affected by the treatments but T2 spodosol followed by T3 in ultisol gave the highest root dry mass.

The enhanced root development is likely triggered by the phytohormone production and nitrogen fixation [41]. Seedlings treated using T2 seemed to have a positive impact on the root development and proliferation. This indicates the effectiveness of PGPR to colonize the roots and promote nutrients solubilization and uptake by the seedlings that result in enhanced growth. *B. tequilensis* contained in BFA was reported by Dastager et al. [42] to produce indole-3-acetic acid (IAA) which promoted the proliferation and elongation of the black pepper roots for the uptake of nutrients. Even though the roots

development of seedlings in spodosol was much better than seedlings in oxisol and ultisol, the majority of seedlings depicted poor growth. This could be due to poor drainage and soil acidity in association with using spodosol [43]. Furthermore, poor root development was seen in oxisol and ultisol especially in the sole treatment with CF but there seems to be an increase root volume when the seedlings are treated with biofertilizers. Poor root development in soil can be caused by the soil bulk density, with less pore space and soil aeration [20] which gave less space for the roots proliferation. Another possible explanation could be that both oxisol and ultisol have higher bulk density, thus uprooting the seedlings was harder as compared to spodosol and histosol which were less dense. Seedlings in more compacted soils such as oxisol and ultisol might produce less primary and secondary roots, but this might be compensated by the production of longer and thicker tertiary and quaternary roots as observed by Yahya et al. [44]. Our study shows that different soil orders play a different role in oil palm seedlings nutrient uptake especially on the stimulatory efficiency of the bacterial inoculant contained in both BFA and BFB which may be important for successful root inoculation and plant growth promotion. The efficiency of the treatment and inoculation on agricultural crops also depends on few factors such as the ability of the bacteria to colonize roots, compounds exuded by the roots that enhance the colonization, and soil health [45]. Furthermore, the differences in the responses towards fertilization could be due to several factors such as the water regime [46], temperature, wind speed, soil texture, and soil depth [47].

4.4. Chlorophyll Content

The decrease in chlorophyll content in spodosol and ultisol under T1 plots could be due to the fact that CF alone failed to sustain N needs of the oil palm seedlings and this is shown in the N status of the seedlings which was the lowest among other soil orders. N is responsible to enhance the chlorophyll content [10]. Furthermore, the decrease could be due to few factors such as exposure to environmental stresses, herbicides, light irradiance or might be due to a source-sink relationship during the plant growth in later stages [48,49]. Reduction in the leaf chlorophyll content of oil palm seedlings treated with PGPR has also been reported by Amir et al. [12]. The decrease may also be influenced by time of day and underlying changes in solar irradiance where standardization of time and irradiance condition when taking chlorophyll measurements is recommended [50].

4.5. Soil Macronutrient Status

N is responsible for the cellular synthesis of chlorophyll and other components for the plant growth [51]. Soil N status plays a role in determining the effect of the PGPR inoculation especially on the nitrifying and denitrifying communities where the crop could affect the soil N dynamics within the rhizosphere and influence the type and level of mineral N available [52]. Although the addition of biofertilizer alongside with the NPK fertilizer can save up to 48% of the total N requirement in oil palm seedlings [53], from the present study there is a need to study the N rate application on crops to predict the N fertilizer requirement to avoid N rates that exceed the plant requirement [54] as seen in the histosol under the T1. The effect of PGPR also may be varied depending on the N source, N rate, and soil fertility [55]. The low N content in both oxisol and ultisol across all treatments could be an indication that essential macronutrients including P and K are taken up by the seedlings for survival [49]. Phosphate fertilizers applied to soils with lower pH are often precipitated immediately by aluminium and iron after application which makes P not available for the uptake by the crops, thus the practice of using soil pH correctors such as gypsum and liming are common in Malaysia [22]. Another alternative of increasing the P availability for the uptake by the crops is via the use of phosphate-solubilizing bacteria (PSB). Although the soils used in the study were in the acidic range, the addition of biofertilizers was found to improve the available P especially in oxisol and ultisol and histosol but no significant differences were observed. However, a revision on the rates of the applied NPK fertilizers on the histosol must be done due to excessive available N and P in the soil as the chemical fertilizer added was 500 mg P/kg in T1, 420 mg/kg soil in T2 and T3, respectively when the oil

palm seedlings growth can be optimized from as low as 90 mg L^{-1} of P_2O_5 [56]. Through the reduction of NPK rate, the farmers or the oil palm plantation companies can optimize the production cost of oil palm seedlings, and reduce the negative environmental impacts due to the excessive use of chemical fertilizer [57]. Therefore, further studies on the increased rate of biofertilizers and reduced mineral fertilizer must be done to avoid over-fertilization.

4.6. NPK Uptake by the Oil Palm Seedlings

The plant N status correlates with the SPAD chlorophyll readings [58] and in the present study there is a positive correlation between the N status of the seedlings with chlorophyll readings. The inoculation with PGPR can enhance the uptake of N in the early phase of the oil palm cultivation. N-fixer such as *Bacillus sphaericus* could increase the fixed N in the soil for the uptake by the plants especially to the most nutrient demanding part in oil palm such as rachis and leaflets [38]. The microbial inoculant in the biofertilizers consisting of the N-fixer has the ability to increase the nutritional assimilation (total N) and increase the growth and at the same time improve the soil properties [59]. PGPR such as *Azospirillum* contributes about 70% of the total N requirement of the host plant [60]. The low solubilization and precipitation of phosphorus in the soil might be caused by several factors such as the pH and the type of soil used [51]. However, combined treatment with BFB resulted in higher phosphate solubilization as *Bacillus* strains such as *Bacillus brevis* strains, *B. polymyxa*, *B. thurengiensis*, *Paenibacillus*, and *B. subtilis* in BFB were considered as some of the important strains applied to soils to enhance phosphorus solubilization and uptake in plants [61]. *B. tequilensis* is a phosphate solubilizer and it also has shown to improve the macronutrient (NPK) uptake of the black pepper in both acidic and alkaline conditions [42]. The oil palm seedlings growth could be enhanced if the dose of the chemical fertilizer is reduced [62]. Essential nutrients such as the NPK must be present in biofertilizers [9] but it might be in a minute amount for the uptake by the roots. Thus, the major source of K in all treatments might be from the fertilization with chemical fertilizer, not fully by the K solubilization by the PGPR. PGPR contained in the BFB are important to the plant nutrition by increasing the N and P uptake by the plants [63]. This study also shows that although the nutrient supply and fertility are the limiting factors especially in oxisol and ultisol [64], the practice of combining biofertilizers with chemical fertilizers can enhance and optimize the yield for oil palms in these soils.

5. Conclusions

From the present study, the addition of biofertilizers alongside with chemical fertilizers have shown not only enhanced oil palm seedlings growth in terms of the height, girth size, and chlorophyll, it also improves the nutrient uptake of the seedlings and soil nutrient status at a reduced rate of chemical fertilizer. Reduction on the rate of the chemical fertilizer may be needed to avoid over-fertilization of the oil palm seedlings.

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