Pertanika J. Trop. Agric. Sci. 48 (5): 1457 - 1470 (2025)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

# Phosphate Solubilizing Bacteria (PSB) and Commercial Rock Phosphate: An Effective Combination for Oil Palm Nursery

**Ding Haoran, Tan Geok Hun* and Susilawati Kasim**

*Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

## ABSTRACT

The growth of oil palm trees depends on phosphate availability. Phosphate-solubilizing bacteria (PSB) enhance the availability of phosphorus from phosphate rock by its phosphorus solubilizing ability. Phosphate solubilizing bacteria (PSB) can improve the available phosphorus content provided by phosphate rock through its phosphorus solubilization ability. In the management of palm tree nurseries, it is vital to carefully choose the appropriate type and application rate of phosphate fertilizers based on the growth requirements and soil characteristics of palm saplings to achieve optimal growth outcomes. The purpose of this study was to determine how well oil palm nursery could benefit from the use of commercial rock phosphate in conjunction with phosphate-solubilizing bacteria (PSB). *Bacillus marisflavi* and *Bacillus aryabahattai* populations in 1 g of compost were found to be $2 \times 10^9$ and $1 \times 10^8$, respectively, using the plate count method. As seen by leaf count, oil palm size, and frond height, the development rate and quality of oil palm seedlings are greatly enhanced by the combination of Morocco rock phosphate (MRP) and PSB. This study demonstrated that a combination of PSB and commercial phosphate rock is beneficial to oil palm seedlings and sought to determine its efficacy in oil palm nurseries.

*Keywords*: *Bacillus aryabahattai*, *Bacillus marisflavi*, commercial rock phosphate, oil palm, phosphate solubilizing bacteria (PSB)

ARTICLE INFO

*Article history:*
Received: 17 October 2024
Accepted: 21 February 2025
Published: 29 August 2025

DOI: https://doi.org/10.47836/pjtas.48.5.07

*E-mail addresses*:
dinghaoran0@gmail.com (Ding Haoran)
geok_hun@upm.edu.my (Tan Geok Hun)
susilawati@upm.edu.my (Susilawati Kasim)
* Corresponding author

## INTRODUCTION

The monoecious oil palm (*Elaesis guineensis* Jacq.) is a member of the *Aracaceae* family and belongs to the genus *Elaeis* (Corley & Tinker, 2015). Although it is widely grown in tropical nations like Malaysia, Indonesia, Nigeria, and Thailand, it is native to West and Central Africa. The oil palm, a tropical tree, is cultivated for its oil-rich

e-ISSN: 2231-8542

fruit. (Rogers & Kadner, 2022). Fruit oil is a versatile substance that finds application in a range of industries, such as food, cosmetics, and biofuel production. Nearly 40% of all vegetable oil produced is palm oil, making it the most frequently used vegetable oil in the world (Myzabella et al., 2019; Singh et al., 2013). With contributions to global oil palm production and exports of 25.8% and 34.3%, respectively, Malaysia is the second-largest producer and exporter of the crop. The principal importers are from United States, India, the European Union, China and Pakistan (Parveez et al., 2022).

Phosphorus is an essential component for oil palm reproductive development, especially for the formation of flowers and fruits. Fruit set, size, and oil content can all be improved with an adequate supply of phosphorus (Etesami, 2019). Only a small amount of the phosphorus in the soil is available to plants, and the majority is unavailable. Phosphate is typically not able to be utilised directly by plants due to its non-bioavailability in soil. Only free, readily available forms of phosphorus can be used by plants from the soil. Soil microbes or plant roots facilitate the availability of soil phosphates (Barroso & Nahas, 2005; Richardson & Simpson, 2011).

One of the major non-renewable resources in the world, phosphorus is found in phosphate rock, a type of non-detrital sedimentary rock that is mostly formed in igneous, metamorphic, and sedimentary rocks. In most minerals, phosphorus takes the form of orthophosphate (Ouabid & Raji, 2023). There are several applications for phosphate rock, including the production of phosphate fertilisers, phosphoric acid, pure phosphorus (yellow and red), and other chemical raw materials. The primary ingredient in high-efficiency phosphate fertiliser and phosphate is phosphoric acid (Khan et al., 2022). Phosphate rock deposits are distributed in a somewhat concentrated manner; over 70% of the world's reserves are found in Morocco (Elamrani & Lemtaoui, 2016).

The group of rhizobacteria that promote plant growth (PGPR) includes phosphate solubilizing bacteria (PSB). According to Satyaprakash et al. (2017), PSBs have the capacity to solubilize phosphate, changing insoluble phosphatic compounds into soluble forms in soil that are then available for plant absorption. By dissolving and absorbing non-bioavailability P, phosphate solubilizing bacteria (PSB) transform it into bioavailability P to meet plant needs (Chen & Liu, 2019). However, the kind and number of organic acids produced determine the solubilization impact (Tucher et al., 2017).

The effectiveness of PSB in improving phosphorus availability depends on various factors, including the type and quantity of organic acids produced. Limited research has explored the combined application of commercial rock phosphate and PSB in enhancing phosphorus uptake, growth, and nutrient utilization in oil palm nurseries. This gap highlights the need to investigate the synergistic effects of rock phosphate and PSB on the growth and nutrient dynamics of oil palms, addressing both the immediate agricultural requirements and the broader sustainability challenges associated with phosphorus use.

This study investigated the effects of commercial rock phosphate and Phosphate Solubilizing Bacteria (PSB) on oil palm growth and nutrient utilisation in oil palm nurseries.

## MATERIALS AND METHODS

### Production Process of Compost

Various organic materials, such as decanter cake, cocoa shells, and coffee grounds were selected for the composting process. All Cosmos Industries Sdn. Bhd. supplied these organic components, which are commonly used in organic fertilizer production. A compost column was filled with twenty metric tonnes of the different kinds of organic materials that were chosen and mixed well. To speed up the breakdown process, a solution of microorganisms that break down was sprayed onto the thoroughly combined organic materials. The entire composting process took 14 days, with each compost column being stirred once a day by a self-propelled compost turner. Based on the wet weight, 10% of the two PSB bacterial inoculants ($1 \times 10^{8\text{-}9}$ cfu/ml) were uniformly sprayed into the compost on day 14.

### Quality Checking of Compost Samples

We gathered compost samples from All Cosmos Industries Sdn. Bhd. As a result, the generated compost's quality was examined. The moisture content, particle size, pH, carbon to nitrogen ratio, total N, P, K, Mg, and B content, as well as total organic materials, were all measured for each sample. Using a Shimadzu moisture analyser M0C63u model, the moisture content was determined. The product's moisture content must be less than 8% (Richard et al., 2002). The compost's particle size was measured using a 3.2 mm perforated plate sieve. The acidity or alkalinity of a substance is indicated by its pH value.

In a sterile beaker, 5 g of the sample were weighed. The sample was then stirred with a glass rod and 50 mL of distilled water added. The pH metre was calibrated using three buffer solutions with pH values of 4.0, 7.0, and 10.0 prior to pH testing. The material was placed in a suspension of distilled water (5 g/50 ml) and left for two hours to measure its pH. Using the dry oxidation or combustion method and a CHNS analyser, the total organic carbon and total nitrogen were calculated. Samples were crushed, weighed, and freeze-dried before was placed in a tin capsule containing vanadium pentoxide and burned in a reactor at 1000°C. After the sample and container melted together, the tin sped up a strong reaction (flash combustion) in an oxygen-improved environment for a limited period (Ge et al., 2022).

Total P, K, Mg, and B contents were determined using the inductively coupled plasma atomic emission spectrometry method in accordance with (ICP-AES). A porcelain crucible containing one gramme of the material was filled, heated to 500°C for two hours, and then removed. After adding 3 mL of $HNO_3$, the sample was roasted on a hot plate at 100°C until it was dry. After being reinserted into the muffle furnace, the crucible was muffled for a further hour at 500°C. After allowing the crucible to cool, 10 mL of HCl were added.

The material was transferred and then diluted in a 50 mL volumetric flask using deionized water. AOAC Method 985.01 analysis was performed on the sample ash solution that was collected (Hemidat et al., 2018).

The total organic matter was determined using the loss-on-ignition method (Chai et al., 2013). A 2 g oven-dried sample was weighed and placed it in a silica crucible and heated it to 550°C for 24 hours in a muffle furnace. The weight difference between the initial sample, the burned sample, and the leftover ash was used to quantify the amount of organic matter.

### Survival of PSB Cultures in the Compost

To create compost, two PSB cultures were introduced ($1 \times 10^{8\text{-}9}$ cfu/ml for each strain). The plate count method was used to determine the total number of PSB in the sample. The term "total viable count" describes the entire number of colonies. The 95 ml of sterile phosphate buffer saline (PBS) was placed within a conical flask, into which 10 g of fertiliser samples were weighed and dissolved. After that, the conical flask was put in a shaking incubator set to 200 rpm for ten to fifteen minutes. Sterile distilled water was used for serial dilution up to a dilution factor of $10^{-9}$. For the purpose of phosphate solubilization bacteria growing media, Pikovskaya agar medium was produced. The glass hockey stick was then used to distribute the suspension across the agar plate surface. After that, plates were incubated at room temperature 27°C for up to 7 days, the colony forming units (CFU) were counted (Chung et al., 2005).

### Field Study

The Tenera oil palm of three months old were planted in the field of Ladang 10, Faculty of Agriculture with different treatment (Table 1).

The size and shape of the oil palm seedlings chosen as the experimental samples were comparable. A thin layer of soil was placed inside 20 × 20 black polybags housing oil palm seedlings. Then, each group - aside from the control group—received 100 g of the matching therapy (Figure 1). Finally, soils were placed into the polybags (Chao, 2018). Vegetative measures, chlorophyll readings, period yield records, and the phosphorus content of leaves and rachis were all observed and documented as parameters for comparison across all treatments. A total of 120 oil palm seedlings were arranged in a Randomized Complete Block Design (RCBD), with five replications each treatment and six oil palm samples each replication, as shown in Figure 2.

Table 1
*Treatment for oil palm nursery*

| Treatment | Detail of fertilizer application |
| --- | --- |
| T1 | Control, no fertilizer |
| T2 | Moroccan Rock Phosphate (MRP) |
| T3 | 30% Compost + Phosphate Solubilizing Bacteria (PSB) + 70% Moroccan Rock Phosphate (MRP) |
| T4 | Compost + Phosphate Solubilizing Bacteria (PSB) |

*Figure 1*. Oil palm seedlings in black polybags

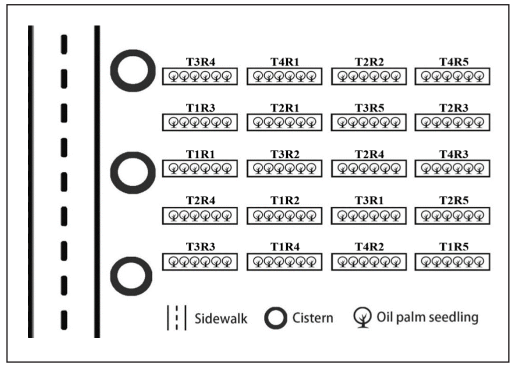


*Figure 2*. Experimental design

## Data Analysis

Five replicates of each treatment were used in the analysis, which followed the RCBD experimental design. The Prism 9 statistical analysis system and the analysis of repeated measures ANOVA were used to analyse the field data. There were notable variations ($P<0.05$) between the treatments.

# RESULTS

## Formulation of Compost

The composition of the compost used in this investigation is presented in Table 2. Analysis of the compost sample revealed that the concentrations of all six heavy metals were below the detection limits of the instrument. Additionally, no *Salmonella*, *Escherichia coli*, or other pathogens were detected in the compost.

Table 2
*Physicochemical and microbiological analysis of compost*

| Test Parameter | Unit | Result |
|---|---|---|
| pH | - | 6.7 |
| Electrical Conductivity | mS/cm | 3.16 |
| C/N ratio | - | 15.01 |
| Organic Matter | % | 66.7 |
| Moisture Content | % | 40.1 |
| Total Nitrogen, N | % | 2.4 |
| Phosphorus, P | % | 0.2 |
| Potassium, K | % | 1.1 |
| Calcium, Ca | % | 0.6 |
| Magnesium, Mg | % | 0.3 |
| Nickel, Ni | ppm | N.D (<0.01) |
| Chromium, Cr | ppm | N.D (<0.01) |
| Lead, Pb | ppm | N.D (<0.01) |
| Cadmium, Cd | ppm | N.D (<0.01) |
| Arsenic, As | ppm | N.D (<0.01) |
| Mercury, Hg | ppm | N.D (<0.01) |
| *Escherichia coli* | MPN/g | N.D (<0.01) |
| *Salmonella* | - | absent |

*Note*. N.D mean not detected

## Survival Test of Two PSB in Compost

In this study, the PSB inoculant was evenly sprayed on the compost. The results for PSB populations in the compost, which were reconfirmed, are shown in Table 3.

Table 3
*PSB population in 1 g of compost*

| Phosphate Solubilizing Bacteria | CFU/g |
|---|---|
| *Bacillus marisflavi* | $2 \times 10^9$ |
| *Bacillus aryabhattai* | $1 \times 10^8$ |

### The Effectiveness of Phosphate Solubilizing Bacteria on Oil Palm at the Nursery

Three months after planting, the data was first gathered (3 MAP). Thereafter, planting took place for six months (6 MAP) and eight months (8 MAP). An appendix contains a summary of all the data. Three-month-old oil palm plants showed no discernible variations in the majority of the characteristics. This is explained by the fact that oil palm seedlings with comparable sizes and shapes were chosen for the experiment.

Figure 3 describes the size of the oil palm at three, six, and eight months old in relation to its vegetative growth. When the oil palm was three months old, there were no appreciable differences between the four treatments. Nevertheless, variations in growth are only discernible between the ages of 6 and 8 months.

At 6 months, oil palm size in T2, T3, and T4 was significantly greater than in the control group (T1). Notably, T3 outperformed both T2 and T4, suggesting that the treatment with 30% compost + Phosphate Solubilizing Bacteria (PSB) + 70% Moroccan Rock Phosphate (MRP) is more effective in promoting growth. A similar pattern was observed at 8 months, although T2 did not show a significant difference compared to T1.

Significant differences were observed when comparing the heights of the first and third fronds in most plot samples, with the exception of the first and third frond heights at three months, as shown in Figures 4 and 5. Regarding the height of the first frond at 6 months, T2, T3, and T4 demonstrated significantly better performance compared to the control group (T1). Interestingly, T3 outperformed T2 and T4, indicating that the treatment with 30% compost + Phosphate Solubilizing Bacteria (PSB) + 70% Moroccan Rock Phosphate (MRP) is more effective in these treatments. Similarly, at 8 months, the same trend was observed, though T2 did not show a significant difference compared to T1. Furthermore, the height of the third frond followed a similar trend in group mean scores to that of the first frond at both six and eight months.

To conclude, the oil palms in the treatment without PSB-mixed compost, aged 6 and 8 months, have shorter fronds compared to those in the treatment with it. Additionally, the treatment with MRP shows a noteworthy distinction from the group without MRP in oil palms of the same ages.

Figure 6 shows the results of chlorophyll content in oil palm leaves. At six months, a comparison of the group mean scores revealed that T1, T3, and T4 demonstrated significantly higher efficacy than T2. The chlorophyll content in the 8-month-old oil palm leaves followed the same trend. In summary, the group with only MRP showed lower values compared to the others.

Figures 7 and 8 displayed the width and thickness of the third frond data analysis for oil palm. The thickness of the oil palm third frond in both six months and eight months were found to the comparison of mean scores among groups T1, T3, and T4 showed significantly higher efficacy than T2. The six months shows the extreme significant difference which

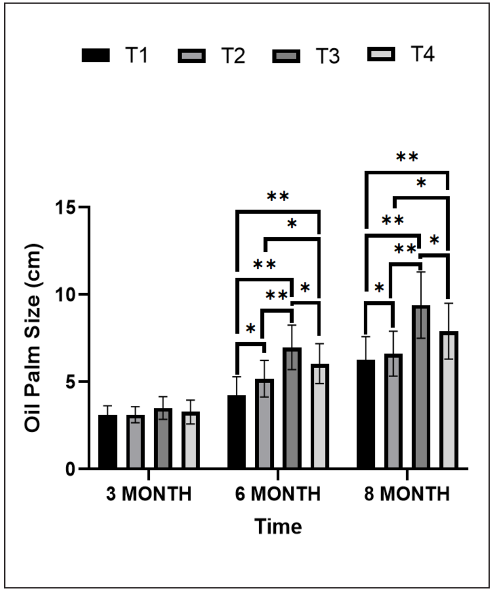


*Figure 3*. The size of 3 months, 6 months, 8 months old oil palm seedling

*Note*. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

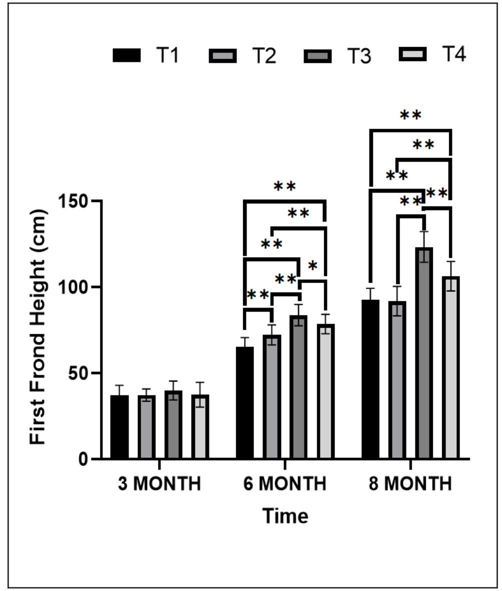


*Figure 4*. The height of 3 months, 6 months, 8 months old oil palm first frond

*Note*. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

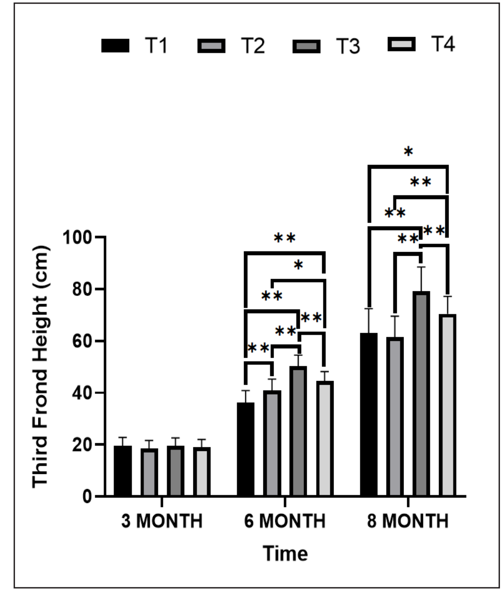


*Figure 5*. The height of 3 months, 6 months, 8 months old oil palm third frond

*Note*. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

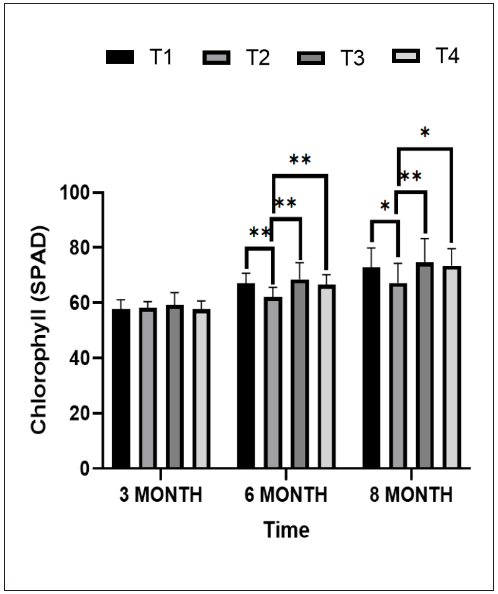


*Figure 6*. The chlorophyll content in 3 months, 6 months, 8 months old oil palm leaves

*Note*. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm by SPAD502. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

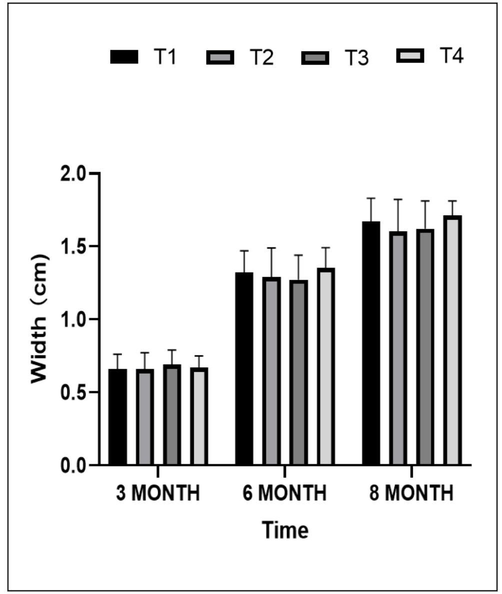


*Figure 7*. The width of 3 months, 6 months, 8 months old oil palm third frond

*Note*. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

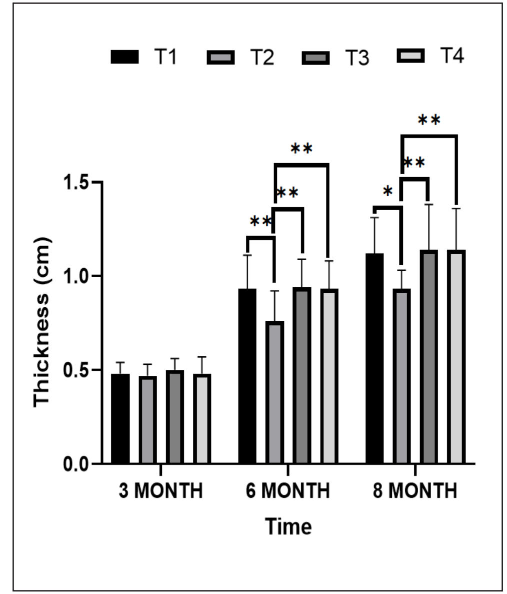


*Figure 8*. The thickness of 3 months, 6 months, 8 months old oil palm third frond

*Note*. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments the p-value less than 0.01, however the thickness of the eighth-month oil palm third frond only exhibited a p-value less than 0.05. The width of the oil palm's third frond, however, did not provide a noteworthy outcome.

The significant increase in frond thickness in treatments with RP and PSB (T1, T3, T4) is indicative of better plant health, enhanced nutrient availability, and overall improved growth, especially at the early stages of development (six months). This supports the hypothesis that frond thickness is a key indicator of the plant response to fertilizer treatments, which can influence its long-term productivity and oil yield. The lack of significant results for frond width further suggests that thickness is a more reliable measure of plant efficacy in this context.

Only after six months did the quantity of oil palm leaf significantly different in Figure 9. The group mean score comparison results with evident differences were the effectiveness of T2, T3, and T4 was significantly greater than that of control group (T1). T3 which possesses PSB and MRP showed significantly higher efficacy than T2 and T4 which only possess single ingredient. There are observed significant differences among all the treatments in 6 months old oil palm trees.

Eight-month-old oil palm trees were divided into rachises and leaves after eight months of growth. After drying, these plant portions were transported to the laboratory for a study of their phosphorus content. The findings of the laboratory analysis are displayed in Figure 10. There were no notable variations found between the other treatment groups, with the exception of the phosphorus content discrepancy between the rachises of T1 and T3. Variations in phosphorus absorption levels could be the cause of the notable differential in phosphorus concentration between T3 and T1 in the rachises.

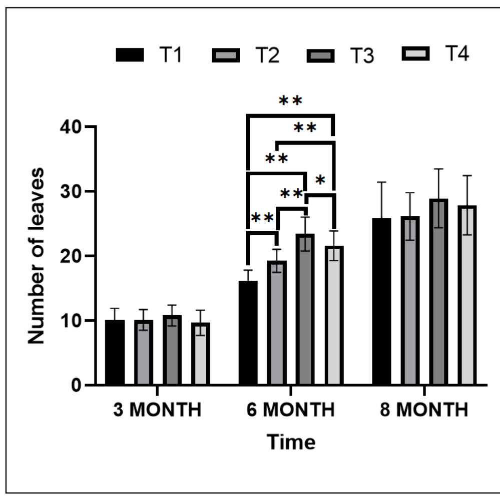


*Figure 9*. The number of 3 months, 6 months, 8 months old oil palm leaves

*Note*. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

## DISCUSSION

### Survival Test of Two PSB in Compost

Table 3 shows that there were two PSBs in 1 g of compost, with respective populations of $2 \times 10^9$ and $1 \times 10^8$. It demonstrates that two

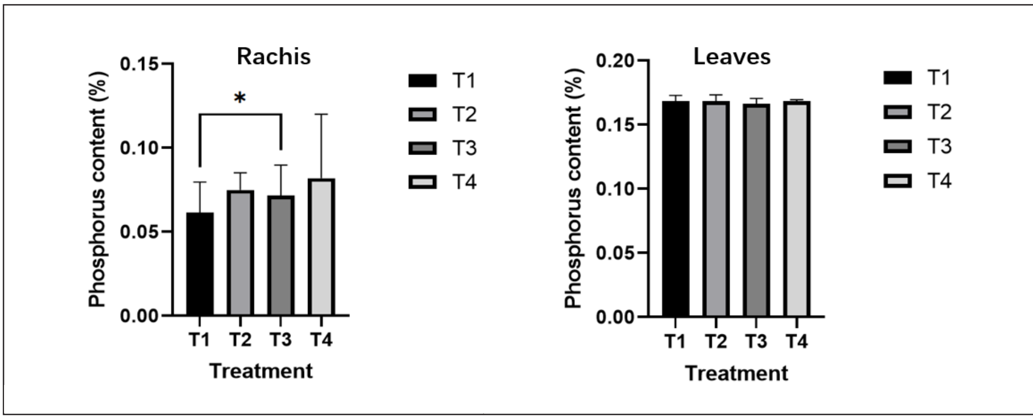


*Figure 10*. The phosphorus content of 8 months old oil palm rachis and leaves *Note*. Analysis data was from the dried rachis and leaves of 8 months old oil palm. The repeated measured one-way ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (p<0.05). The lack of zigzag line represents no significant difference between treatments

PSBs could live and procreate in compost. This suggests that composting offers the PSBs the perfect habitat for growth and proliferation. Additionally, this research might provide insightful information for managing and optimising composting operations. Bacteria that solubilize phosphates can break down and make use of phosphorus molecules. They have the ability to change organic phosphorus into inorganic forms, increasing compost's phosphorus concentration. This gives compost a higher nutritional content and gives plants the phosphorous minerals they need. Understanding these microbes' functions in their natural habitats and how they interact with other creatures depends on this research (Timofeeva et al., 2022). PSB are essential environmental microbes that have strong ecological flexibility in their natural environments and a wide range of potential uses in the fields of biological control and environmental restoration. They also aid in the breakdown of organic contaminants, the synthesis of antibiotics, and the decomposition of organic materials (Numan et al., 2018). Compost maturation can be accelerated by modifying the growth and reproduction of phosphate-solubilizing bacteria through manipulation of composting parameters, including temperature, moisture, and ventilation.

**Field Data Analysis**

The oil palm seedlings treated with MRP and PSB compost in our experiment showed noticeably quicker growth rates and outperformed the control group. Malhotra et al. (2018) claimed the ability of phosphorus to promote early branching and budding, accelerate the growth of plant stems and roots, and enhance seed germination. Oil palms absorb phosphorus most quickly in their seedling stage, therefore a phosphorus deficit at this time can negatively impact the oil palm's ability to expand in the future (Ajeng et al., 2020; Lovelock et al., 2006). According to Abidemi et al. (2006), most seedlings' sizes were considerably enlarged when they were given phosphorus biofertilizer. An essential nutrient for plant growth is phosphorus. Khattab et al. (2019) showed Plants that are poor in phosphorus display signs like stunted growth, short stature, delayed production of flower buds, and increased fruit and blossom drop. Variations in the absorption of total phosphorus could affect the height of the fronds. The results of Kumar et al. (2020) also demonstrated that seed germination, as well as other plant parameters such as height and weight, significantly increased in plants treated with PSB. Additionally, the frond height of the control group, which includes MRP and PSB at 6 and 8 months, shows a significant difference compared to the other groups. This indicates that the combination of MRP and PSB positively impacts frond height in oil palms. The results also show that treatments with MRP and PSB significantly improved chlorophyll content, frond thickness and highest leaf quantity in oil palm at early stages of development, outperforming other treatments. These findings align with research by Etesami (2019), which highlighted the positive impact of PSB on nutrient availability and plant growth.

Plants with phosphorus deficit may develop without branches and shed their leaves early. Heppell et al. (2015) proved older leaves close to the base of the stem exhibit the first signs of phosphorus deficit, which then spread upward. This could help to explain the notable variations in all treatments that were seen in oil palm plants that were six months old. Plants typically contain between 0.1% and 0.5% of phosphorus in their dry weight. The movement of growth centres inside a plant and metabolic activities are intimately linked to the dispersion of phosphorus. Phosphorus is predominantly found in young shoots and root tips during the vegetative growth phase, which contributes to the plant's pronounced apical dominance (Gaiero et al., 2020; Viegas et al., 2021). This could account for why leaves have more phosphorus than rachises. Rachises sustain leaves, flowers, and fruits inside a defined area in addition to acting as a conduit for nutrients and water. Stems can occasionally carry out photosynthesis, store nutrients, and aid in reproduction. As a result, the rachises can show differences in their amounts of phosphorus absorption.

## CONCLUSION

By phosphate solubilization, phosphate-solubilizing bacteria increase the amount of phosphorus that is available in the soil. They greatly raise the soil's total phosphorus levels when paired with MRP. Phosphate solubilizing bacteria (PSB) and Morocco rock phosphate (MRP) work in concert to promote oil palm seedling growth, which improves growth rates and quality. In addition, it improves soil conditions and increases oil palms' ability to absorb and utilise phosphorus. As a result, using MRP and PSB together while planting oil palms works well to improve the yield and quality of the oil palm. *Bacillus* *marsflavi* and *Bacillus aryabhattai* are the two PSB cultures which showed a high rate of survival. The benefits in terms of leaf count, oil palm size, and frond height are enhanced effectively when Morocco rock phosphate (MRP) and phosphate solubilizing bacteria (PSB) are combined. Oil palm seedlings have been shown to benefit from commercial production.

## ACKNOWLEDGEMENTS

We thank the All Cosmos Industries Sdn. Bhd. for providing funding support for this study.

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