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DUAL APPLICATION OF PALM OIL MILL EFFLUENT (POME) AND BENEFICIAL MICROBE ISOLATES FOR *Oryza sativa* GROWTH UNDER GLASS HOUSE CONDITION

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ABSTRACT

Oryza sativa *is the primary staple food in Asia. However, the high demand for rice leads to the overuse of chemical fertilisers and requires an alternative. Thus, this study aimed to investigate the impact of the combination of palm oil mill effluent (POME) and plant growth-promoting rhizobacteria (PGPR) on the growth of paddy cultivation. PGPR was isolated from lemongrass-cultivated soil and tested on nitrogen fixation, phosphate solubilisation and potassium solubilisation plates. POME, a byproduct from the palm oil milling process, has been combined with PGPR in powder form with different ratio combinations. The combination of POME: PGPR treatments was used in the following ratios: T0 = (0 g: 0 g), T1 = (5 g: 0 g), T2 = (0 g: 5 g), T3 = (5 g: 10 g) and T4 = (10 g: 5 g) and applied it alternately once in a week for 30 days in glasshouse condition. Treatment T4 produced the highest result in rice plant height (42.6 cm), rice plant dry weight (0.777 g) and root dry weight (0.700 g). Specifically, these values represent a 4.39 fold increase in plant height, 1.17 fold increase in plant dry weight and 3.70 fold increase in root dry weight compared to control (T0). These findings show that the combination of POME and PGPR may be a potential organic biofertiliser as an alternative to chemical fertilisers.*

Keywords: biofertiliser, *Oryza sativa*, palm oil mill effluent, plant growth-promoting rhizobacteria.

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INTRODUCTION

Rice is the primary food source in Malaysia and the government prioritises it. However, unequal distribution of fertiliser is contributing to the decline in paddy production despite extensive cultivation. Previous study show that farmers are unable to afford costly fertilisers, pesticides and herbicides, leading to reduced productivity. Othman et al. (2022) confirm that rice is the primary staple food in many developing countries, with approximately 90% of the world's rice production concentrated in 15 countries. Malaysia's rice cultivation area in Southeast Asia is approximately 0.70 million hectares, the smallest in the region. According to Dohlman et al. (2022), Indonesia, Vietnam, and Thailand produced 11.50, 7.54 and 10.83 million hectares of rice, respectively.

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Paddy crops require essential nutrients to flourish and achieve high yields. Chemical fertilisers gradually diminish soil fertility, leading to groundwater pollution and reduced soil productivity, which negatively impacts rice cultivation. Organic fertilisers, while contributing to soil health, are insufficient in providing necessary nutrients for growth. Saputro and Kurniawati (2024), found that there are intricate relationships among soil, plants and microbes that regulate the movement of micronutrients, leading to enhanced soil fertility. Several studies indicate that biofertilisers play a crucial role in integrated nutrient management and the promotion of sustainable agriculture.

In general, many countries rely on palm oil products, which will produce palm oil mill effluent (POME), a form of waste. Thus, managing POME may enhance the quality of water, air and the environment. According to Wu (2009), it is recommended to utilise POME as a cost-effective organic fertiliser as an alternative to the excessive use of chemical fertilisers. POME enhances soil structure and contains essential plant-growth nutrients such as phosphorus, potassium, magnesium and calcium, thereby enhancing root health, soil productivity and food yield. It is non-toxic due to its chemical-free composition. Also, beneficial bacteria are crucial for the well-being and growth of plants. Plant rhizospheres Harbour plant growth-promoting rhizobacteria (PGPR), which engage with root crops to influence their growth and yield. According to Andy et al. (2020), PGPR refers to biofertilisers, biocontrol chemicals, or microbial inoculants. Rhizobia are beneficial microorganisms that facilitate the growth and flourishing of plants. Nazma et al. (2023) highlighted that investigating advantageous PGPR as the symbionts in the symbiotic relationship in rhizospheres of plants that possess the ability to impede infections and enhance plant growth is a more convenient, expeditious and cost-effective approach. This study aims to investigate the effects of POME and beneficial PGPR on the growth of *Oryza sativa* as it is a cost-effective and environmentally friendly alternative to chemical fertilisers.

MATERIALS AND METHODS

Isolation of Beneficial Microbes

Approximately 5 g of soil sample obtained from lemongrass cultivation was placed into a Falcon tube, to which 10 mL of distilled water was added (Othman et al., 2022). Three replicates were prepared. The samples were left undisturbed for 24 hr. Bacterial isolation was performed using nutrient agar plates.

Characterisation of Plant Growth-Promoting Bacteria

Nitrogen fixation activity. The soil sample was agitated on a nutrient agar medium using a glass spreader, and then placed in an incubator for 24 hr. A nitrogen fixation test was done to observe the nitrogen fixation activity from the isolated PGPR. This activity required to prepare nitrogen-free malate plate with the following composition, per litre of distilled water containing K_2HPO_4 (5.00 g), NaCl (0.50 g), $MgSO_4{\cdot}7H_2O$ (0.10 g), $CaCl_2{\cdot}2H_2O$ (0.20 g), micronutrient solution (0.02 g), bromothymol blue solution (2 mL), FeEDTA solution (2 mL), KOH (4.50 g), agar (15.00 g). The solution was adjusted to 6.5 of pH and autoclaved in 20 min with 120°C. The solution then was poured into four replicates of petri dishes and set aside until it cool and harden. The pure culture of PGPR was inoculated onto the nitrogen-free malate plate, using the streak plate method under sterilised condition. The plates were then incubated for 24 hr, at 30°C in the incubator.

Phosphate solubilising activity. Phosphate solubilising activity was tested to ensure that the PGPR isolated can provide phosphate to the plant. This activity was inoculated on National Botanical Research Institute's (NBRIP) phosphate growth medium, with the composition prepared per litre of distilled water – glucose (10.00 g), $(NH_4)_2SO_4$ (0.10 g), KCl (0.20 g), $Ca_3(PO_4)_2$ (5.00 g), $MgCl{\cdot}6H_2O$ (5.00 g), $MgSO_4{\cdot}7H_2O$ (0.25 g) and agar (15.00 g). The solution was homogenised and autoclaved. The solution was then standardised and sterilised using an autoclave. The selected bacteria were introduced on the phosphate plate and allowed to incubate for 48 hr.

Potassium solubilisation. The potassium solubilising test was conducted by using Alexandrov medium agar. Mica powder is provided as insoluble potassium. The autoclaved media was then poured onto petri dishes and set aside to harden. Then, the selected PGPR was placed on the potassium plates and cultured for 48 hr.

Indole compound content. A colourimetric technique was used to quantify the synthesis of indole-3-acetic acid (IAA) in each isolate. The Van Urk Salkowski reagent was employed for this purpose, following Salkowski's method as originally described by Ehmann in 1977. A total of 1.0 mL of PGPR culture was added to 100 mL of nutrient broth, and the solution was enhanced with 5.0 mL of L-tryptophan. The solution was then incubated at 28°C. After one day of incubation, 1.5 mL of PGPR culture was added into the microcentrifuge tube, and then centrifuged at 7,000x g for 7 min. About 1.0 mL of the supernatant was transferred carefully into a cuvette. To detect the indole component's presence, four mL of Salkowski reagent was added, and a pink colouration was observed within 20–30 min. The concentration of the indole compound was determined using a spectrophotometer.

Encapsulation of Plant Growth-Promoting Rhizobacteria

About 13 g of nutrient broth powder was mixed with 1 L of distilled water. The solution was thoroughly mixed using a stirrer and vigorously shaken. Then, the solution was transferred into a bottle and autoclaved at 121ºC for 2 hr. After that, the pure PGPR culture was introduced into a nutritional broth using a sterilised inoculating loop in a sterilised laminar airflow. The PGPR were cultured for 24 hr at 30ºC in the incubator. Then 1 g of sodium alginate, and 50 mL of PGPR culture with 100 mL of sterilised distilled water were mixed in a Schott bottle. Then, a calcium chloride solution was prepared by mixing 30 g of $CaCl_2$ with 100 mL of distilled water. The sodium alginate solution was injected into the calcium chloride solution to catalyse its transformation into gel beads. After filtration, the gel beads were positioned on top of a tray. A layer of encapsulated beads was thinly spread on a tray and placed in an incubator set at 121ºC. The PGPR was allowed to dry for 3–4 days. Subsequently, the desiccated beads were pulverised into a fine powder using a grinding apparatus and a mortar and pestle. The procedure was repeated until the gel beads achieved a refined texture.

Preparation of POME in Powder Form

The POME powder was prepared by first collecting the liquid effluent and allowing it to undergo sedimentation for 48 hr to reduce the solid content. This method was adjusted based on Ismail et al. (2014). The supernatant was then placed in aluminium trays in batches and subjected to drying at 100°C for 24 hr in an oven. The dried POME flakes obtained were manually ground using a commercial grinder to achieve a fine powder that could pass through a 100 micron sieve. This powder was stored in air-tight containers.

Dual Application of PGPR and POME on Rice Cultivation

POME was obtained from a palm oil mill in Perak, Malaysia. Rice seeds were obtained from Batu Merah Agrofarm in Perak. Rice seedlings come in packets of 50 seeds. The variety used was MRIA1. The seedlings were then soaked in water for two days, which speeds up germination and boosts the percentage of seeds that germinate successfully. The moist tissue approach was used to germinate the seeds, which took around a week to see the results. After the seeds germinated, the uniformed size seedlings were selected to be transferred into 16′ x 16′ pots. The rice cultivation was monitored for 30 days after sowing (DAS) under nursery conditions. The PGPR and POME were alternately applied once a week during the cultivation period. Plants were watered twice a day. The design of this experiment was a complete randomised design (CRD), with three replications, as the plant requirements are uniform. The treatments involved; T0 – control, T1 – 5 g of POME, T2 – 5 g of PGPR, T3 – 5 g of POME + 10 g of PGPR and T4 – 10 g of POME and 5 g of PGPR.

Vegetative Growth Measurements

Growth parameters of rice plants were taken during the cultivation period. Plant height, fresh weight, dry weight and root length were measured using tape and digital weighing scales, respectively. The chlorophyll content was measured using a SPAD meter (SPAD-502 plus) (Konica Minolta, Japan).

Statistical Data Analysis

All the collected data was analysed using SPSS (Version 2021). At a confidence level of 95%, the differential of the data was examined using one-way ANOVA, followed by Tukey's B test.

RESULTS AND DISCUSSION

Isolation of Bacterial Isolates as Plant Growth-Promoter

Four isolates of PGPR were successfully isolated from lemongrass soil and roots and cultured in triplicate on nutrient agar plates (*Figure 1*). Prior investigations by Halimursyadah et al. (2022), have shown that distinct colonies of rhizobacteria can be isolated and differentiated by their varied densities (e.g., hard and dry, butter, slime or dull). In this study, the isolates showed a slimy appearance, milky white and accompanied by an unpleasant odour. This observation is consistent with the findings of Silva et al. (2021), where PGPR has been isolated from chromium-contaminated, noting similar characteristics. These PGPR can fix nitrogen and enhance nutrients in maize, highlighting the potential of these bacteria to thrive under extreme conditions. The milky white and slimy appearance in this study may suggest that these PGPR consist of similar adaptive traits that enable them to support plant growth. This similarity underscores the potential of the isolates from lemongrass soil to contribute as an effective biofertiliser.

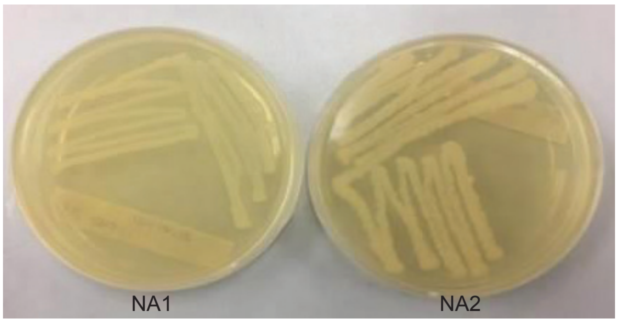


Figure 1. Two plates of microbial isolates were obtained from the soil in which lemongrass was planted, namely NA1 and NA2.

Characterisation of Plant Growth-Promoting Rhizobacteria

The positive results were achieved from the nitrogen fixation test, phosphate solubilising test and potassium solubilising test for the PGPR isolates derived from the lemongrass soil and lemongrass root (*Figure 2*). After 24 hr of incubation, the PGPR activity transformed the nitrogen-free malate agar from green to blue, validating its characterisation. The outcome is indicative of nitrogen fixation which transformed nitrogen-free malate agar from green to blue which confirms the ability of these isolates to convert nitrogen in the atmosphere into ammonia, making it available to plants (Yilihamu et al., 2020). The findings are in concurrence with (Wang et al., 2020), who reported similar nitrogen-fixing capabilities in a variety of soil bacteria, by emphasising the significance of these microorganisms for long-term and sustainable agriculture.

The phosphate solubilisation test showed the presence of a halo zone around the colonies on the NBRIP plate which indicates effective phosphate solubilisation. Purwaningsih et al. (2022) found that different PGPR isolates may exhibit varying degrees of phosphate solubilisation efficiency. Some of the isolates produced large zones of solubilisation. This variability highlights the potential for selecting specific strains that can optimise phosphate availability in soils because this is crucial for root growth. Furthermore, behind phosphate solubilisation, lies a mechanism where the production of organic acids can cause low soil pH, which facilitates the release of bound phosphates (Halimursyadah et al. 2022). This is aligned with the study by Othman et al., 2022, who demonstrated that *Acinetobacter* sp. effectively solubilised phosphate through organic acid production which increases the phosphate availability in soil.

The potassium solubilisation activity of PGPR was evidenced by the formation of transparent zones (*Figure 2c*) which surround the colonies of isolates on Aleksandrive media, indicative of positive and effective potassium solubilisation. Potassium solubilising microorganisms play an important role in enhancing plant growth and mitigating the environmental effects in agriculture practice as highlighted by Ashfaq et al. (2020). Similar to these findings, previous studies have shown that potassium solubilising bacteria, which was isolated from the rhizosphere, may produce soluble zones on the silicate media, with diameters ranging from 0.65–1.50 cm, depending on the specific isolates (Sood et al., 2023). This further supports that potassium solubilising bacteria can leverage the potassium availability in soil and can encourage plant development (Joshi et al., 2023)

Moreover, a qualitative IAA production test was conducted by using the Salkowski reagent. The IAA test results indicated that indole compounds have been successfully exhibited on the test, by investigating the transformation of solution, from yellow pale to pink colour (*Figure 2d*). This result is consistent with Adhyaningtas et al. (2023) who reported that IAA concentrations in a specific range, suggest that the isolates from lemongrass may have varying IAA production capabilities. However, the findings were contradictory to the previous study in which the IAA concentrations, in the range of 21.66–83.38 ppm created by rhizobacteria were indicated by a light-yellow colony (Halimursyadah et al., 2022).

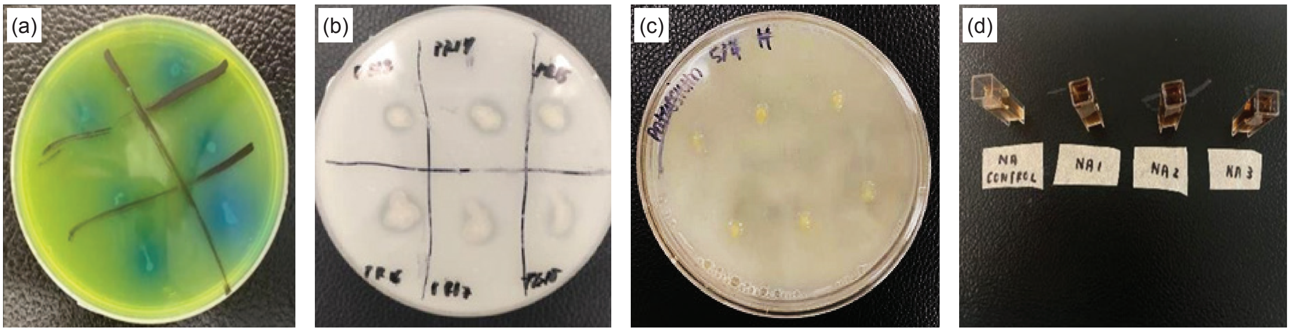


Figure 2. The compilation of plant growth-promoting rhizobacteria characteristics tests for positive results of (a) nitrogen fixation, (b) phosphate solubilisation, (c) potassium solubilisation and (d) IAA production.

Effect of POME and PGPR Combination on Plant Height

The results of plant height data indicate that Treatment T4, consisting of a 10 g: 5 g mixture of POME and PGPR, produced the most substantial plant growth after a four-week period of observation at $p < 0.05$ (*Figure 3*). The weekly measurements were 9.700, 17.800, 4.367 and 42.600 cm respectively. Statistically significant analysis confirmed significant differences were identified across the five treatment groups. Treatment T4 resulted in the highest plant height, with a 4.39 fold increase compared to the control (T0). This indicates synergistic effects of POME and PGPR can lead to enhanced plant growth compared to treatment lacking in this combination. The application of 10 g POME introduces a rice source of organic matter and essential nutrients such as potassium, phosphorus and magnesium, which are important for plant height. Palm oil mill effluent is known for its high nutrients contents, which support plant growth, a finding in concurrence with Alam et al. (2022) who reported improved growth and performance in Brazilian spinach (*Alternanthera sissoo*) following POME application. In addition to POME, the inclusion of PGPR enhances the nutrient profile by introducing beneficial microbes that can fix nitrogen, solubilise phosphate and potassium and produce IAA (Othman et al., 2022). This aligns with the findings by Ray et al. (2024), which demonstrated the synergistic effects of PGPR and organic matter which promoted spinach growth. The ratio of 10 g of POME to 5 g of PGPR appears to be optimal for PGPR to effectively colonise plant roots and enhance nutrients and growth. Puri et al. (2020) also found that the presence of PGPR can convert unavailable form of organic matter into available forms, as observed in Pinaceae trees under nutrient-limited conditions. Similarly, the findings of this study show that the 10 g: 5 g of POME and PGPR ratio provides a balanced nutrient supply from POME while ensuring sufficient microbial inoculants to stimulate plant growth effectively.

Effect of POME and PGPR Combination on Chlorophyll Content

The mean value of chlorophyll content was similar to all treatments at $p < 0.05$ (*Figure 4*). It is important to note that although the combination of POME and PGPR has improved plant height, it does not necessarily lead to an increase in chlorophyll content. Ghasemi et al. (2020) found that, the presence of nutrients alone does not guarantee increase of chlorophyll content as other physiological processes may contribute to the increment.

Effect of POME and PGPR Combination on Plant Fresh Weight and Dry Weight

The fresh and dry weight of rice plants across the different treatments indicate an obvious trend, with Treatment T4 (10 g POME: 5 g PGPR) obtaining the highest plant fresh and dry weight. The mean value of plant fresh weight was not significantly different from the other treatments ($p > 0.05$) (*Table 1*). In contrast, Treatment T4 produced the highest dry

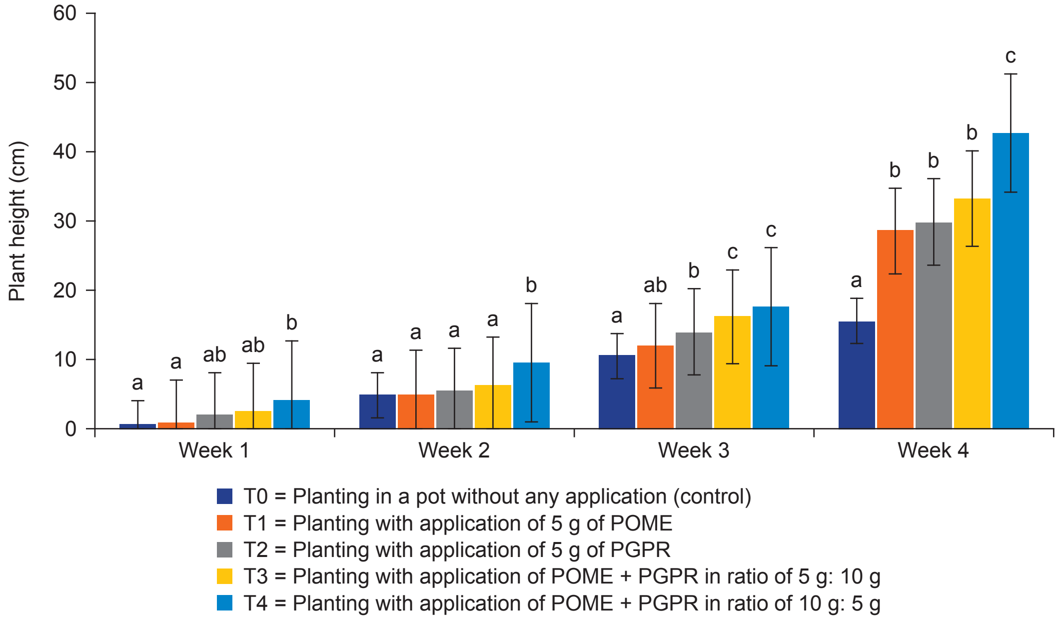
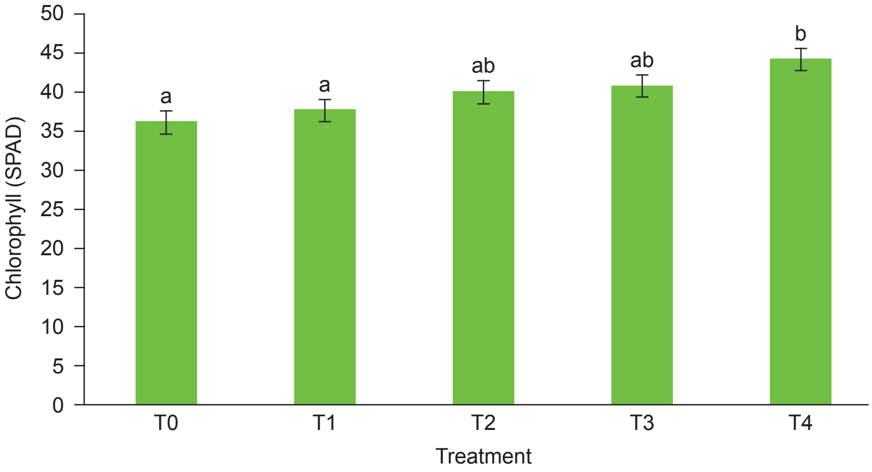


Figure 3. Plant height measurements of different treatments application based on weekly progress at p *< 0.05.*



Note: T0 - control, T1 - Application of 5 g POME; T2 - Application of 5 g PGPR; T3 - Application of POME + PGPR in ratio of 5 g: 10 g; T4 - Application of POME + PGPR in ratio of 10 g: 5 g.

Figure 4. Chlorophyll content of all treatments rice plant differences at 95% of significance level.

TABLE 1. THE MEAN VALUE OF PLANT FRESH WEIGHT AND PLANT DRY WEIGHT OBSERVATION AFTER FOUR WEEKS AT 95% SIGNIFICANCE LEVEL

Growth Parameter	Treatment T0	Treatment T1	Treatment T2	Treatment T3	Treatment T4
Fresh weight (g)	0.577 ± 0.07a	1.343 ± 0.55ab	1.890 ± 0.33ab	2.713 ± 0.35bc	3.933 ± 1.12c
Dry weight (g)	0.667 ± 0.08a	0.210 ± 0.04b	0.343 ± 0.05c	0.470 ± 0.06d	0.777 ± 0.09e

Note: T0 - control; T1 - Application of 5 g POME; T2 - Application of 5 g PGPR; T3 - Application of POME + PGPR in ratio of 5 g: 10 g; T4 - Application of POME + PGPR in ratio of 10 g: 5 g.

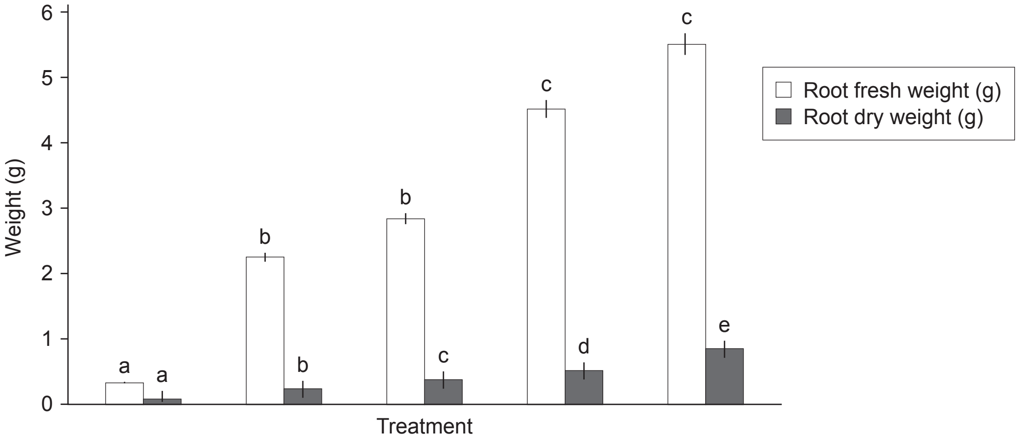
weight at 0.777 g ($p < 0.05$) compared to the other treatments, with a 1.17 fold increase compared to the control (T0). The observed increment in biomass, especially dry weight can be attributed to the synergistic effects of nutrient management in POME and the application of PGPR. This is consistent with findings from Midya et al. (2021) who found that integrated nutrient management, involving both microbial inoculants and organic amendments has led to improved biomass production which is dry weight in the lower rice field of Indo-Gangetic Plain, India while also increasing the soil fertility. The enhancement of biomass in Treatment T4 is most likely resulting from the improved nutrient availability and improved soil structure due to organic matter content in POME.

Effect of POME and PGPR Combination on Root Fresh Weight and Root Dry Weight

The combination of POME and PGPR significantly enhanced root dry weight only (*Figure 5*). For root fresh weight, Treatment T4 showed similar results to Treatment T3 and both were higher than other treatments (*Figure 5*). In terms of root dry weight, Treatment T4 recorded the highest dry weight (0.700 g), with a 3.70 fold increase compared to control (T0) ($p < 0.05$). This outcome aligns with Astuti et al. (2021), who reported that the nutritional composition in POME can support the growth of beneficial microorganisms especially PGPR, which in turn promotes *Lantara camara* root growth by *Bacillus thuringiensis*-induced carrier composition. Similarly, Hastuti et al. (2022) found that the application of PGPR in conjunction with POME significantly improved the growth of oil palm seedlings, with PGPR alone showing fewer effective results for growth and root parameters. Our findings further suggest that PGPR can mitigate some of the potential negative effects of POME's nutrient load, improving root weight and biomass and overall helping plant growth.

CONCLUSION

In conclusion, the isolation of beneficial microbe (PGPR) from the lemongrass soil and crop was successful. Two isolates show positive results for the nitrogen fixation test, phosphate and



Note: T0 - control; T1- Application of 5 g POME only; T2 - Application of 5 g PGPR only; T3 - Application of POME + PGPR in ratio of 5 g:10 g; T4 - Application of POME + PGPR in ratio of 10 g: 5 g.

Figure 5. *The mean value of root fresh weight and root dry weight observation after 4 weeks at 95% difference of significance level.*

potassium solubilisation test and IAA production test. Significant enhancements were noted in multiple parameters such as rice plant height, rice plant dry weight and root dry weight with 4.39, 1.17, and 3.70 fold increases compared to control. The dual application of POME and PGPR presents a promising approach to promote sustainable rice cultivation and sustainable oil palm waste production for reuse and recycle. The findings also support the potential of this combination as an alternative that reduces the reliance on chemical fertilisers for environmental sustainability.

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