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RESEARCH

Soil fertility improvement using *Paenibacillus* spp. as biofertiliser in Paloh Jenang oil palm plantation

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Abstract
Oil palm (*Elaeis guineensis*) is widely cultivated and exported due to its high global demand. However, poor soil fertility can greatly reduce oil palm production. Repetitive soil exploitation and excessive use of chemical fertilizers and pesticides degrade soil quality and introduce harmful substances. This study aims to characterize *Paenibacillus* spp. and assess its potential as a biofertilizer to enhance soil fertility in Paloh Jenang oil palm fields. Molecular techniques, specifically 16S primers, were employed to identify *Paenibacillus* spp. Soil samples from favorable and non-favorable areas of the plantation were collected and treated with *Paenibacillus* spp. granules. The effects on soil fertility were measured by evaluating soil pH, electrical conductivity (EC), and nutrient composition using CNS Analysis and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP–OES). Statistical analysis, including ANOVA, revealed that *Paenibacillus* spp. improved soil fertility, with significant increases in pH and EC. Key nutrients such as total nitrogen, phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S) were higher in treated soils compared to the control. Notably, P2 bacteria showed a significant pH increase after one month of treatment. The study concludes that *Paenibacillus* spp., a Gram-positive bacteria, exhibits strong potential as a biofertilizer, improving soil fertility and enhancing oil palm growth. This research underscores *Paenibacillus* spp.'s role in boosting oil palm yields while promoting environmental sustainability.

Keywords Oil palm · Soil fertility · Biofertilizer · *Paenibacillus* spp.

1 Introduction

Oil palm cultivation is vital for the economies of tropical countries like Indonesia and Malaysia, contributing significantly to poverty alleviation through employment and smallholder farming. The success of oil palm plantations relies heavily on soil fertility, which directly affects production, quality, and yield (Obeng et al. 2020). To maximize profits, oil palms must be cultivated in nutrient-rich soils, which support the growth of high-yielding fruits (Perez-Sato et al. 2023). However, poor soil fertility leads to decreased oil palm quality and reduced production, impacting both farmers and the economy.

The Paloh Jenang oil palm plantation has faced low productivity for about 25 years, primarily due to inadequate soil fertility. The overuse of chemical fertilizers and traditional farming practices have degraded the soil in some plantations. This over-reliance on synthetic inputs raises concerns about the long-term sustainability of soil health, posing challenges for productivity.

To address these issues, biofertilizers have emerged as a sustainable solution for improving soil fertility. *Paenibacillus* spp., a bacterium that fixes atmospheric nitrogen and solubilizes phosphorus, shows promise as an effective biofertilizer (He et al. 2021). Research has demonstrated the potential of *Paenibacillus* spp. to enhance plant development and productivity across various crops, including tomatoes, cucumbers, and wheat (Harahap et al. 2020; Liu et al. 2019).

This study aims to explore the potential of *Paenibacillus* spp. in improving soil fertility in Paloh Jenang oil palm plantations. By employing molecular identification, soil analysis, and efficacy evaluation, the research aims to

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promote sustainable agricultural practices, reduce reliance on chemical fertilizers, and ensure the long-term productivity of oil palm plantations.

2 Materials and methods

2.1 Preparation of of *Paenibacillus* spp.

Multiple strains of *Paenibacillus* spp. were acquired from the Plant Protection Laboratory at the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut, Malaysia. The bacteria were inoculated into Nutrient Agar (NA) (Oxoid Limited, UK) and incubated at 37 °C for a duration of two days for further purposes. The cultivated bacteria were subsequently collected with an inoculation loop and mixed with nutritional broth (Oxoid Limited, UK). Then, it was refrigerated for 24 h (Mohammad et al. 2022).

2.2 Molecular identification of *Paenibacillus* spp.

The selected bacteria were identified using a molecular approach involving DNA extraction, 16S rRNA gene amplification, and sequencing. DNA was extracted from gram-positive bacteria using a modified method from Mohammad et al. (2022). A bacterial colony was cultured overnight in nutrient broth, and DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega, Switzerland). After centrifugation, the cells were treated with lysozyme and EDTA, incubated at 37 °C, and processed with Nuclei Lysis Solution and RNase. The DNA was precipitated using isopropyl alcohol, washed with ethanol, air-dried, rehydrated, and stored at −20 °C. DNA quality and quantity were assessed using agarose gel electrophoresis.

The 16S rRNA gene was amplified via polymerase chain reaction (PCR) using the primers 16S2 (5–GAG TTT GAT CCT GGC TCA–3) and 16SR2 (5–ACG GCT AAC TTG TTA CGA–3). PCR conditions included denaturation at 95 °C, annealing at 50 °C, extension at 72 °C, and a final extension stage at 72 °C across 40 cycles (Weselowski et al. 2016). Amplified products were analyzed on a 1.0% agarose gel, with successful amplification indicated by a single band of the expected size. Sequencing of the PCR products followed, and bacterial identity was determined using a BLAST search in the GenBank database.

2.3 Soil sampling

Soil samples were obtained from the Paloh Jenang oil palm plantation located in Cheneh, Terengganu. Six soil series were identified: Lubok Itek, Gong Chenak, Batu Lapan, Renggam, Serdang, and Bungor. Samples were collected from eighteen sites, comprising three plots for each soil series. Approximately 5 kg of soil was extracted from each plot at a depth of 0–15 cm with a soil hand auger. The soil samples were sieved to discard any other particles, such as rocks and tree branches, and were ground into a finer texture. Before administering the initial treatment, the soil samples underwent comprehensive analysis to evaluate their condition.

2.4 Soil pH and electric conductivity (EC)

A pH meter was used to measure soil acidity or alkalinity, and soil pH was measured using a portable soil-specific pH meter (Takemura DM-15, Takemura Electric Works, Japan), designed for direct insertion into moist soil. Before measurement, soils were adjusted to near field capacity to ensure adequate electrode–soil contact, following the manufacturer's instructions. To ensure reliability, selected samples were cross-validated using the standard soil–water suspension method (1:2.5) in an accredited laboratory. Similarly, soil salinity was measured directly in moist soil using a portable soil EC meter designed for in situ measurements, providing a value representing the electrical conductivity of the soil.

2.5 CNS analysis

The CNS Analyser was used to quantify the macroelements—carbon, nitrogen, and sulfur—in the soil series of the Paloh Jenang oil palm plantation. 30 g of soil samples from each soil series were dispatched to the Forest Research Institute Malaysia (FRIM) for the analysis of the total ion concentration of three soil elements.

2.6 Microwave digestion and Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis

Microwave digestion and inductively coupled plasma optical emission spectrometry (ICP-OES) were utilized to determine the macronutrient and micronutrient content of soil samples. For digestion, 0.5 g of treated soil was placed in reaction vessels with 10 mL of concentrated nitric acid (HNO_3) under a fume hood. The vessels were sealed and heated to 180 °C for 20 min, with the temperature maintained for an additional 20 min to ensure complete digestion (Mohammad et al. 2022). This process enabled precise nutrient analysis using ICP-OES.

ICP-OES measures the emission of light from excited atoms and ions to quantify specific elements. Macronutrients such as phosphorus (P), potassium (K), magnesium

(Mg), calcium (Ca), and sulfur (S) were analyzed alongside micronutrients including boron (B), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn). After digestion, vessel lids were opened to release gases, and the solutions were filtered. 10 mL of each filtrate was diluted to 100 mL with ultrapure water in volumetric flasks. These diluted samples were transferred to 50 mL Falcon tubes for analysis, ensuring accurate and reliable nutrient quantification. The concentration of digested soil sample was calculated as follows in Eqs. (1) and (2):

$$Conc.\left(\frac{mg}{kg}\right) = \frac{C \times V}{W \times s} \quad (1)$$

Where
C = Inst. Value in mg/L (ppm)
V = final volume after preparation (L)
W = wet sample mass (kg)
S = dry weight per 100 (%)

$$\text{Percentage of dry weight } (\%) = \frac{dry\ sample(g)}{sample(g)} \times 100 \quad (2)$$

2.7 Preparation of bacteria granules and treatment of oil palm soil

Bacteria granules of *Paenibacillus* were prepared according to Mohammad et al. (2022) and mixed with the soil sample taken from the oil palm plantation. Approximately 200 g of kaolin and 800 g of wheat flour were autoclaved at 121 psi for 20 min. The materials were then mixed with 200 mL of bacterial spore suspension ($1 \times 10^x 9$ CFU) and 200 g of glucose to produce a homogenous dough, which was rolled to 1 mm thickness, air-dried under laminar airflow for 48 h, and then ground into smaller fragments.

The soil samples were treated with *Paenibacillus* spp. granules by diluting them with water and spraying them onto the soil. Each sample of 1 kg of soil is treated with 1 g of bacterium granules diluted in 100 mL distilled water. Each soil series sample was treated with three different treatments of *Paenibacillus* spp., which are P1, P2 and P3. P1 refers to *Paenibacillus* spp. Type 1, and P2 refers to *Paenibacillus* spp. Type 2, while P3 denotes a mixed culture combining the two *Paenibacillus* species from P1 and P2. The treatment is administered once in the initial month, with data recorded following the first, second, and third months of applying bacterial granules to the soil. Figure 1 shows the dilution of bacteria treatment applied to the soil.

2.8 Statistical analysis

The experiment of untreated (control) and treated soils was conducted using a completely randomized design, and the data were analyzed by analysis of variance (ANOVA) in accordance with the experimental structure via Minitab version 17. ANOVA identified significant differences among group means under varying conditions. A p-value < 0.05 indicated statistically significant changes between treated and untreated soils, highlighting the treatment's impact (Mohammad et al. 2022). Following a significant two-way ANOVA, post-hoc multiple comparison analysis was performed using Tukey's HSD test to identify significant differences among treatment groups.



Fig. 1 *Paenibacillus* spp. treatment for the soil samples

3 Results and discussion

3.1 Identification of *Paenibacillus* spp.

Two distinct strains of *Paenibacillus* spp., labeled P1 and P2, were identified using 16S rRNA gene sequencing. DNA from both strains was successfully amplified, yielding fragments of approximately 1500 bp. A single band indicates the presence of DNA, and more than 10,000 base pair DNA fragments were obtained when P1 and P2 were successfully extracted. However, identification was limited to the genus level rather than the species level. Both bacterial strains, P1 and P2, showed 99% identity, confirming their close genetic relationship within the *Paenibacillus* genus Fig. 2.

3.2 Characterization of soil from oil palm plantation

3.2.1 pH of soil

Soil samples from all six series were highly acidic, reducing fertility and hindering optimal oil palm growth. Ideal pH for oil palm ranges from 5.0 to 5.8 (Kome et al. 2020), but levels in non-favourable (Batu Lapan, Gong Chenak, Lubok Itek) and favourable (Bungor, Renggam, Serdang) soils were below this range. Batu Lapan showed the highest pH but still fell short. This acidity limits nutrient availability, reducing productivity. Figure 3 highlights the pH levels of these soil

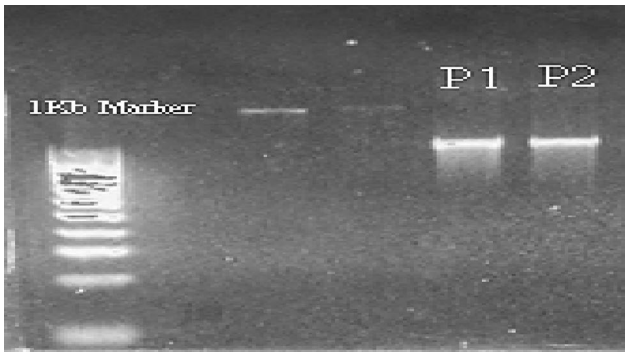


Fig.2 PCR amplification of *Paenibacillus* spp. DNA

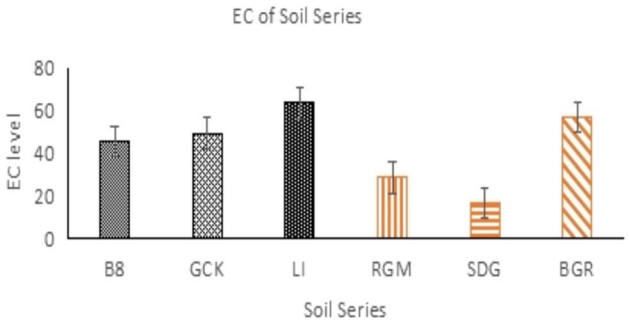


Fig. 4 Electric Conductivity (EC) of the soil series. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)

series from Paloh Jenang plantation, emphasizing the need for pH adjustments to improve soil fertility.

3.2.2 Electric conductivity (EC) of soil

Soil nutrient availability, crucial for plant growth, is influenced by electrical conductivity (EC), which measures total salt concentration in the soil. EC, expressed in µS/cm, should ideally remain below 1000 µS/cm for oil palm soils. As shown in Fig. 4, both non-favourable and favourable soil series achieved optimal EC levels. High EC levels indicate elevated salinity, which inhibits growth, while low EC values often suggest high clay content, limiting nutrient availability due to restricted water movement. Maintaining optimal EC ensures balanced nutrient availability, supporting healthy and productive oil palm growth.

3.3 After treatment with *Paenibacillus* spp. as a biofertilizer

3.3.1 Soil pH analysis

Soil pH greatly impacts nutrient availability and interactions within the soil. At low pH levels, essential nutrients become

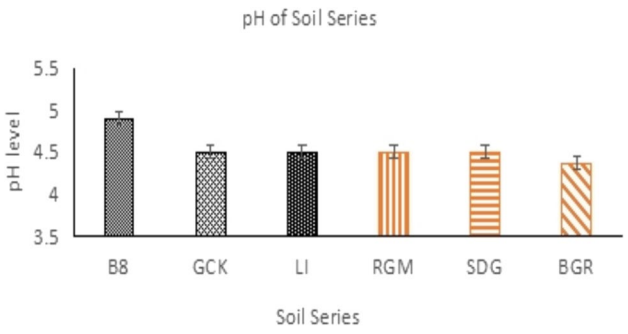


Fig. 3 The pH levels of the non-favourable and favourable soil series. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)

less accessible, while elements like iron, aluminum, and manganese may reach toxic concentrations. Additionally, insoluble complexes of iron, phosphorus, and aluminum can form, further restricting nutrient uptake. Acidic soils often lead to reduced crop yields, as nutrients become unavailable and toxicity impairs plant growth (Aini et al. 2014).

As shown in Fig. 5, soil pH increased to the optimal range (5.0–5.8) for oil palm growth after treatment with *Paenibacillus* spp. Both favourable and unfavourable soils improved significantly compared to untreated controls. Specifically, the Gong Chenak, Lubok Itek, Renggam, and Serdang soil series exhibited significant pH increases ($p < 0.05$) after three months. This rise in pH is attributed to the activity of *Paenibacillus* spp., which can produce organic acids like malic acid, gluconic acid, citric acid, oxalic acid, tartaric acid, lactic acid, and succinic acid, ammonia, and enzymes (chitinase, glucanase, cellulase, nitrogenase) that modify soil chemistry, neutralize acidity, and release essential cations (Dobrzynski and Kulkova 2025; Kim et al. 2023). Achieving optimal pH enhances nutrient availability, promotes beneficial microbial activity, and improves soil health, ultimately promoting better crop growth and yields (Kome et al. 2020; Gondal et al. 2021). However, *Paenibacillus* spp. must adapt to diverse soil textures and compositions, which may require extended treatment durations to achieve consistent, significant effects across all soil series.

3.3.2 Electric conductivity (EC) analysis

Electrical conductivity (EC) measures the presence of ions and salts such as total nitrogen, calcium, sodium, and phosphorus that act as nutrient sources for plants (Wang et al. 2012). Figure 6 illustrates a steady increase in EC values across all soil samples treated with *Paenibacillus* spp. over three months. *Paenibacillus* spp. generally do not directly increase soil EC as a primary function, but rather promote nutrient cycling and organic matter decomposition, which can affect the concentration of ions in the soil

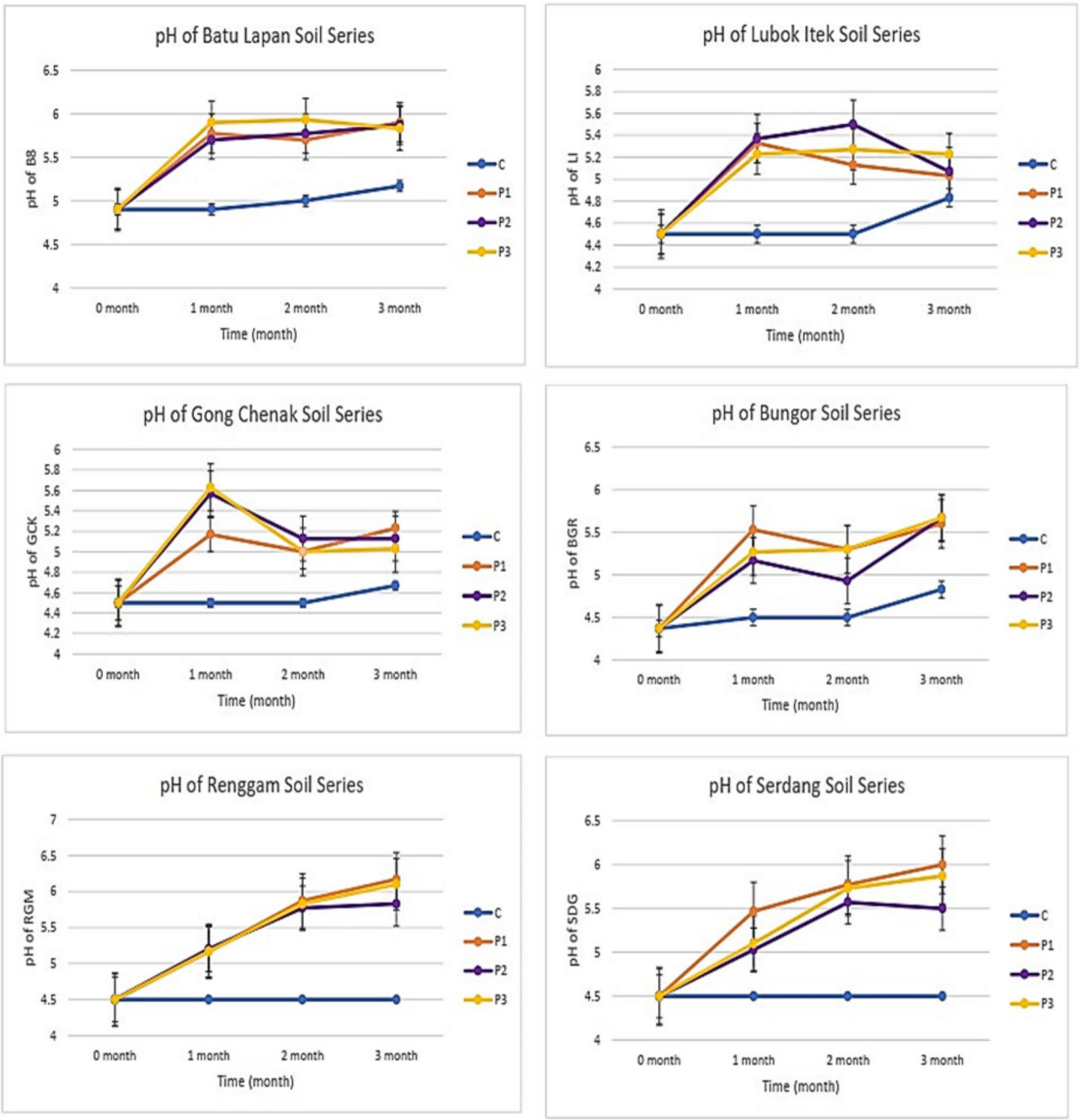


Fig. 5 pH level of soil series after three months of *Paenibacillus* spp. treatments. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)

solution (Tariq et al. 2025; Mohammad et al. 2024). These processes provide fixed nitrogen and nutrients, supporting sustainable soil health, improved plant health, and higher crop yields over time (Wang et al. 2022). Therefore, any observed increase in EC is likely a normal consequence of enhanced nutrient cycling and is usually beneficial, provided it stays within an optimal range for plant growth. However, a longer adaptation period is necessary for these bacteria to establish a stable and significant impact.

3.3.3 Macroelements analysis

A CNS analyzer was utilized to measure total carbon (C), nitrogen (N), and sulfur (S) content in natural solid,

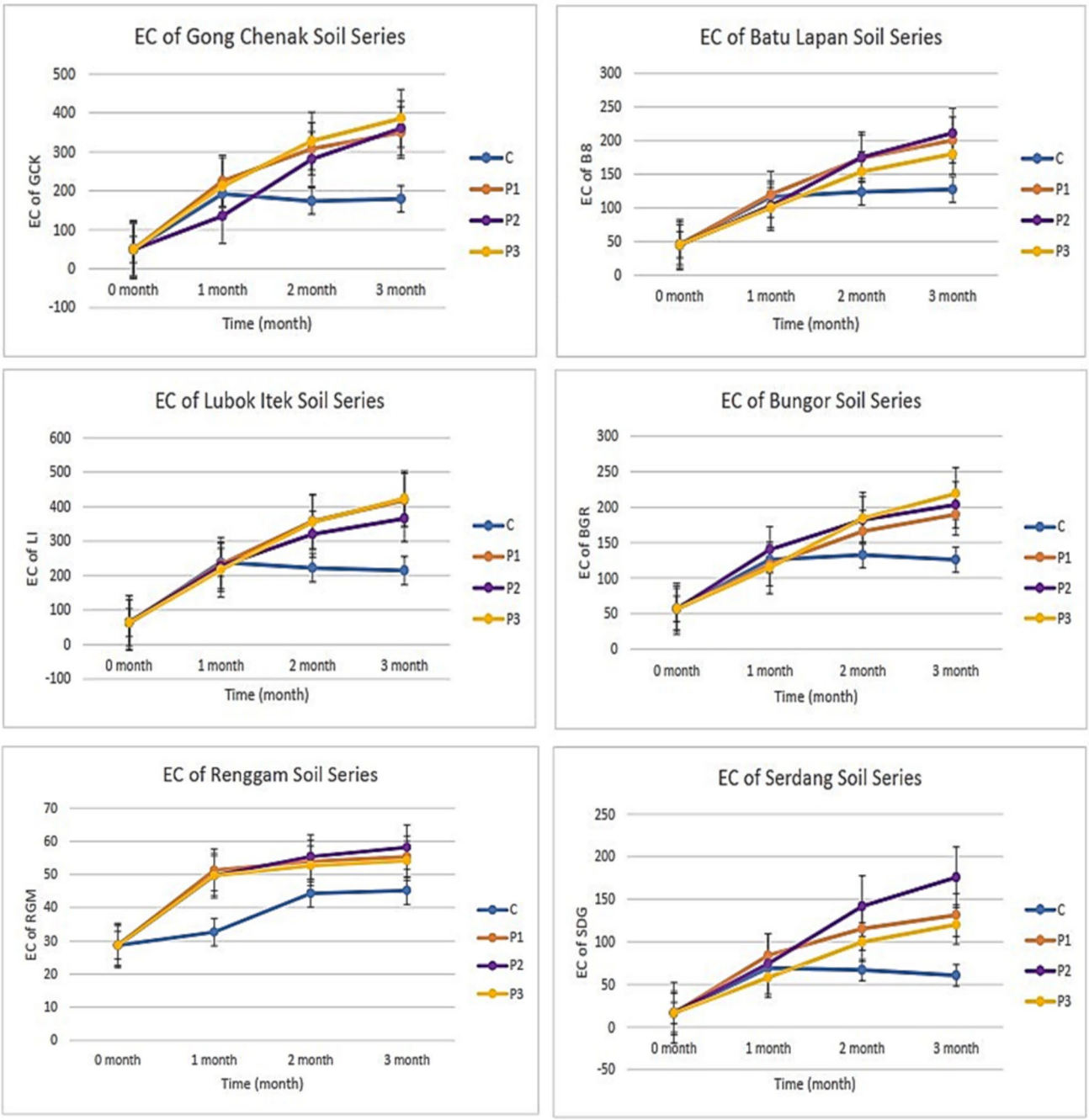


Fig. 6 EC value of soil series after three months of *Paenibacillus* spp. treatments. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)

liquid, and gas samples from various soil series. Carbon is crucial for oil palm soil, supporting health, fertility, and ecological processes. Carbon levels vary depending on climate, farming practices, and soil type (Manning et al. 2019). ANOVA analysis (Fig. 7) revealed significant differences in sulfur, nitrogen, and carbon contents among soil series, with treated soils generally showing higher nutrient levels compared to the control. Tukey's HSD test ($p < 0.05$) indicated significant treatment effects for sulfur and nitrogen, particularly in the Lubok Itek and Batu Lapan soil series, while carbon content showed significant increases in treated soils, especially in Batu Lapan, although some treatments did not differ significantly from one another.

Carbon plays a crucial role in soil organic matter formation, and fractions such as particulate organic carbon

are recognized as sensitive indicators of improved soil quality (Handayani et al. 2020). These fractions fluctuate with oil palm crop age and influence soil fertility and productivity. The observed increases in soil carbon and associated nutrients are linked to microbial activity and organic secretions, including extracellular polysaccharides and organic acids, which enhance nutrient availability and promote soil aggregate formation. Improved aggregation reduces organic carbon degradation and supports its stabilization and long-term retention in the soil (Smail et al. 2025; Shabrina et al. 2025), indicating that *Paenibacillus* spp. application contributes to improved soil quality and supports sustainable oil palm cultivation.

Nitrogen is crucial for plant absorption, with diazotrophic bacteria playing a vital role in converting atmospheric nitrogen (N_2) into plant-available ammonium (NH_4^+). This process promotes plant growth and reduces reliance on nitrogen fertilizers (Liu et al. 2019). As depicted in Fig. 7, nitrogen levels did not significantly differ between untreated soil (control) and soil treated with *Paenibacillus* spp. across all soil series. However, P2-treated soil exhibited the highest nitrogen increase among treatments, suggesting that P2 may enhance nitrogen availability more effectively.

Sulphur, often overlooked compared to nitrogen and phosphorus, is essential for oil palm growth and oil production. It aids in forming lipids, increasing fatty acids and triglycerides in oil palm seeds, and supporting fruit development and protein synthesis. Sulphur also contributes to carbohydrate and sugar metabolism, vital for overall plant health (Sabir et al. 2015). Figure 7 shows increased sulphur levels in treated soils, with P2-treated soil demonstrating the highest increase. *Paenibacillus* spp. oxidize sulphur, enriching the soil's sulphur cycle and improving sulphur availability for plant uptake (Singh et al. 2022).

Macro and micronutrients significantly affect oil palm yield and quality. Key elements like phosphorus (P), potassium (K), and magnesium (Mg) are essential to replenish nutrients lost during the harvest of fresh fruit bunches (FFB) (Alam et al. 2020). Phosphorus, critical for energy transfer, photosynthesis, and membrane synthesis, supports high-yielding oil palms (Wu et al. 2019). Phosphorus is critical for energy transfer, photosynthesis, and membrane synthesis in oil palm, supporting high productivity (Wu et al. 2019). As shown in Fig. 8, phosphorus concentrations increased significantly in treated soils, with the Serdang (SDG) series under P2 treatment exhibiting the highest P content and a significant difference compared with the control, approaching the optimal range of 0.16–0.19 ppm for young palms and 0.15–0.18 ppm for mature palms (Uexkull and Fairhurst 1991).

Magnesium, which is essential for chlorophyll synthesis, protein formation, and enzyme activation (Ishfaq et al. 2022), showed a significant increase in the Batu Lapan (B8) series and Lubok Itek (LI) soil series, particularly under P3 treatment, while other soil series displayed moderate changes, likely due to differences in soil composition and microbial adaptability.

Potassium plays a key role in osmotic regulation, oxidative stress protection, and cellular function, enhancing plant resilience and productivity (Sardans and Peñuelas 2021). Figure 8 indicates a significant increase in potassium concentration in the Bungor (BGR), Gong Chenak (GC), and Serdang (SDG) compared with the control, whereas increases in other soil series were present but not consistently significant.

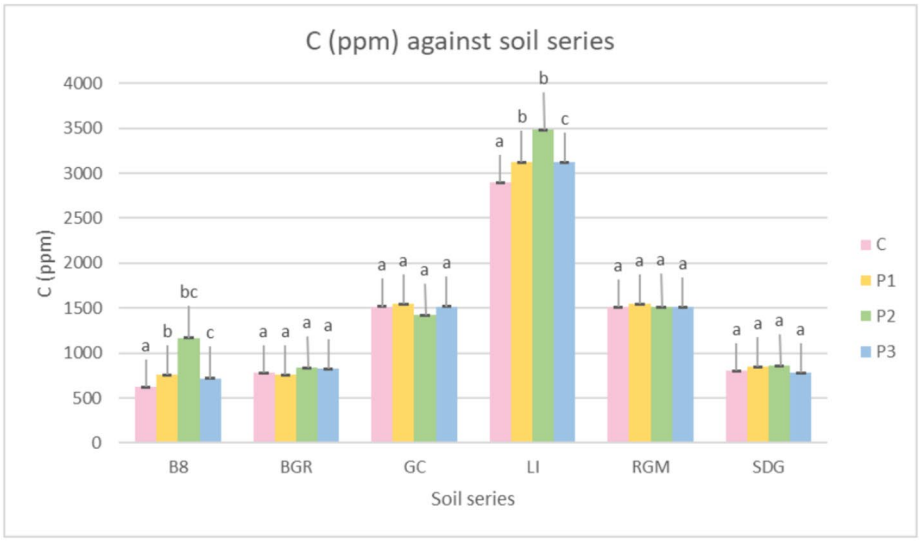
3.3.4 Microelements analysis

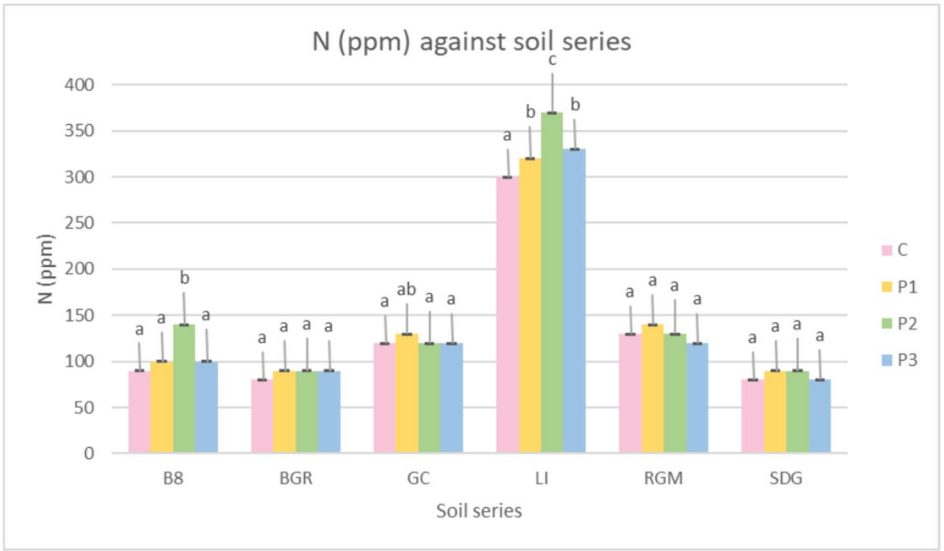
Boron (B) is essential for plant growth, particularly in cell wall biosynthesis, influencing root elongation, tissue differentiation, pollen germination, pollen tube growth, and cell membrane functions (Shireen et al. 2018). Manganese (Mn) supports metabolic processes across plant cell compartments, playing a vital role in growth (Alejandro et al. 2020). As shown in Fig. 9, both boron and manganese concentrations increased in treated soil series, particularly in Lubok Itek, compared to the untreated control. However, these increases were not statistically significant, indicating their supportive role in plant development without significant changes in concentration.

Copper (Cu), crucial for respiration and photosynthesis, is a component of the electron transport chain essential for these processes (Ajeng et al. 2020). In Fig. 9, soil treated with Paenibacillus bacteria showed increased copper levels. Notably, the Batu Lapan and Lubok Itek series exhibited significant increases ($p < 0.05$) compared to the control, with the highest copper increase in the P3 treatment (two bacterial strains). Zinc (Zn), vital for photosynthetic redox processes (Alengebawy et al. 2021), increased significantly in the Lubok Itek series and other series, except for Serdang, though not significantly. Iron (Fe) concentration also rose in Batu Lapan, Lubok Itek, and Renggam series, attributed to clay's capacity to bind Fe ions and immobilize bacteria, enhancing soil Fe content (Cardoso et al. 2023).

3.3.5 Statistical significance analysis

Tukey's HSD test was used to assess statistical differences among treatments. For macroelements, only potassium (K) in the Renggam series significantly differed from





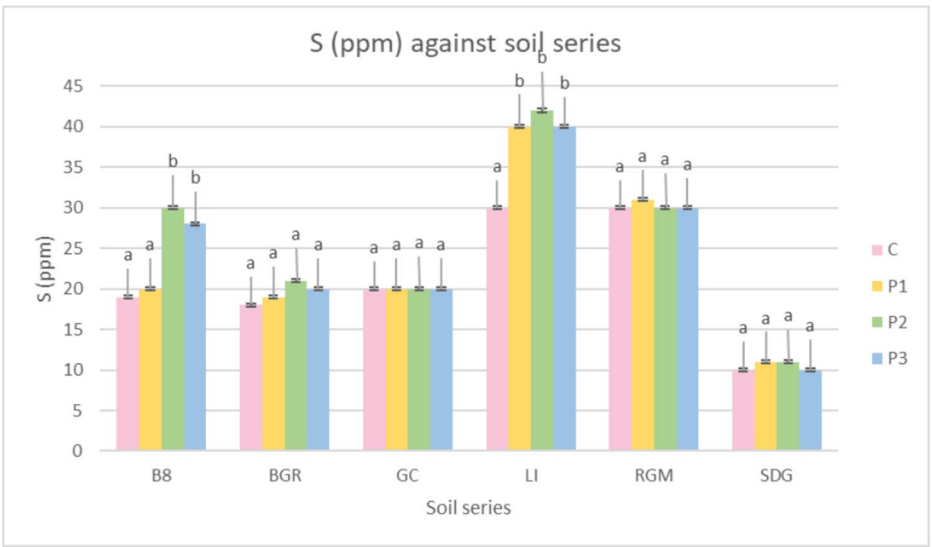
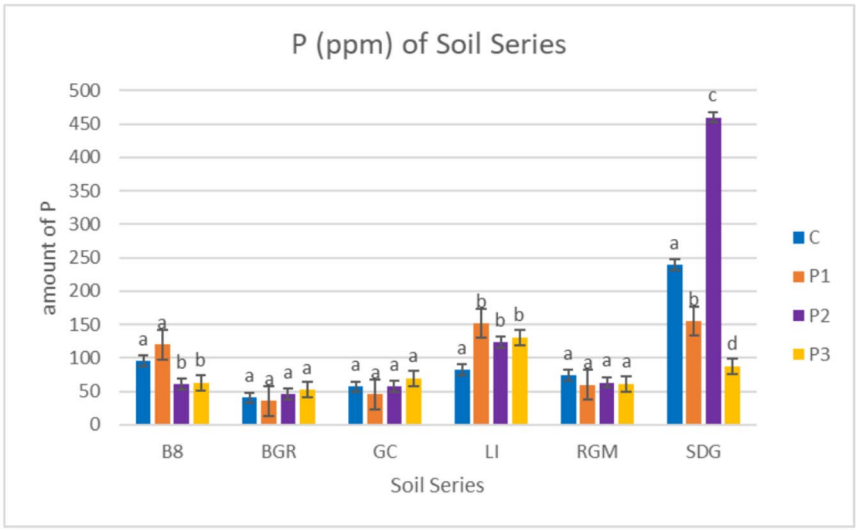
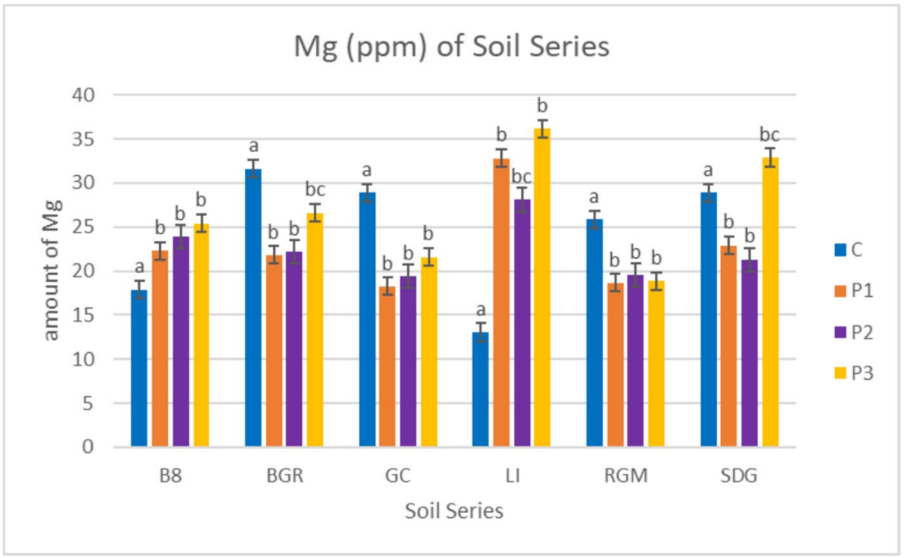


Fig. 7 The concentration of carbon (C), nitrogen (N) and sulphur (S) in soil after three months of *Paenibacillus* treatment. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)





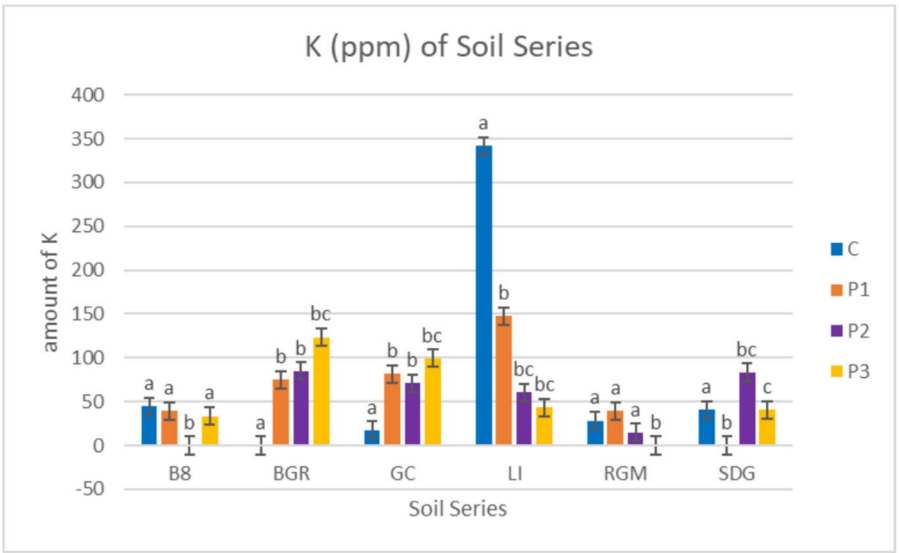


Fig. 8 The concentration of phosphorus (P), magnesium (Mg), and potassium (K) in soil after three months of *Paenibacillus* treatment. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)

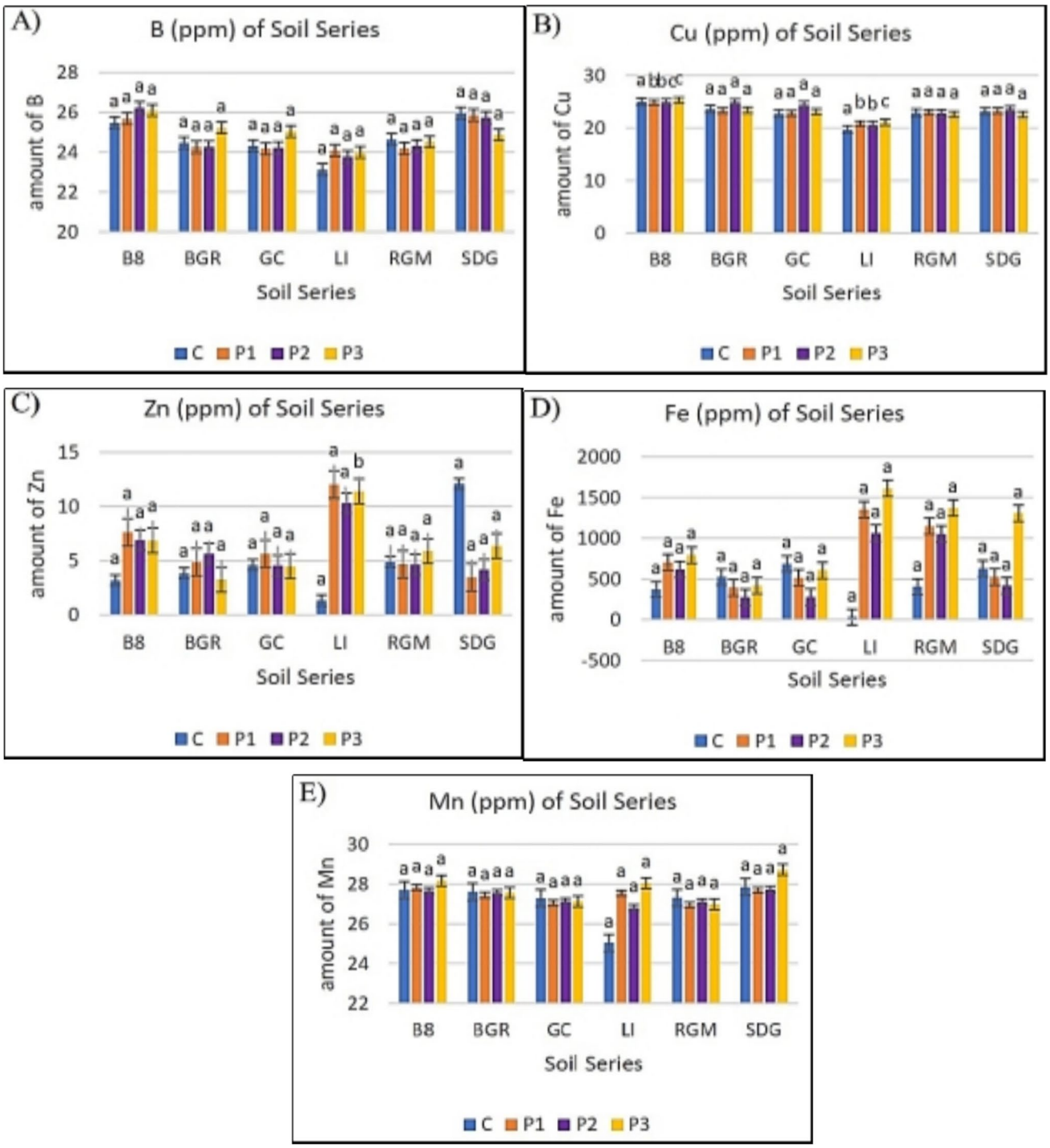


Fig. 9 The concentration of microelements of boron (B), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) in soil after three months of *Paenibacillus* treatment. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)

the control. Among microelements, zinc (Zn) and copper (Cu) showed significant increases ($p < 0.05$). Other macroelements and microelements in treated soils showed no significant differences compared to the control. Table 1 highlights potassium, zinc, and copper as the elements with significant variations, reflecting the impact of *Paenibacillus* treatments on soil nutrient levels, particularly enhancing these specific elements.

Table 1 Concentration of soil elements that show significance. Values are presented as the mean of three replicates, with vertical bars representing the standard error (SE)

Minerals concentration (ppm)				
Soil Series	RGM	LI		B8
Treatments	K	Zn	Cu	Cu
Control (untreated soil)	28.3096[a]	1.3462[a]	19.6923[a]	24.9569[a]
P1-treated soil	39.2135[ab]	11.4231[a]	21.0769[b]	25.2941[b]
P2-treated soil	14.7606[bc]	10.3077[a]	20.5769[bc]	24.8725[b]
P3-treated soil	0.0000[c]	12.0385[b]	20.8077[c]	24.8725[c]

*The different letter in the same column denotes a significant difference among treatments ($p < 0.05$) by the Tukey's HSD test

4 Conclusions

This study investigated the effectiveness of *Paenibacillus* spp. as a biofertilizer to enhance soil fertility in oil palm plantations at Paloh Jenang. Soil analysis revealed significant acidity (low pH), which limits nutrient absorption by oil palm plants. The application of *Paenibacillus* spp. resulted in notable improvements in soil fertility indicators, such as increased pH, electrical conductivity (EC), and nutrient availability. Among the treated soil series, the Lubok Itek series showed the greatest enhancements after 3 months of bacterial treatment. These findings confirm the potential of *Paenibacillus* spp. as an effective biofertilizer for restoring soil fertility. Future studies should explore longer treatment durations to better understand the sustained impact of these beneficial bacteria on soil health and oil palm productivity, paving the way for sustainable agricultural practices.

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Authors contribution Sarina Mat Rosid: Formal analysis, Editing. Nurnabila Kamaruzaman: Methodology, Investigation, Formal Analysis. Noor Afiza Badaluddin: Formal analysis, Supervision Funding acquisition. Mohd Izwan Awang: Formal analysis. Mohammad Moneruzzaman Khandaker: Formal analysis.

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Data availability All data generated or analysed during this study are publicly available in the Soil fertility improvement using Paenibacillus spp. as biofertiliser in Paloh Jenang oil palm plantation, under accession number https://doi.org/10.1007/s44447-026-00137-9.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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