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Synergistic effects of *Bacillus salmalaya* strain 139SI with fertilizer on nutrient uptake and fertilizer use efficiency of oil palm seedlings

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ABSTRACT

Aims: Plant-microbe interaction in the rhizosphere significantly influences nutrient uptake efficiency. Thus, this research was aimed to investigate the potential of *Bacillus salmalaya* strain 139SI in increasing nutrient use efficiency through its synergistic effects with fertilizer application.
Methodology and results: This research analyzed the effects of *B. salmalaya* strain 139SI inoculant, fertilizer and a combination of both on soil nutrients, vegetative growth, chlorophyll level, photosynthetic activities, nutrient uptake and nutrient use efficiency in oil palm seedlings for four months in a nursery setting. At the end of the research, the inoculation of *B. salmalaya* strain 139SI resulted in a significant increase in palm growth, chlorophyll level, photosynthetic activities, nutrient uptake and nutrient use efficiency compared to the untreated group. Soil nutrient analysis demonstrated that the inoculation of *B. salmalaya* strain 139SI led to a notable increase in available nitrogen within the rhizosphere soil. The findings of this research also indicated a noteworthy synergistic effect between the *B. salmalaya* strain 139SI inoculant and fertilizer. The most promising outcomes for plant growth performance and nutrient uptake were observed when the *B. salmalaya* strain 139SI inoculant was added to the fertilized palm.
Conclusion, significance and impact of study: This research shows that *B. salmalaya* strain 139SI may work synergistically with fertilizer to enhance nutrient absorption and increase fertilizer usage efficiency. Integrating *B. salmalaya* into the nutrient management of oil palm seedlings can potentially reduce reliance on synthetic fertilizers, offering advantages to both farmers and the ecosystem.

Keywords: Indole 3-acetic acid, nitrogen fixation, nutrient use efficiency, phosphate solubilization, plant-microbe interaction

INTRODUCTION

Palm oil is one of the most consumed vegetable oils in the world. Malaysia currently stands as the second largest palm oil producer, with the total area cultivated with oil palm in 2022 reaching 5.67 million hectares, a decrease of 1.1% compared to the previous year (MPOB, 2019). Many factors determine the productivity of an oil palm plantation, the most important of which is the quality of the transplanted oil palm seedlings produced at the nursery stage. Besides the significant genetic improvement of the seedling derived from the cross-pollination of selected parent palms, proper agronomic practices, particularly fertilizer application, could enhance the production of high-quality planting materials (Kushairi *et al*., 2018). However, nutrient inputs are one of the significant costs in oil palm management due to the high demand for nutrients by the seedlings (Darras *et al*., 2019; Siang *et al*., 2022). In addition, fertilization is also known to cause environmental damage, such as greenhouse gas emissions, water pollution, and nutrient losses through leaching (Hassler *et al*., 2015; Kurniawan *et al*., 2018). Thus, the best nutrient management practice must be designed to optimize fertilizer use efficiency and protect the environment.

The integration of inorganic fertilizer application with organic materials such as plant growth-promoting rhizobacteria (PGPR) is one option that can benefit both agronomic practice and ecosystems. Various studies have documented the mechanisms of the action of PGPR in promoting plant growth. The role of PGPR in biological nitrogen fixation (BNF), nutrient chelation, solubilization, the production of growth hormones and biocontrol agents can improve soil fertility and facilitate plant growth

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(Ahemad and Kibret, 2014). There were also several reports on the potential of various species of PGPR inoculant in reducing the use of chemical fertilizer without compromising plant growth and yield (Zainuddin *et al*., 2022). However, it is important to thoroughly explore the impacts of PGPR integration with fertilizer, as many previous studies reported that PGPR produced inconsistent effects on nutrient uptake and plant growth. For example, Ijaz (2019) found that PGPRs such as *Bacillus* sp. and *Pseudomonas* sp. could reduce 50% of chemical fertilizer applications by increasing fertilizer use efficiency.

On the other hand, Yu *et al*. (2012) reported that inoculation of N-fixing and phosphate solubilizing bacteria consisting of *Bacillus megaterium* and *Arthrobacter pascens* into soil amended with rock phosphate fertilizer has an insignificant effect on nutrient uptake of the plant. Meanwhile, some PGPR effects depend on the rate of fertilizer applied. For instance, high doses of N reduce the beneficial effects of *Serratia* spp., as mentioned by Nascente *et al*. (2019).

Thus, further research is needed to explore the potential of combining *B. salmalaya* strain 139SI inoculant with inorganic fertilizer applications to enhance nutrient uptake by oil palm seedlings during the nursery stage. Inoculating the rhizosphere soil with this microbe at an early stage can promote its population growth and colonization in the root system. This can protect the seedling against diverse soil stress conditions following transplantation into the plantation area. Our previous study found that *B. salmalaya* strain 139SI exhibited several promising plant growth-promoting features, such as producing plant growth hormones IAA, iron chelation compounds, converting atmospheric nitrogen into ammonia and solubilizing phosphate. The result of scanning electron microscope (SEM) analysis also found that this strain has shown its ability to migrate from rhizosphere soil to colonize plant roots (Azri *et al*., 2018). Based on that information, we hypothesized that inoculation of oil palm seedlings with this strain could increase nutrient availability for plant absorption in the immediate vicinity of the root, thus enhancing oil palm seedling growth. This study also aimed to investigate the synergistic effects of inoculating *B. salmalaya* strain 139SI with inorganic fertilizer on nutrient uptake and plant physiology. On top of that, it is also essential to determine whether the promising features of this strain in enhancing nutrient availability found based on the laboratory results can be replicated in the actual unsterilized nursery condition.

MATERIALS AND METHODS

Bacterial strain

Bacillus salmalaya strain 139SI was provided by the Molecular and Bacteriology Laboratory, University of Malaya. The strain 139SI was initially isolated from rhizosphere soil obtained from an oil palm plantation in Selangor, Malaysia (2.99917° N, 101.70778° E) (Ismail *et al*., 2012). The species classification of this strain was based on phenotypic characteristics, phylogenetic analysis and 16S rRNA G+C characterization (Gen Bank accession No: JF825470; ATCC BAA-2268) (Ismail and Dadrasnia, 2015). The strain was screened for plant growth-promoting features and found positive for N-fixing activity using the method of Baldani *et al*. (2014) and able to produce indole acetic acid (IAA) (18.5 ± 0.4 µg/mL) based on the method described by Gang *et al*. (2019). The *B. salmalaya* strain 139SI also gave a positive result for phosphate solubilization, as evaluated with the National Botanical Research Institute's Phosphate (NBRIP) plate culture (Mehta and Nautiyal, 2001) and siderophore production based on the chrome-azurol S approach (Louden *et al*., 2011).

For inoculum preparation, two loops of the strain from 2-day-old cultures were transferred onto a brain heart infusion (BHI) plate, followed by incubation at 30 °C for 48 h. Bacterial cells were scraped from the plate and suspended in sterile, distilled water. The inoculum was diluted to a final concentration of approximately 1×10^9 CFU/mL based on the optical density at 600 nm (UV-VIS Spectrophotometer, Thermo Fisher Scientific), which was confirmed by plate counting.

Plants growth conditions

Oil palm (*Elaeis guineensis* Jacq.) seedlings (dura × pesifera), all three months of age, were obtained from Sime Darby Seed Sdn. Bhd., Malaysia. The seedlings were transplanted into a polythene bag (38 cm × 51 cm) containing a horticultural soil formulation of 3:2:1 peat, clay and sand as a growth medium. The thoroughly mixed growth medium was sampled, oven-dried at 60 °C for 48 h, then finely ground before passing a 1-mm sieve size for macro- and micronutrient content analysis. The available nitrogen (N) was determined using the micro-Kjeldahl method, and the content of other nutrients was analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Soil pH was measured with a scientific pH meter. The percentage of elements in the 1g soil sample was N: 0.10% ± 0.07, P: 0.1% ± 0.03, K: 0.06% ± 0.01, Mg: 0.03% ± 0.02, Ca: 0.34% ± 0.02, S: 0.09% ± 0.01 and pH = 6.18 ± 0.15. Watering was done twice daily throughout the experimental period. All seedlings were allowed to stabilize with the nursery climate conditions for 30 days after being transplanted in the polythene bag.

Plants treatment

After acclimatization, a total of 40 palm seedlings were selected for the experiment based on their uniformity in size and free from any disease. The seedlings were clustered into four groups of treatments. The palm in treatment 1 (C1) was untreated. Fertilizer was given to the palm in treatment 2 (C2). Other treatment groups included palms inoculated with *B. salmalaya* strain 139SI (T1) and palms inoculated with *B. salmalaya* strain 139SI + fertilizer (T2). Palm seedlings in C1 and C2 served as

Table 1: Application rates of NPK fertilizers and *B. salmalaya* strain 39SI inoculant.

Month	NPK (15:15:6) Fertilizer	*B. salmalaya* strain 139SI inoculant (mL)
1	3.0	20.0
2	5.0	30.0
3	7.0	40.0
4	9.0	50.0

controls. A commercial fertilizer (NPK yellow) 15:15:6 comprising urea as the source of N, triple superphosphate as the source of P and muriate of potash as the source of K was given to the palm in C2 and T2. The oil palm seedlings were inoculated with the *B. salmalaya* strain 139S1 through a root zone drenching method. The amount of inoculant and the dose of fertilizers applied were based on the recommended fertilizer rate requirements of oil palm and biofertilizer application methods described by Lim *et al*. (2018), with minor adjustments (Table 1). The experiment was conducted in a glasshouse at the Institute of Biological Sciences nursery, Faculty of Science, University of Malaya (3° 7'24.63" N, 101°39'15.61" E) for four months [mean temperature: 31/25 °C (day/ night), relative humidity: 66-81%]. The plants were laid out in a completely randomized design (CRD) with ten replications.

Growth and vegetative measurements

The determinants of plant growth were quantified using parameters such as plant height, stem diameter, leaf count, leaf surface area and dry weights of both the shoot and root systems. Plant height and stem diameter were measured monthly over a four-month period. Plant height was recorded as the distance from the base of the plant to the apex of the newest fully mature leaf. The stem diameter was measured approximately 1 cm above the soil level using a vernier caliper. At the end of the treatment period, the leaf count and leaf surface areas were recorded. For leaf surface area measurements, images of the leaves were captured using a Casio Exilim 6-megapixel digital camera. The leaf surface area was then determined by analyzing the scaled photos of the leaves using the ImageJ image processing program. Lastly, the plants were harvested after four months, and the soil was carefully washed off from the roots. The lengths of the roots and shoots for each treatment were measured. Subsequently, the shoots and roots were labeled according to their respective treatments and oven-dried at 70 °C to determine the plant biomass.

Determination of photosynthetic rate, chlorophyll content and chlorophyll fluorescence

After four months, the photosynthetic rate, chlorophyll fluorescence and chlorophyll content were determined in five fully expanded leaves (leaf no. 3 to 7) per palm, replicated in ten palm seedlings per treatment. The photosynthetic rate was measured using the portable photosynthesis system (Model LI-6400XT, LICOR, USA) equipped with the built-in light source set at 1,800 µmol photons $m^{-2}sec^{-1}$. The level of CO_2 supplied to the leaf was controlled by using the built-in CO_2 injection system of the photosynthesis unit and was adjusted at approximately 400 µmol. The photosynthesis rate was measured between 11:00 am and 15:00 pm at a leaf temperature between 28-30 °C. The portable photosynthesis system utilizes gas exchange principles to measure the photosynthetic rates of plants. Net photosynthesis rates are expressed as rates of CO_2 uptake (µmol m^{-2} sec^{-1}).

Leaf greenness due to chlorophyll content was measured using a portable chlorophyll meter (SPAD-502, Minolta Co., Japan). Chlorophyll fluorescence was measured using a Plant Efficiency Analyzer (PEA, model LH36/2R, Hansatech Instrument Limited, England). A leaf clip was attached to the leaves to keep the leaf area in the dark for 40-45 min of dark adaptation. The fluorescence signal of the leaf was measured for a duration of 3 sec upon exposure to light, after which the quantum yield was determined under specific conditions of temperature (28 °C) and time range (10 µ sec to 1 sec). Then, the maximum fluorescence (Fm) and minimum fluorescence (Fo) values were obtained. The yield of variable fluorescence (Fv) was calculated as Fm - Fo, and the calculation of chlorophyll fluorescence was determined according to the ratio Fv/Fm, which represents the quantum yield of photosynthesis.

Analysis of nutrient concentration in soil and plant samples

Samples of soil in the vicinity of the plant's roots were obtained at depths of 0-20 cm from each pot at the end of the experiment. Each individual plant and soil sample from all treatments was subjected to oven-drying at 70 °C and subsequently finely ground to pass through a 1 mm sieve for further analysis.

Soil samples from each treatment group were suspended in Milli-Q water (1:4, soil: water) and the pH of the soils was measured with a scientific pH meter. The available N in soil samples collected from each treatment was determined using the micro-Kjeldahl. Five g of soil samples were added to 25 mL of Mehlich 1 extraction solution (0.05 mol/L H_2SO_4 + 0.05 mol/L HCL) in a flask and shaken for 5 min on a shaker. The mixture was centrifuged at 3000 rpm for 10 min and then filtered through Whatman No. 2 filter paper. The concentration of available nutrients in soil was analyzed using ICP-OES.

Plant samples were digested with H_2SO_4 and K_2SO_4, and then the total N was determined by the micro-Kjeldahl method. One g of plant samples was also digested with HNO_3 at a temperature of 115 °C for 120 min. After completing the digestion process, hydrochloric acid (HCl) was added and the samples were subjected to further digestion at the same temperature for another 120 min. The resulting mixture was adjusted to a final volume of 100 mL by adding distilled water to each tube. Subsequently, the mixture was filtered and subjected to analysis for nutrient content via ICP-OES (Perkin Elmer Optima 2100 DV) (Masson *et al*., 2010). Plant nutrient uptake was estimated using the formula described by Sharma *et al*. (2020):

Nutrient uptake = (Nutrient content × Total biomass)/100

Nutrient use efficiency (NUE) for different treatments was calculated using the following formula:

Nutrient use efficiency (NUE) = (Nui - Nuc)/(Nfi - Nfc)

Nui and Nuc are the total nutrient uptake by the plant in different treatments and control. Nfi and Nfc are the total nutrients (N, P and K) applied as fertilizer in different treatments and controls, respectively (Arif *et al*., 2017).

Statistical analysis

The plants were laid out in a completely randomized design (CRD) with ten replications. All parameters were analyzed by one-way analysis of variance (ANOVA) performed with SPSS version 22. Significant differences between means were compared using a Tukey range test at $P \leq 0.05$. Pearson correlation of coefficient test was performed to estimate the relationships between nutrient uptakes with palm growth and photosynthetic activities.

RESULTS

Plant vegetative growth

Based on the data presented in Table 2 and Figures 1 and 2, there is clear evidence of a synergistic effect between *B. salmalaya* strain 139SI and inorganic fertilizer, which results in improved growth of oil palm seedlings. The findings of the study indicate that the incorporation of *B. salmalaya* strain 139SI inoculant with inorganic fertilizer resulted in a significant increase in the overall biomass of oil palm seedlings compared to those treated with solely inorganic fertilizer. After four months of treatment, seedlings inoculated with *B. salmalaya* strain 139SI and receiving fertilizer at the same time (T2) produced the highest shoot dry weight, followed by C2 (fertilizer) > T1 (*B. salmalaya* strain 139SI) > C1 (untreated). Similarly, the result of root dry weight displayed the same pattern. Oil palm seedlings from T2 recorded the highest root dry weight, 285.70%, 36.17% and 22.43%, which were heavier compared to the corresponding C1, C2 and T1, respectively. An interesting observation was made regarding the root dry weight of oil palm seedlings in C2 and T1. In contrast to the shoot dry weight, the average root dry weight of T1 palm seedlings was discovered to be 11.22% greater than that of C2.

The measurements of stem height and diameter presented in Figures 1 and 2 indicate that *B. salmalaya* strain 139SI had a noticeable growth-promoting effect on the fertilized oil palm seedling. The height of the stem of oil palm seedlings from T2 significantly outpaced the growth of palm seedlings from C1, C2 and T1 by 118.3%, 14.1% and 29.1%, respectively. Similarly, oil palm seedlings from T2 also produce larger stems compared to those in C1 (136.4%), C2 (14.7%) and T1 (25.4%), respectively.

Observation of the development of leaves found that the palm seedlings of all treatment groups possess five bifurcated leaves per palm at the early stage of treatment. As the palm seedling grows, it produces an average of one new leaf per month. At seven months, a pinnate leaf began to appear in the palm seedlings of C2, T1 and T2. In general, the oil palm seedlings of C2, T1 and T2 possess an average of ten leaves per plant at the end of treatment. In contrast, the growth and development of the leaf in C1 oil palm seedlings exhibited significantly slower progress. At the end of the treatment, palm leaves from the untreated C1 only produced eight small sizes of the bifurcated palm leaf with mild chlorotic symptoms (Figure 3). Consequently, the number of leaves produced by the oil palm seedling has significant effects on the total leaf area in each group of treatments. The total leaf area value ranged from 635.64 cm^2 to 1767.69 cm^2, with the T2 group having the highest value, followed by C2>T1 and C1 (Table 2).

Table 2: Effects of strain 139SI inoculation on shoot and root dry weight, number of leaf and total leaf area of oil palm seedling after 4 months of treatment.

Treatments	Growth parameter				
	Shoot dry weight. (g plant^{-1})	Root dry weight (g plant^{-1})	Total plant biomass (g plant^{-1})	No. of leaf	Total leaf area (cm^2)
Untreated (C1)	25.49 ± 6.40^{d}	8.18 ± 2.10^{c}	33.67 ± 7.15^{d}	8.0 ± 0.43^{b}	635.64 ± 51.31^{b}
Fertilizer (C2)	92.57 ± 4.60^{b}	23.17 ± 4.20^{b}	115.73 ± 7.15^{b}	10.0 ± 0.33^{a}	1618.54 ± 54.60^{a}
B. salmalaya strain 139SI (T1)	78.70 ± 7.00^{c}	25.77 ± 2.00^{b}	104.47 ± 6.89^{c}	10.0 ± 0.33^{a}	1532.20 ± 57.11^{a}
B. salmalaya strain 139SI + Fertilizer (T2)	111.50 ± 19.84^{a}	31.55 ± 3.80^{a}	143.05 ± 19.70^{a}	10.0 ± 0.33^{a}	1767.69 ± 79.48^{a}

The individual values are depicted as mean ± standard error of ten replicates. Different letters indicate significant differences between treatments according to the Tukey test ($P<0.05$).

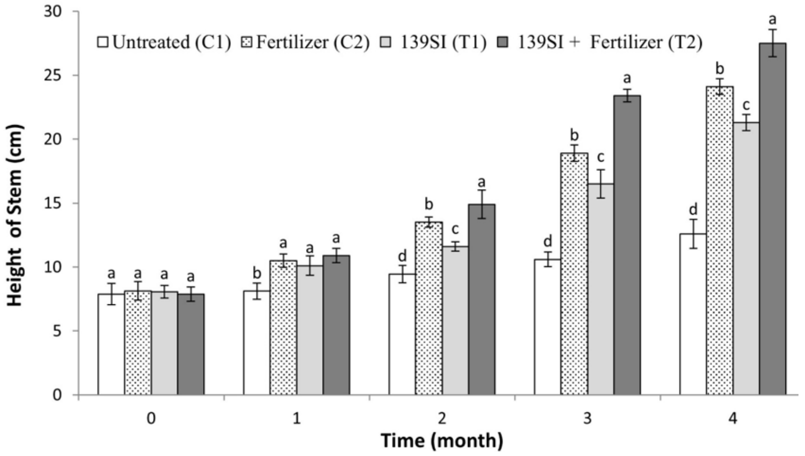

Figure 1: Effects of *B. salmalaya* strain 139SI inoculation on oil palm seedling stem height over four months of treatment. The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to the Tukey test (*P*<0.05).

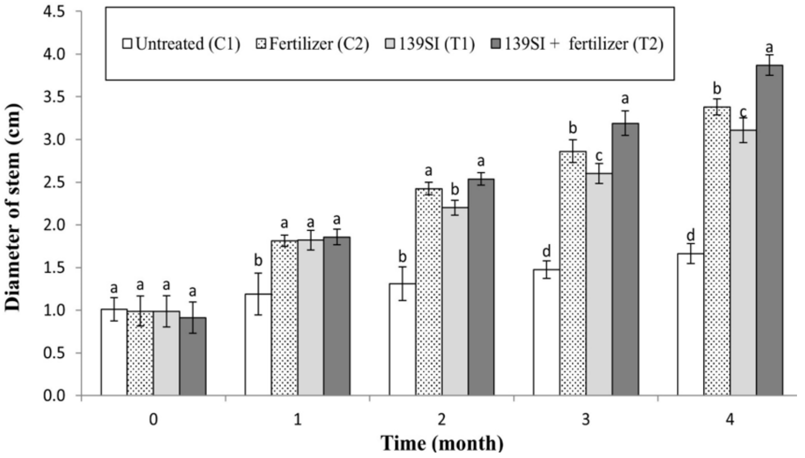

Figure 2: Effects of *B. salmalaya* strain 139SI inoculation on oil palm seedling diameter of stem over four months of treatment. The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to the Tukey test (*P*<0.05).

Photosynthetic rates, chlorophyll content and chlorophyll fluorescence

Inoculation of *B. salmalaya* strain 139SI in the fertilized palm seedlings (T2) resulted in significantly higher chlorophyll contents than in C2 and T1, exhibiting increases of 1.54% and 1.61%, respectively (Table 3). However, these values were statistically insignificant. In contrast, the chlorophyll content reading for the palm seedlings in C1 was the lowest, which confirms the earlier observation of chlorosis symptoms. Results of this study also found that the amount of chlorophyll content in the leaf had a significant effect on the photosynthetic rate, as the value shows the rate is directly proportional to the chlorophyll content. Photosynthetic rate values did not differ between C2, T1 and T2, with values ranging from 8.44 to 8.46 µmol $m^{-2}sec^{-1}$. The same pattern of results was also observed for chlorophyll fluorescence values, in which the different treatments received by the seedlings had an insignificant effect on the readings. The chlorophyll fluorescence values ranged between 0.819 µmol $m^{-2}sec^{-1}$ in T2 and 0.804 µmol $m^{-2}sec^{-1}$ in C1.

Analysis of nutrient content in soil

Inoculation of *B. salmalaya* strain 139SI increases the acidity of the soil. The result shows that the soil pH of T2 samples decreases to 5.14 compared to 6.73 in C1 Table 4). The application of fertilizer in C2 brought the soil pH even lower compared to C1 and T1, as the results show the soil pH of T2 and C2 were 4.74 and 4.72, respectively. Soil analysis also reveals that inoculation with strain 139SI and fertilizer application significantly changed the N concentration in soils. At the beginning of

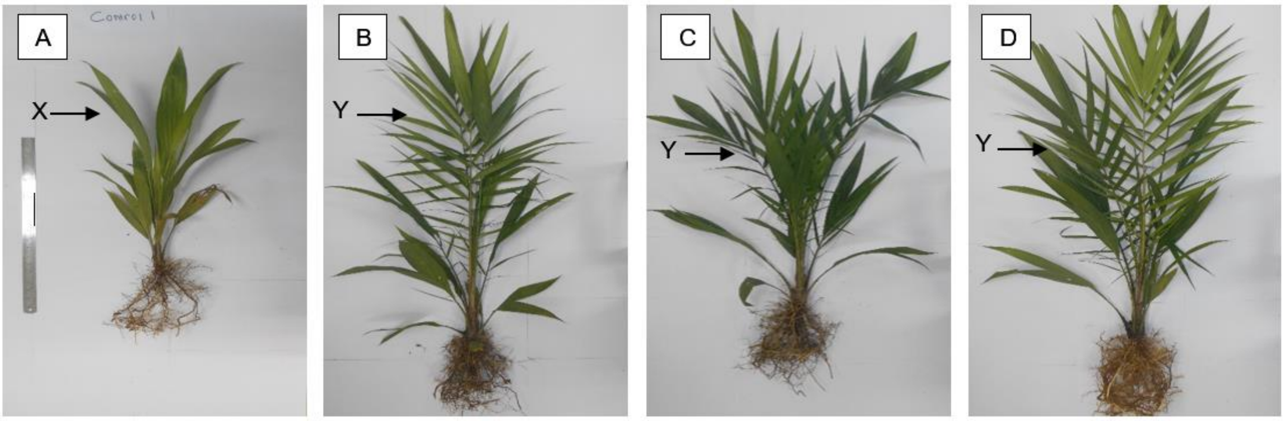

Figure 3: Oil palm seedling in A) untreated control (C1), B) fertilized control (C2), C) *B. salmalaya* strain 139SI inoculation (T1) and D) *B. salmalaya* strain 139SI inoculation + fertilizer (T2) after four months of treatment. In contrast to the stunted palms observed in C1, the vegetative growth of palms in C2, T1 and T2 showed enhancement. Palms in C1 produced small, bifurcated leaves (X), whereas palms in C2, T1 and T2 exhibited mature pinnate leaves (Y).

Table 3: Effects of *B. salmalaya* strain 139SI inoculation on photosynthetic activities of oil palm seedlings after four months of treatment.

Treatments	Photosynthetic parameter		
	Chlorophyll content (nmol/cm^{2})	Photosynthetic rate (µ m^{-2} sec^{-1})	Chlorophyll fluorescence
Untreated (C1)	59.24 ± 1.04^{b}	8.08 ± 0.27^{b}	0.804 ± 0.01^{a}
Fertilizer (C2)	62.90 ± 1.50^{a}	8.44 ± 0.24^{a}	0.817 ± 0.01^{a}
B. salmalaya strain 139SI (T1)	62.86 ± 1.08^{a}	8.46 ± 0.20^{a}	0.816 ± 0.01^{a}
B. salmalaya strain 139SI + Fertilizer (T2)	63.87 ± 4.01^{a}	8.45 ± 0.22^{a}	0.819 ± 0.01^{a}

The individual values are depicted as mean ± standard error. Different letters indicate significant differences between treatments according to the Tukey test ($P<0.05$).

the treatment, the concentration of N was 0.10% and inoculation of *B. salmalaya* strain 139SI significantly elevated the amount to 0.19%. The concentration of available N in soil sample T1 was also significantly higher than the N concentration in sample C1 (0.13%). However, without fertilizer, the inoculation of *B. salmalaya* strain 139SI had no significant effects on other nutrient concentrations in the soil.

Nutrient analysis of root samples

Inoculation of *B. salmalaya* strain 139SI improves the nutrient concentration in the root of the palm samples displayed in Table 5. The result of the nutrient analysis shows the concentration of primary macronutrients N, P and K in the root sample of T1 was 1.11%, 0.23% and 0.90%, respectively, a significant increase of 46.15%, 53.33% and 28.57% compared to the concentration of N, P and K in C1. However, the nutrient enhancement was still lower than the value recorded on palm-received fertilizer, C2. The best improvement in the percentage of nutrient concentration was recorded in T2. The increase of N, P, K, Mg and Fe in this group was the highest compared to the other treatments. Meanwhile, the mean differences in concentrations of Ca, S and Cu among all groups of treatments were found to be statistically insignificant.

Nutrient analysis of shoot samples

Analysis of the nutrient concentration of the upper parts of the palm revealed that the nutrient concentration in the palm sample T1 was higher compared to C1 (Table 6). The shoot samples of T1 demonstrated a significant increase in the concentrations of N, P and K, with percentage increments of 238%, 310% and 31%, respectively, compared to those of C1. Inoculating *B. salmalaya* strain 139S1 also significantly enhanced the concentration of other nutrients in shoot samples of T1, except for S, where the increase was marginal compared to C1. It also found that the enhancement in the concentration of N, Mg, Ca, Cu and Fe in the palm upper part of T1 was comparable to C2. On the other hand, the concentration of P, K and S content in the shoots sample of T1 was significantly lower than in C2. The positive effects of adding *B. salmalaya* strain 139SI inoculant to the fertilized palm could be observed in the outcome of nutrient concentration analysis in samples of T2. A higher nutrient concentration in palm shoots of T2 than in the fertilized palm shoots of C2 was recorded in all nutrients

Table 4: Effects of *B. salmalaya* strain 139SI inoculation on pH and nutrient availability in rhizosphere soil after four months of treatment.

Treatment	pH	Elements (mg/kg soil)					
		N	P	K	Mg	Ca	S
Untreated (C1)	6.73 ± 0.14^{c}	0.13 ± 0.00^{c}	0.20 ± 0.00^{b}	0.07 ± 0.01^{b}	0.03 ± 0.00^{a}	0.39 ± 0.00^{a}	0.09 ± 0.01^{a}
Fertilizer (C2)	4.72 ± 0.05^{a}	0.25 ± 0.02^{a}	0.46 ± 0.01^{a}	0.26 ± 0.01^{a}	0.03 ± 0.00^{a}	0.39 ± 0.00^{a}	0.08 ± 0.01^{a}
B. salmalaya strain 139SI (T1)	5.24 ± 0.10^{b}	0.19 ± 0.00^{b}	0.21 ± 0.00^{b}	0.07 ± 0.01^{b}	0.03 ± 0.00^{a}	0.39 ± 0.03^{a}	0.10 ± 0.01^{a}
B. salmalaya strain 139SI + Fertilizer (T2)	4.74 ± 0.06^{a}	0.26 ± 0.00^{a}	0.43 ± 0.01^{a}	0.25 ± 0.02^{a}	0.03 ± 0.00^{a}	0.38 ± 0.04^{a}	0.08 ± 0.00^{a}

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to the Tukey test ($P<0.05$).

Table 5: Nutrient content of oil palm seedling root samples after four months of treatment with *B. salmalaya* strain 139SI inoculant.

Treatment	Percentage (%)							
	N	P	K	Mg	Ca	S	Cu	Fe
Untreated (C1)	0.80 ± 0.04^{c}	0.15 ± 0.00^{c}	0.70 ± 0.04^{b}	0.13 ± 0.00^{b}	0.19 ± 0.01^{a}	0.23 ± 0.01^{a}	0.05 ± 0.01^{a}	0.01 ± 0.01^{c}
Fertilizer (C2)	1.32 ± 0.02^{a}	1.28 ± 0.00^{a}	1.59 ± 0.05^{a}	0.17 ± 0.01^{a}	0.18 ± 0.01^{a}	0.23 ± 0.02^{a}	0.05 ± 0.00^{a}	0.04 ± 0.01^{a}
B. salmalaya strain 139SI (T1)	1.11 ± 0.03^{b}	0.23 ± 0.01^{b}	0.90 ± 0.01^{b}	0.17 ± 0.01^{a}	0.18 ± 0.02^{a}	0.23 ± 0.02^{a}	0.05 ± 0.01^{a}	0.02 ± 0.01^{b}
Fertilizer + *B. salmalaya* strain 139SI (T2)	1.54 ± 0.04^{a}	1.32 ± 0.01^{a}	1.58 ± 0.04^{a}	0.17 ± 0.01^{a}	0.18 ± 0.00^{a}	0.24 ± 0.03^{a}	0.06 ± 0.01^{a}	0.04 ± 0.01^{a}

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to the Tukey test ($P<0.05$).

Table 6: Nutrient content of oil palm seedling shoot samples after four months of treatment with *B. salmalaya* strain 139SI inoculant.

Treatment	Percentage (%)							
	N	P	K	Mg	Ca	S	Cu	Fe
Untreated (C1)	0.72 ± 0.03^{c}	0.20 ± 0.02^{c}	0.70 ± 0.06^{c}	0.13 ± 0.01^{c}	0.18 ± 0.00^{b}	0.20 ± 0.02^{b}	0.05 ± 0.01^{b}	0.03 ± 0.01^{d}
Fertilizer (C2)	2.73 ± 0.17^{b}	1.31 ± 0.02^{a}	1.73 ± 0.04^{a}	0.26 ± 0.03^{b}	0.72 ± 0.11^{a}	0.43 ± 0.01^{a}	0.07 ± 0.01^{a}	0.06 ± 0.00^{ab}
B. salmalaya strain 139SI (T1)	2.44 ± 0.05^{b}	0.82 ± 0.00^{b}	0.92 ± 0.01^{b}	0.22 ± 0.02^{b}	0.46 ± 0.04^{a}	0.27 ± 0.01^{b}	0.08 ± 0.01^{a}	0.05 ± 0.01^{b}
Fertilizer + *B. salmalaya* strain 139SI (T2)	3.31 ± 0.01^{a}	1.38 ± 0.00^{a}	1.84 ± 0.01^{a}	0.34 ± 0.01^{a}	0.88 ± 0.01^{a}	0.57 ± 0.02^{a}	0.08 ± 0.02^{a}	0.07 ± 0.00^{a}

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to the Tukey test ($P<0.05$).

measured. However, a statistically significant increase in the concentration of nutrients was recorded in N and Mg only. The results of nutrient concentration analysis also indicate that most of the nutrients were higher in the palm's upper part than the root.

Analysis of plant nutrient uptake and nutrient use efficiency

In general, inoculation of *B. salmalaya* strain 139SI significantly affects the nutrient uptake of oil palm seedlings (Table 7). In contrast to the percentage of nutrient concentration in the palm samples, the difference in increment of nutrient uptake in T1 palm seedlings was statistically significant, as the results showed that nutrient uptake in this group increased dramatically compared to C1. The N uptake of palm seedlings in T1 recorded the highest increment by sixfold, followed by Mg and Cu by more than threefold compared to C1. Nevertheless, the increment of nutrient uptake by the palm seedlings of T1 was not as high as that recorded in the control treatment of C2. As anticipated, the fertilized palm inoculated with the *B. salmalaya* 139SI strain showed a more efficient nutrient absorption compared to the palms in C2 and T1.

Table 7: Effects of *B. salmalaya* strain 139SI inoculation on nutrient uptake of oil palm seedlings.

Treatments	Plant nutrient uptake (g/plant)							
	N	P	K	Mg	Ca	S	Cu	Fe
Untreated (C1)	0.512 ± 0.04^{d}	0.12 ± 0.0 3^{d}	0.47 ± 0.03^{d}	0.09 ± 0.00^{c}	0.12 ± 0.00^{d}	0.14 ± 0.01^{d}	0.03 ± 0.00c	0.01 ± 0.00^{d}
Fertilizer (C2)	4.69 ± 0.50^{b}	3.00 ± 0.11^{b}	3.84 ± 0.11^{b}	0.50 ± 0.01^{b}	1.04 ± 5.03^{b}	0.76 ± 0.02^{b}	0.14 ± 0.00^{b}	012 ± 0.01^{b}
B. salmalaya strain 139SI (T1)	3.71 ± 0.11^{c}	1.10 ± 0.12^{c}	1.90 ± 0.12^{c}	0.41 ± 0.00^{b}	0.67 ± 0.02^{c}	0.52 ± 0.01^{c}	0.14 ± 0.01^{b}	0.07 ± 0.01^{c}
B. salmalaya strain 139SI + Fertilizer (T2)	6.94 ± 0.14^{a}	3.86 ± 0. 31^{a}	4.89 ± 0.12^{a}	0.73 ± 0.02^{a}	1.52 ± 0.01^{a}	1.16 ± 0.03^{a}	0.20 ± 0.01^{a}	0.16 ± 0.01^{a}

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to the Tukey test (*P*<0.05).

Table 8: Effects of *B. salmalaya* strain 139SI inoculation on nutrient use efficiency of oil palm seedlings.

Treatments	g biomass/ g applied nutrient		
	N	P	K
Fertilizer (C2)	0.372 ± 0.06^{b}	0.461 ± 0.03^{b}	0.268 ± 0.01^{b}
B. salmalaya strain 139SI (T1)	0.285 ± 0.03^{c}	0.157 ± 0.02^{c}	0.113 ± 0.02^{c}
B. salmalaya strain 139SI + Fertilizer (T2)	0.573 ± 0.04^{a}	0.600 ± 0. 03^{a}	0.351 ± 0.02^{a}

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to the Tukey test (*P*<0.05).

Table 9: Pearson's correlation coefficients of plant growth, chlorophyll content and photosynthetic rate with nutrient uptake between all treatments.

Plant physiology	Nutrient uptake						
	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Magnesium (Mg)	Sulfur (S)	Copper (Cu)	Iron (Fe)
Stem height	0.968*	0.974*	0.955*	0.941*	0.969*	0.805*	0.979*
Stem diameter	0.979*	0.982*	0.956*	0.954*	0.978*	0.933*	0.984*
Leaf area	0.926*	0.969*	0.889*	0.916*	0.934*	0.922*	0.958*
Total plant biomass	0.929*	0.933*	0.894*	0.896*	0.937*	0.818*	0.957*
Chlorophyll content	0.858*	0.816*	0.836*	0.875*	0.876*	0.844*	0.867*
Photosynthetic rate	0.839*	0.824*	0.840*	0.744*	0.817*	0.812*	0.817*

Notes: *significant at levels of *P*<0.01.

The results of this study indicated that T2's palms exhibited significantly higher nutrient absorption than those in other treatment groups. The primary nutrients required in large amounts that restrict plant growth are referred to as essential macronutrients. The availability of these elements is very closely related to the nutrient supply in most agroecosystems. The efficiency of nutrient utilization can be improved by using *B. salmalaya* strain 139SI, as demonstrated by this study (Table 8). The effects of inoculating fertilized oil palm seedlings on nutrient use efficiency were significant. The result shows that T2 has a nutrient use efficiency value of 0.573 ± 0.04, which is 55% higher than T1.

Pearson correlation of coefficient analysis

In the present study, the amount of nutrient uptake by the palm was found to positively correlate with palm growth and photosynthetic activities (Table 9). Based on the Pearson correlation coefficient test, the palm growth parameters such as stem height, diameter, leaf area and total biomass were significantly and positively correlated with nutrient uptake, as shown by the high correlation coefficient values. The correlation analysis also revealed that the chlorophyll content of oil palm seedlings was found to positively correlate with the amount of nutrient uptake, especially N, Mg, S and Fe, with correlation coefficient values of 0.858, 0.875, 0.876 and 0.867, respectively. A significant positive correlation between nutrient uptake and the photosynthetic rate was also recorded, with correlation coefficients ranging from 0.744 (Mg) to 0.840 (K).

DISCUSSION

Generally, PGPR enhances plant growth by increasing the nutrient availability for the plant, acts as phytostimulation by producing growth hormone or serves as a biocontrol agent by reducing the adverse effects of various pathogens on plant growth and development (Hayat *et al*., 2010; Ahemad and Kibret 2014; Kumari *et al*., 2019). There has been a surge of interest in inoculant preparations containing multiple PGPRs or rhizobia in recent years, with the aim of improving their potential for promoting plant growth. Nonetheless, this approach may cause competition among PGPR for the same niches in the rhizoplane and sometimes mask the growth-promoting characteristics of specific PGPR, as discussed by Shah *et al*. (2021). Using a solitary strain of PGPR with multiple traits that promote plant growth provides a significant advantage compared to utilizing numerous strains of PGPR in an inoculant.

In the present study, integrating the application of *B. salmalaya* strain 139SI inoculant with fertilizer on oil palm seedlings produced profound synergistic effects on various growth parameters, especially the biomass of the palm. This interesting feature is noticed based on the booster effects of *B. salmalaya* strain 139SI inoculant on palm growth with the presence of inorganic fertilizer. The group treatment that involved the application of both *B. salmalaya* strain 139SI inoculant and fertilizer yielded significantly enhanced palm vegetative growth and nutrient concentration as opposed to the group that received only fertilizer. Significant enhancement of the plant's nutrient use efficiency had a notable impact on the increase in oil palm seedling biomass recorded in T2. According to Hawkesford *et al*. (2016) nutrient use efficiency is defined as how well plants use the available mineral nutrients. It can be measured as biomass per unit input of nutrient content in fertilizer. Thus, the efficiency of nutrient use by the plants depends on their ability to take up the nutrients from the soil. Nutrients supplied through fertilizers are considered efficient when maximum plant biomass is obtained with the minimum possible amount of fertilizer application. The outcomes of the nursery trial are consistent with our previous investigation, which unveiled several beneficial traits of the *B. salmalaya* strain 139SI that enhance plant growth. These traits include involvement in nitrogen fixation, phosphate solubilization, production of indole-3-acetic acid (IAA) and siderophore production (Azri *et al*., 2018). All these capabilities of the *B. salmalaya* strain 139SI contribute to improved nutrient uptake and utilization by the plant, ultimately leading to enhanced growth, as seen in T1 and T2.

Obviously, inoculation with *B. salmalaya* strain 139SI also induced massive root growth in oil palm seedlings, as observed in T1 and T2. The enhanced root development of the 139SI-inoculated palm seedlings is manifested by the increase in the root's dry weight compared to the fertilized palm. This strongly suggested the influence of the IAA produced by the *B. salmalaya* strain 139SI on root growth. Multiple findings from the preceding research have indicated that IAA-producing PGPR plays a significant role in facilitating the enhanced development of both deeper and more extensively distributed root systems in plants. This physiological adaptation enables plants to effectively access greater reservoirs of nutrients, thereby leading to an overall improvement in nutrient use efficiency. This advantage arises from the plants' ability to capitalize on a greater soil volume through the expansion of their root architecture (Thilagar *et al*., 2016; Calvo *et al*., 2017; Panigrahi *et al*., 2020).

Interestingly, inoculation of *B. salmalaya* strain 139SI also induced acidification of rhizosphere soil by 1.5 pH units, as observed in T1. In general, this feature is associated with the use of phosphate-solubilizing bacteria, especially in soils with a neutral or alkaline pH. Phosphate-solubilizing bacteria lower the pH of rhizosphere soil by producing low molecular weight organic acids such as gluconic and ketogluconic acids to dissolve soil P, as discussed by Liu *et al*. (2013) and Timofeeva *et al*. (2021). The significant rise in P uptake observed in inoculated oil palm seedlings as compared to untreated ones in the present study indicates that the combined effect of IAA phytostimulation on root growth and P solubilization can assist in the acquisition of P by the plant. Meanwhile, according to Miransari (2013), only a small portion of P from fertilizer is available for the plant, as the rest becomes inaccessible due to precipitation. Incorporating phosphate solubilizing PGPR with fertilizer is an effective approach to enhancing the availability of P in the soil. This is due to the ability of such PGPR to convert the insoluble form of P into soluble forms (Oufdou *et al*., 2016; Ateş *et al*., 2022). A slight decrease in rhizosphere soil pH also can help increase the solubility of nutrients and consequently improve plant nutrient uptake (Gómez-Suárez *et al*., 2020). Thus, the combination of the rhizosphere acidifying potential resulting from *B. salmalaya* 139SI inoculation, which could further increase the availability of sparingly soluble nutrients such as P and other micronutrients, together with the highly significant stimulation of root growth, can induce synergistic effects on nutrient acquisition and nutrient use efficiency. Moreover, the Pearson correlation test revealed significant positive results, indicating a robust association between root growth and nutrient uptake. This finding further elucidates the higher nutrient content recorded in the inoculated T1 compared to the untreated control despite both treatments receiving no fertilizer.

The application of inorganic fertilizer resulted in a decrease of two units in soil pH for both C2 and T2. Urea in inorganic fertilizers must undergo a transformation process before it becomes accessible to plants. This process involves the hydrolysis of urea in the soil into ammonium and CO_2 gas. The formation of hydrogen ions (H^+) during the nitrification of ammonium in fertilizers will cause soil acidification in the rhizosphere. Furthermore, ammonium also induces acidification of rhizosphere soil due to proton excretion by root cells. The hydrogen ion is secreted during ammonium absorption by plant roots to maintain charge balance across the membranes of the plant cell walls (Xin *et al*., 2019). Acidic soil conditions can negatively impact nutrient availability and microbial activity, potentially limiting plants' uptake of essential nutrients. Previous observations reported by Liu *et al*. (2013) and Arif *et al*. (2016) showed that the addition of a non-endophytic associative PGPR inoculant significantly increased fertilizer use efficiency, thus minimizing the detrimental effects of inorganic fertilizer application, such as soil acidification. Based on their findings, the parameters related to root development, such as root dry weight or length, exhibited significant increases, indicating that the improvement in root system development facilitates enhanced nutrient absorption from the fertilizer. Improved nutrient acquisition due to the enhanced root growth-promoting effects of the inoculant was also clearly demonstrated by oil palm seedlings of T2. Thus, integrating PGPR with fertilizer could be a potential strategy in nutrient management systems to increase crop production and, at the same time, achieve sustainable practices in agriculture.

Improving soil fertility by inoculating beneficial microbes, such as the *B. salmalaya* strain 139SI used in this study, is environmentally friendly and economically feasible to reduce synthetic N fertilizer application. In the present study, the increased N concentration of T1 rhizosphere soil can be hypothesized due to the ability of *B. salmalaya* strain 139SI to fix the atmospheric N_2. Enhanced N concentration in the vicinity of plant roots due to PGPR inoculant was also reported previously (Adesemoye *et al*., 2010; Prasanna *et al*., 2016). In other reports, using the ^{15}N isotope dilution technique, Kuan *et al*. (2016) suggest that non-symbiotic PGPR can provide crops with significant quantities of N derived from the atmosphere. In contrast, Biswas *et al*. (2000) suggested that the boost in N absorption resulting from the use of specific rhizobia strains was probably caused by alterations in growth physiology or root morphology rather than biological nitrogen fixation (BNF). This is because the BNF process requires a high amount of energy, and non-symbiotic bacteria have low metabolic activity, which restricts their ability to fix substantial amounts of N for plant utilization as they compete for root exudates in the rhizosphere (Martínez-Viveros *et al*., 2010). Furthermore, Pham *et al*. (2017) and Roley *et al*. (2019) found that the amount of N obtained from biological nitrogen fixation (BNF) in the tissues of N-fixer-inoculated plants was negligible. This provides evidence to support the idea that BNF did not significantly contribute to the plants' N content. Considering the widely acknowledged challenges in translating observations of rhizobacteria's BNF properties from artificial media to the conditions present in the rhizosphere, it is suggested that the observed boost in N uptake in T1 and T2 palm trees following inoculation is more likely the outcome of IAA phytostimulation-induced improvement in root morphology. Further validation using the ^{15}N isotope technique is required to substantiate the capability of the *B. salmalaya* strain 139SI to augment the proportion of N derived from the atmosphere in plant tissue.

At the end of treatment, a mild chlorotic symptom was observed on the leaves of untreated palms, and subsequent analysis verified that these palms had lower chlorophyll content than the other treatments. Observations also revealed that chlorophyll levels directly affected the photosynthetic process, as the palm that was adequately nourished recorded a higher photosynthetic rate than the malnourished palm. It is suggested that the increase in chlorophyll levels of palm leaves is the result of the improved uptake of nutrients from the soil, a notion reinforced by the results of a correlation analysis that revealed a significant association between chlorophyll levels, photosynthetic parameters and nutrient uptake, particularly N, Mg and Fe. As discussed in detail in the other report, these elements play a critical role in the chlorophyll synthesis and photosynthesis processes. N and Mg are components in the molecular structure of chlorophyll, which explains the positive correlation observed between N and Mg levels and chlorophyll contents (Shi *et al*., 2021). Additionally, Mu and Chen (2020) revealed that N uptake influences leaf expansion and photosynthetic activity. Besides that, the Mg ion is involved in the activation of ribulose bisphosphate carboxylase, an enzyme that catalyzes the carbon fixation process in the dark phase of photosynthesis (Bloom, 2019).

The observed positive correlation between chlorophyll levels in palm leaves and Fe uptake is consistent with previous research that showed a decrease in photosynthetic pigments due to low Fe uptake (Osório, *et al*., 2014; Gama *et al*., 2016; Li *et al*., 2021). Fe is an essential mineral element for plants. Several cellular processes rely on Fe, including respiration, chlorophyll biosynthesis and photosynthesis (Kroh and Pilon, 2020; Schmid *et al*., 2020). Mushtaq *et al*. (2022) and Kerbab *et al*. (2021) reported that plants treated with a siderophore extracted from PGPR exhibited an increase in Fe content and an improvement in chlorophyll synthesis. In addition, Radzki *et al*. (2013) reported that treating Fe-starved tomato plants with the siderophore-producing bacteria *Chryseobacterium* C138 was effective in providing a bioavailable form of Fe, which led to a significant increase in chlorophyll levels and Fe content. Therefore, the increased Fe content of the palm inoculated with the *B. salmalaya* 139SI strain indicates that the siderophore produced by the strain and the induction of root growth through IAA phytostimulation may aid in Fe uptake.

CONCLUSION

This nursery experiment successfully demonstrated that the inorganic fertilizer with the *B. salmalaya* strain 139SI inoculant enhanced nutrient uptake and plant growth. Inorganic fertilizer increased soil nutrients, while the inoculant stimulated root growth, resulting in better nutrient acquisition. The strain's ability to increase nutrient availability in the soil through siderophore production and phosphate solubilization also contributes to enhanced nutrient uptake. This synergy between inorganic fertilizers and *B. salmalaya* strain 139SI holds promise for optimizing plant nutrient uptake, making this a cost-effective and eco-friendly way to improve nutrient availability in soil.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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