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Biodegradable plant pots made from dried banana pseudo-stems enriched with a *Bacillus* sp.-biochar composite as an eco-friendly alternative to plastic pots

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ABSTRACT Agricultural plastic waste is a major environmental pollutant due to its non-biodegradable nature. This study discusses the production of biodegradable pots (bio-pots) using a biochar composed of banana pseudo-stems and *Bacillus* sp. The isolated *Bacillus* sp. produced indole-3-acetic acid (IAA), solubilized potassium and phosphate, and secreted siderophores immobilized in banana pseudo-stem biochar. X-ray diffraction analysis revealed $CaCO_3$ and KCl as the major elements, aside from carbon, released to the soil. Bio-pots were made from banana pseudo-stem biochar mixed with a *Bacillus* sp.–biochar composite at various formulations: 0%, 1%, 3%, 5%, and 10%. Mechanical testing indicated that the porous structure of the biochar contributed to low pot density and tensile strength. Moreover, the air-filled spaces within the biochar enhanced water absorption, correlating with the amount of biochar used. Marigolds were cultivated outdoors in the bio-pots to assess growth and yield. Our findings showed that those grown in biopot-4 (10%) displayed improved growth and yield compared to the control group (grown in the ground). After 10 weeks, the control plants became infected with fungi and aphids, whereas those grown in biopot-4 remained unaffected. In summary, bio-pots incorporating 10% *Bacillus* sp.–biochar are eco-friendly, reducing the need for chemical fertilizers, fungicides, and insecticides, while contributing to environmental sustainability. Moreover, the combination of biochar and *Bacillus* sp. is more effective than an unmixed form, since *Bacillus* sp. can inhabit and propagate in biochar pores if the conditions are otherwise unsuitable for growth.

KEYWORDS *Bacillus* sp.; Biochar; Biodegradable pot; Marigold; PGPR

1. Introduction

Due to global warming, many countries have launched campaigns focused on reducing, reusing, recycling, and promoting the use of renewable materials. A notable area of concern is the reduction of plastic waste, which decomposes slowly. Plastic pots and polybags are major environmental pollutants, particularly in agriculturally intensive regions. They are primarily composed of petroleum-based polymers and are favored for their cost-effectiveness and desirable mechanical properties, including reusability, durability, lightweight nature, and resistance to extreme environmental conditions. However, less than 10% of plastic pots are recycled, and their accumulation in the ground contributes to reduced soil porosity, air circulation, microbial activity, and soil fertility (Tomadoni et al. 2020). To address these environmental concerns, biodegradable pots made from organic materials have emerged as a sustainable alternative for agriculturists and consumers. Previously, biodegradable pots have been produced using various organic materials, such as water hyacinth, banana pseudo-stems, textile and paper waste, mushroom lump, and palm bunches (Kaewmanee 2021; Juanga-Labayen and Yuan 2021). These pots exhibited desirable mechanical and physical properties such as high water absorption and moisture content, as well as resistance to tensile strength and elongation during break tests. Additionally, biodegradable pots can be planted directly into the soil, saving agriculturists time by reducing cleanup efforts and eliminating the need for pot disposal. This planting method also promotes root elongation and nutrient accumulation in plants; upon decomposition, the pots contribute organic matter to the soil and act as a natural fertilizer, supporting plant growth (Tomadoni et al. 2020; Fuentes et al. 2021).

Thailand is one of the world's major agricultural producers, cultivating numerous crops such as rice and fruits. However, these extensive agricultural practices often result in considerable agricultural waste. Banana is a commercially important crop in Thailand, given its ease of cultivation and numerous varieties available for purchase.

Post-harvest, the banana pseudo-stem is typically discarded or used as a slow-decomposing fertilizer. In this study, we aimed to produce biodegradable pots (bio-pots) using biochar derived from banana pseudo-stems. Biochar is characterized by its porous structure, which enhances water absorption, moisture retention and air flow, essential for plant growth. Because of its carbon-rich composition, biochar can also be exploited as a carbon sink for improving soil quality and crop yield. Leng et al. (2021) have shown that the pores in biochar were suitable as habitats for microorganisms such as bacteria (0.3–3 µm), protozoa (7–30 µm) and fungi (2–80 µm). Therefore, this study combined plant growth-promoting rhizobacteria (PGPR) with biochar to produce biodegradable pots designed to improve plant growth.

PGPR are beneficial bacteria such as *Bacillus* sp. and Actinomycetes that facilitate plant growth through direct (e.g., nitrogen (N) fixation, phosphorous (P) and potassium (K) solubilization, siderophore production, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and IAA production) and indirect (e.g., producing cell wall degrading enzymes and antibiotics or inducing systemic resistance to protect plants against pathogens and improve growth and yield) mechanisms. PGPR are increasingly used in sustainable agricultural practices over fertilizers and pesticides, despite the long time needed to implement these strategies. Previous studies have shown that PGPR can enhance phytohormone (e.g., IAA) levels, promote antioxidant enzyme (e.g., super oxide dismutase, polyphenol oxidase and peroxidase) activity, and reduce disease incidence (e.g., blast disease in rice), leading to improved yield in crops such as rice and mung bean (Hussein et al. 2016; Rais et al. 2017, 2018; Wagi and Ahmed 2019; Zhou et al. 2021). So, PGPRs are valuable for agricultural soil to promote plant growth and improve soil health, leading to be sustainable agriculture. Since, PGPRs act as a green substance instead of chemical inputs by producing plant hormones, IAA and solubilizing and chelating minerals for plant to be easy uptake, moreover, PGPRs increase plant tolerance to biotic and abiotic stresses and defeating soil-borne diseases. Then, the present study aimed to produce bio-pots using banana pseudo-stem-derived biochar enriched with *Bacillus* sp. to improve marigold growth and yield while limiting the use of fertilizers and pesticides.

2. Materials and Methods

2.1. PGPR screening and molecular identification

Soil samples (10 g) were collected at a depth of 5–10 cm from several sites in Lop Buri, Thailand. A 10-fold dilution was set up using 1 g of each soil sample and distilled water. The mixture was homogenized before spreading on nutrient agar (NA) and incubating at 37 °C for 24 h (Hashmi et al. 2025). Next, single-colony cultures were grown and subjected to colony identification, gram staining, and catalase testing. Positive *Bacillus* sp. colonies were screened for plant growth promoting properties, including IAA production, phosphate solubilization, potassium solubilization, and siderophore production.

Colonies selected as positive for having all PGPR properties were subjected to DNA extraction. PCR was performed using 16s rDNA primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The reaction setup was as follows: one cycle of denaturing at 94 °C for 5 min, 30 cycles, each at 94 °C for 30 min (denature stage), 58 °C for 30 min (annealing stage), and 72 °C for 1 min (extension stage), followed by a last extension stage for one cycle at 72 °C for 10 min. The PCR products (5-µL volumes) were analyzed on 1% (w/v) agarose gel in 0.5× TBE buffer. The PCR products were sequenced at AGCT company, Thailand, and the results were compared against the NCBI Database (http://www.ncbi.nlm.nih.gov). Phylogenetic trees were constructed using the MEGA X software (Devi et al. 2023).

2.2. IAA and siderophore production

All isolates were incubated in 10 mL of nutrient broth (NB) supplemented with 0.2% tryptophan. The cultures were shaken at 200 rpm for 48 h before centrifugation at 8,000 rpm for 15 min. The supernatant was added to 2 mL of Salkowski reagent and kept in a dark box for 30 min. Isolates producing IAA would turn pink, and the intensity of color was measured at 530 nm to quantify the amount of IAA produced. Each isolate was tested in three replicates (Devi et al. 2023).

Each isolate was spread (approximately 2 cm) on the Chrom azurol S (CAS) agar and incubated at 35 °C for 7 days. A color change of the CAS agar from blue to orange indicated siderophore activity. Each isolate was tested in triplicate (Sham et al. 2020).

2.3. Phosphate and potassium solubilization

Isolates were inoculated into NB medium and incubated for 24 h. Next, 0.1 mL of each of the inoculated sample was dropped on Pikovskaya's agar and incubated at 35 °C for 7–14 days. A clear zone appeared around each positive colony. Each isolate was tested in triplicate (Devi et al. 2023).

Each inoculated sample (0.1 mL) in NB was dropped on Alexsandrow agar and incubated at 35 °C for 7–14 days. A clear zone appeared around each positive colony. Each isolate was tested in triplicate (Raji and Thangavelu 2021).

2.4. Biochar preparation, scanning electron microscopy, and X-Ray diffraction

Dried banana pseudo-stems were sliced into small pieces, and the raw material was subjected to pyrolysis under low oxygen conditions. A small-scale biochar producing unit consisted of the inner circle tank (11 cm in diameter and 23 cm in height) and outer circle tank (20 cm in diameter and 30 cm in height). The raw material was placed in the

inner circle tank, and the outer circle tank was filled with firewood and closed with the lid. Pyrolysis was carried out at 400 °C for 4 h, which were the conditions required to completely burn cellulose and hemicellulose.

The biochar surface morphology was analyzed via scanning electron microscopy (SEM). Each sample was coated with platinum and crossed-sectioned before being observed under the scanning electron microscope. Individual elements on the biochar surface were analyzed using wavelength dispersive X-ray spectroscopy (WDX).

X-ray diffraction analysis was performed using a multipurpose X-ray diffraction system (SmartLab®SE). Biochar samples were crushed and sieved to achieve a size less than 50 microns. Then, 20 mg of sample was tested over two ranges spanning 10–120°, using a step size of 0.02°. Calculations were performed using the following equation (Fatimah et al. 2022):

$$D = \frac{K\lambda}{B\cos\theta} \quad (1)$$

D is the mean size (nm)
K is shape factor about 0.9
λ is X-ray wavelength
B is the full width at half the maximum (FWHM) in radians of x-ray diffraction peak
θ is the Bragg's angle

2.5. Bacillus–biochar composite preparation and biodegradable molding

Biochar was crushed, sieved, and sterilized at 121 °C and 15 psi for 20 min before mixing with *Bacillus* sp. inoculated in NB medium for 72 h. Biochar (50 g) was mixed with 100 mL of *Bacillus* liquid culture, and the mixture was shaken at 150 rpm for 3 h. Subsequently, the mixture was transferred on sterile plates to dehydrate in the oven at 50 °C for 2–3 h until dry. The dried mixture stored at 4 °C until further use.

Dried banana pseudo-stems were sliced to small pieces, crushed, and sieved through a 2-mm plate. The raw material was incubated at 105 °C for 3 h, then stored at room temperature for cooling Gelatin-based composite was prepared by mixing 100 g of casava starch with 1 L of distilled water before heating at 60 °C for 15 min using a screw speed of 40 rpm until the texture was a homogenous gel.

This study used a randomized complete design with one factor of *Bacillus*-biochar formulation ratio that consisted of 5 levels: Biopot-0 (control), Biopot-1 (1% Bacillus–biochar composite), Biopot-2 (3% Bacillus–biochar composite), Biopot-3 (5% Bacillus–biochar composite), and Biopot-4 (10% Bacillus–biochar composite).

Biodegradable pots were molded into a conical shape 10 cm high and 1 cm thick, with an upper diameter of 9.5 cm and a lower diameter of 7 cm. First, each formulation was mixed with 200 g of gelatin and homogenized to make a ready-mold paste. The ready-mold paste was shaped into pots manually, and the pots were dried at 60 °C for 3 h for constant weight. Each formulation was prepared with five replications.

2.6. Testing pot properties

2.6.1 Density

Following the method described by (Jaya et al. 2022), bio-pot specimens were dried in an oven, weighed (W), and measured using a Vernier caliper. Volume (V) was calculated by multiplying the area and thickness of the specimen. The density of bio-pots was calculated using the following equation:

$$Density\ (g/cm^3) = \frac{W}{V} \quad (2)$$

2.6.2 Expanding test and water absorption test

Following the method described by (Kaewmanee 2021), three square specimens (5 × 5 cm) of each formulation were dried in a desiccator. Then, their thickness (T_0) and weight (W_0) were determined. The specimens were then immersed in distilled water for 1 h before being weighed again (W_1). For the expanding test, excessive water was removed from the surface of the specimens. Each specimen was placed on dried surface area for 1 h before weighing (T_1). Expansion and water absorption were calculated using the following equations:

$$Expansion\ (\%) = \frac{(T_1 - T_0)}{T_0} \times 100 \quad (3)$$

$$Water\ absorption\ (\%) = \frac{(W_1 - W_0)}{W_0} \times 100 \quad (4)$$

2.6.3 Mechanical test

Bio-pots were subjected to the tensile test to determine their mechanical properties. Five bio-pots of each experiment were tested using a constant crosshead speed of 1 mm/sec; the distance between a crosshead and the sample was approximately 30 cm (Testure analyzer, stable micro system, UK). A four-point tensile test was performed once at the bottom and three times at the body of each bio-pot.

2.7. Plant-biopot test

The five bio-pot types were tested in outdoor conditions. The experiment was performed from November 2024 to January 2025. One marigold (*Tagetes* spp.) plant was placed into a bio-pot filled with soil before being transferred to the soil, while control plants were directly planted in soil without any containers. Control plants and bio-pot plants were watered once every two days. After 12 weeks after planting, we measured the plant height, canopy width, stem diameter, root length, stem fresh weight, root fresh weight, and root and stem dry weight of marigold plants from each treatment group. The number of floral buds, medium-opened flowers, fully opened flowers were counted. Total flower number, flower diameter, and the fresh and dried weights of flowers were also measured.

3. Results and Discussion

3.1. PGPR screening

Thirty-three isolates were obtained from 10 soil samples in Lop Buri, Thailand. The isolates were identified as Gram-positive rods capable of forming endospores and tested positive for the catalase enzyme. All colonies on NA were creamy white, rough, dry, and irregularly edged. Next, all isolates were tested to determine characteristics associated with PGPR.

3.1.1 IAA production

All isolates showed potential auxin production capabilities when grown in Salkowski's reagent supplemented with tryptophan; however, IAA production levels ranged from low (approximately 7.23 in Ba28) to high (36.76 in Ba18). Isolates Ba18, Ba11, Ba21, and Ba10, produced auxin at high concentrations, i.e., at approximately 36.76, 32.77, 31.59, and 30.92 µg/mL, respectively (Table 1).

3.1.2 Phosphate solubilization

Isolates were tested for phosphate solubilization on Pikovskaya's agar. A halo zone around colonies meant their ability to solubilize inorganic phosphorus and phosphate. The results showed that seven isolates (Ba3, Ba5, Ba24, Ba25, Ba31, Ba32, and Ba33) had phosphate solubilization abilities (Table 1).

TABLE 1 Properties of 33 isolates analyzed for various plant growth-promoting characteristics.

Isolate No.	IAA (µg/mL)	Phosphate solubilization	Siderophore	Potassium solubilization
Ba1	17.61	–	–	–
Ba2	20	–	+	–
Ba3	19.23	+	–	+
Ba4	24.64	–	–	–
Ba5	16.84	+	–	–
Ba6	21.99	–	–	–
Ba7	13.56	–	+	–
Ba8	14.51	–	+	–
Ba9	20.64	–	–	+
Ba10	30.92	–	–	+
Ba11	32.77	–	–	+
Ba12	16.68	–	+	+
Ba13	15.24	–	+	–
Ba14	15.27	–	–	–
Ba15	14.02	–	+	–
Ba16	10.48	–	–	+
Ba17	13.56	–	–	–
Ba18	36.76	–	–	+
Ba19	17.98	–	+	–
Ba20	18.46	–	+	+
Ba21	31.59	–	–	–
Ba22	15.24	–	+	–
Ba23	14.68	–	+	–
Ba24	9.34	+	+	+
Ba25	11.36	+	+	+
Ba26	17.47	–	+	–
Ba27	8.91	–	–	–
Ba28	7.23	–	–	+
Ba29	9.52	–	+	–
Ba30	11.01	–	–	–
Ba31	13.03	+	+	+
Ba32	18.46	+	+	+
Ba33	15.32	+	–	+

(+) = Isolate produced PGPR characteristics (–) = Isolate did not produce PGPR characteristics

3.1.3 Siderophore production

The CAS blue medium was used to test for siderophore production. A clear zone appeared around sixteen colonies, namely Ba2, Ba7, Ba8, Ba12, Ba13, Ba15, Ba19, Ba20, Ba22, Ba23, Ba24, Ba25, Ba26, Ba29, Ba31, and Ba32 (Table 1).

3.1.4 Potassium solubilization

Thirty-three isolates were tested for potassium solubilization on Aleksandrow agar. A clear zone was observed around fourteen isolates, namely Ba3, Ba9, Ba10, Ba11, Ba12, Ba16, Ba18, Ba20, Ba24, Ba25, Ba28, Ba31, Ba32, and Ba33 (Table 1).

3.1.5 Molecular identification

The Ba32 isolate exhibited all characteristics associated with PGPR and was selected for bioformulation with banana biochar. Then, Ba32 was verified via PCR using 16S rDNA primers, and the phylogenetic tree grouped the Ba32 to *Bacillus subtilis* clade with a bootstrap value of 76 (Figure 1).

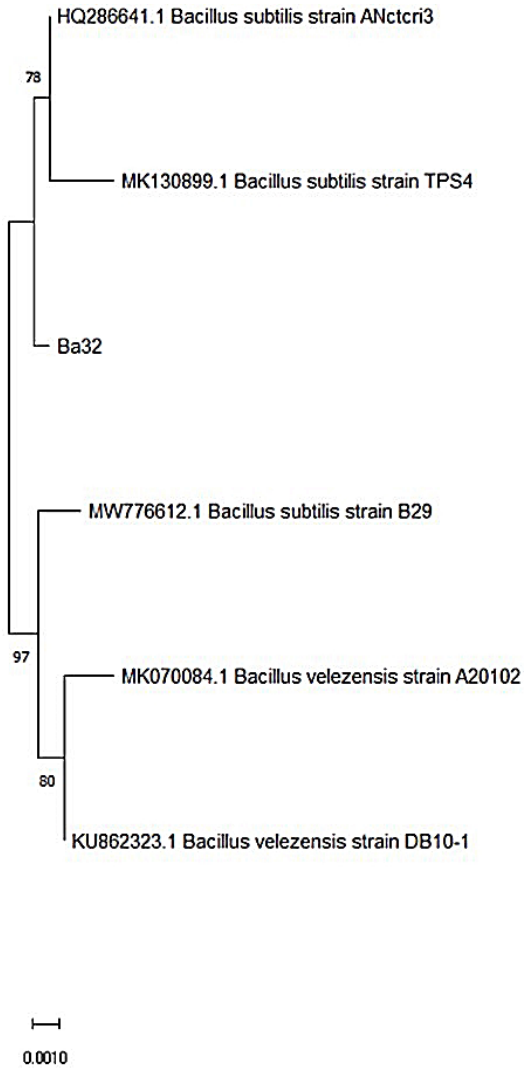


FIGURE 1 Phylogenetic tree based on the 16S rRNA gene of *Bacillus* sp., constructed for comparison with related *Bacillus* sp. species using the neighbor-joining method in MEGA X.

3.2. Biochar characterization

Following pyrolysis of dried banana stalk at 400 °C for 4 h, SEM and XRD were used to determine the pore size of the biochar and its crystallization properties, respectively. The SEM analysis revealed a pore size of approximately 20 µm, suggesting that the biochar could provide a suitable habitat for microorganisms (Figure 2a). XRD analysis showed that $CaCO_3$ and KCl were the major elements of the biochar (Figure 2b).

3.3. Biochar production

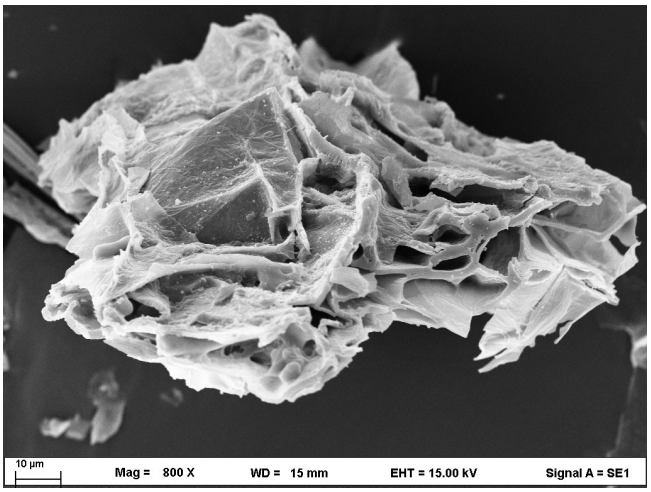
All biopots were tested to determine their physical properties. Biopot-0 (control) had the highest density (0.54 g/cm^3) and tensile strength (66.53 N), while biopot-4 exhibited the highest expansion and water absorption (approximately 365.14% and 128.38%, respectively). The results for all tested biopots are shown in Table 2.

3.4. Marigold cultivation trial using bio-pots

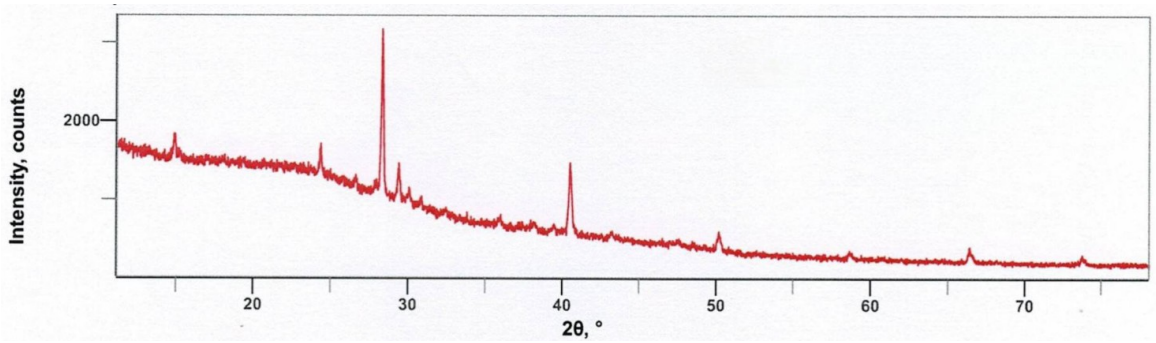
Marigolds were planted in bio-pots for 12 weeks. Table 3 and 4 shows the results obtained after planting. Marigolds planted in biopot-4 showed the highest plant height, canopy, stem diameter, root length, as well as fresh and dried weights of stems and roots, followed by plants cultivated directly in the soil without bio-pots. Moreover, plants grown directly in the soil showed superior growth than plants from the remaining groups (Figure 3). According to fluorescence, marigold plants in biopot-4 showed the highest flower number, diameter flower, and fresh and dried flower weights. Although the growth and development of plants grown directly in soil normal growth were almost identical to those grown in biopot-4, marigold plants were attacked by aphids and began to display damaged and slow growth after 10 weeks.

3.5. Discussion

Plastic pots are a major form of agricultural waste. They are used mainly during the nursery stage and to transport soil due to their lightweight nature and resistance to adverse environmental conditions and microorganisms. However, because they are primarily composed of polyethylene, these pots can be degraded over long periods of time and can inhibit root growth by limiting access to nutrients and water. To reduce plastic waste and promote plant growth, bio-pots were developed using organic materials that serve as biofertilizer once the pot degrades in the soil. In this study, bio-pots were made using banana pseudo-stem and its biochar supplemented with PGPR, specifically *Bacillus* sp. Previous studies have reported that biochar with pore sizes ranging from 0.3 µm to 3 µm can provide a suitable bacterial habitat (Leng et al. 2021). Here, the pore size of banana pseudo-stem biochar was approximately 20 µm, indicating its suitability for plant



(a)



(b)

FIGURE 2 Surface porosity of banana pseudo-stem biochar after pyrolysis at 400 °C for 4 h (a). X-ray diffractograms of banana pseudo-stem biochar (b).

growth promoting bacteria (PGPB). Moreover, the biochar exhibited desirable water absorption and carbon sequestration capabilities. Finally, XRD analysis showed that the banana pseudo-stem biochar contained the major elements $CaCO_3$ and KCl, which could be released to the plant as nutrients.

Several PGPR exist, including *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas*, and *Serratia*. Characteristics of PGPR include IAA production, phosphate and potassium solubilization, ammonia and hydrogen cyanide (HCN) synthesis, and siderophore production. In this study, *Bacillus* sp. was isolated and identified as a PGPR as it showed the highest potential among the tested strains (Kaki et al. 2013). Thirty-three isolates were screened and only one isolate, Ba32, was selected to be combined with the biochar. PCR and phylogenetic analyses showed that Ba32 was grouped in the *Bacillus subtilis* clade (Figure 1).

Five biopot types underwent mechanical testing, and the results showed that Biopot-4 exhibited the highest levels of water absorption and expansion, in relation to the addition of biochar. An increased biochar content correlated with larger pore size, which inversely affected density and tensile strength. The presence of biochar increased pore spaces within the bio-pot; therefore, biopot-0, which contained no biochar, was more compact than the others and exhibited the highest density and resistance to tensile strength tests. By contrast, the other biopots, which contained varying ratios of biochar, exhibited a slight reduc-

TABLE 2 Mechanical analysis of bio-pot.

Treatment	Density (g/cm^3)	Expansion (%)	Water absorption (%)	Tensile strength (N)
Biopot-0	0.54 ± 0.04^{a}	207.56 ± 10.05^{c}	69.79 ± 1.80^{d}	66.53 ± 4.61^{a}
Biopot-1	0.53 ± 0.02^{a}	221.85 ± 11.82^{c}	73.81 ± 2.82^{cd}	49.91 ± 6.62^{b}
Biopot-2	0.50 ± 0.02^{ab}	296.95 ± 19.82^{b}	80.00 ± 5.00^{c}	38.39 ± 0.87^{c}
Biopot-3	0.50 ± 0.01^{ab}	346.85 ± 48.66^{ab}	109.95 ± 3.28^{b}	35.30 ± 1.68^{c}
Biopot-4	0.48 ± 0.02^{b}	365.14 ± 51.76^{a}	128.38 ± 7.75^{a}	33.35 ± 3.73^{c}

**Means in the same column with different superscript letter are significantly different by DMRT at $p \leq 0.05$.

TABLE 3 Effect of various treatments on the growth of marigold (measured at 12 weeks).

Treatments	Plant height (cm)	Canopy width (cm)	Stem diameter (cm)	Root length (cm)	Stem fresh weight (g/plant)	Stem dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)
Control	$42.14^{ab} \pm 3.72$	$27.14^{b} \pm 2.48$	$0.86^{a} \pm 0.11$	$14.11^{ab} \pm 3.10$	$61.76^{ab} \pm 22.83$	$10.26^{a} \pm 3.02$	$2.71^{ab} \pm 0.84$	$0.63^{ab} \pm 0.16$
Biopot-0	$31.70^{c} \pm 5.63$	$18.50^{c} \pm 3.24$	$0.46^{c} \pm 0.15$	$12.50^{b} \pm 2.48$	$15.49^{c} \pm 9.83$	$2.02^{b} \pm 1.25$	$1.00^{c} \pm 0.32$	$0.15^{c} \pm 0.07$
Biopot-1	$31.60^{c} \pm 7.19$	$19.40^{c} \pm 3.52$	$0.44^{c} \pm 0.11$	$15.38^{ab} \pm 3.00$	$16.93^{c} \pm 8.75$	$2.23^{b} \pm 1.06$	$0.91^{c} \pm 0.34$	$0.17^{c} \pm 0.06$
Biopot-2	$32.29^{c} \pm 7.48$	$20.00^{c} \pm 5.77$	$0.52^{bc} \pm 0.04$	$14.75^{ab} \pm 5.13$	$23.50^{c} \pm 6.47$	$3.19^{b} \pm 0.79$	$1.23^{c} \pm 0.21$	$0.24^{c} \pm 0.05$
Biopot-3	$38.25^{bc} \pm 4.40$	$24.00^{bc} \pm 2.27$	$0.66^{b} \pm 0.13$	$15.25^{ab} \pm 3.25$	$39.69^{bc} \pm 20.69$	$5.39^{b} \pm 3.03$	$1.92^{bc} \pm 0.62$	$0.34^{bc} \pm 0.15$
Biopot-4	$48.00^{a} \pm 3.51$	$35.14^{a} \pm 5.61$	$0.93^{a} \pm 0.11$	$18.47^{a} \pm 1.78$	$73.77^{a} \pm 20.62$	$11.08^{a} \pm 3.98$	$3.18^{a} \pm 0.87$	$0.79^{a} \pm 0.34$

**Means in the same column with different superscript letter are significantly different as shown by DMRT at $p \leq 0.05$.

TABLE 4 Effect of various treatments on the yield of marigold (measured at 12 weeks).

Treatments	Floral bud number (flowers/plant)	Medium-opened flower number (flowers/plant)	Opened flower number (flowers/plant)	Total flower number (flowers/plant)	Diameter of flower (cm)	Flower fresh weight (g/plant)	Flower dry weight (g/plant)
Control	$13.29^{ab} \pm 6.73$	$0.71^{a} \pm 0.95$	$8.86^{ab} \pm 4.85$	$22.86^{ab} \pm 11.94$	$4.73^{a} \pm 0.70$	$3.20^{a} \pm 1.08$	$0.66^{a} \pm 0.09$
Biopot-0	$4.20^{b} \pm 2.28$	$1.20^{a} \pm 1.10$	$2.00^{c} \pm 1.87$	$7.40^{b} \pm 3.65$	$4.83^{a} \pm 0.40$	$4.30^{a} \pm 0.61$	$0.61^{a} \pm 0.12$
Biopot-1	$7.60^{b} \pm 4.83$	$1.00^{a} \pm 1.22$	$1.20^{c} \pm 1.10$	$9.80^{b} \pm 5.22$	$4.87^{a} \pm 1.37$	$4.58^{a} \pm 3.00$	$0.68^{a} \pm 0.27$
Biopot-2	$6.57^{b} \pm 6.08$	$0.57^{a} \pm 0.79$	$2.86^{c} \pm 2.04$	$10.00^{b} \pm 7.75$	$5.27^{a} \pm 1.07$	$5.13^{a} \pm 2.29$	$0.69^{a} \pm 0.25$
Biopot-3	$10.88^{ab} \pm 6.38$	$1.13^{a} \pm 1.73$	$4.63^{bc} \pm 2.45$	$16.64^{b} \pm 9.24$	$4.87^{a} \pm 0.77$	$4.39^{a} \pm 1.91$	$0.66^{a} \pm 0.26$
Biopot-4	$21.57^{a} \pm 10.53$	$3.14^{a} \pm 3.67$	$10.29^{a} \pm 3.55$	$35.00^{a} \pm 14.45$	$5.38^{a} \pm 0.52$	$5.70^{a} \pm 1.37$	$0.84^{a} \pm 0.17$

**Means in the same column with different superscript letter are significantly different as shown by DMRT at $p \leq 0.05$.

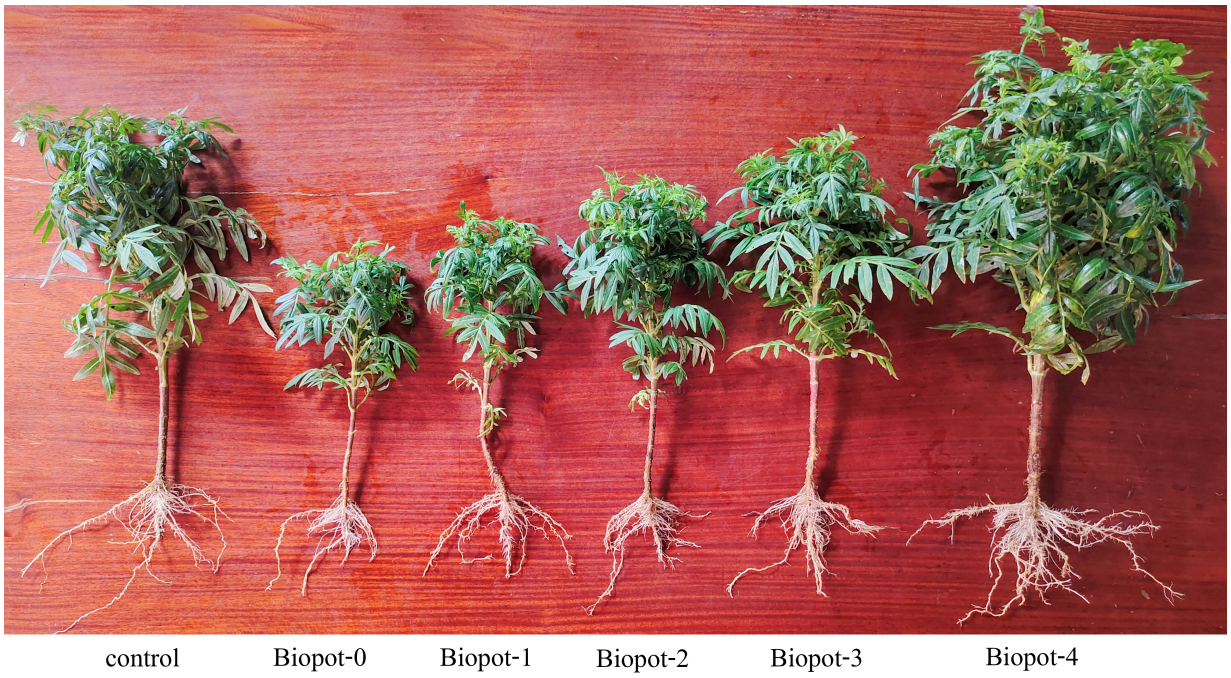


FIGURE 3 Effect of various treatments on the growth of marigold measured at 12 weeks of cultivation.

tion in density and tensile strength due to the air-permeable nature of the biochar pores. Moreover, biochar pores promoted water absorption, which was linked to increasing expansion values. Biopot-4, containing 10% *Bacillus* sp.–biochar composite, exhibited the highest water absorption and expansion because of its high *Bacillus* sp.–biochar composite ratio. Biochar can store water in external and internal pores since water molecules form hydrogen bond with oxygen and nitrogen on the biochar's surface improving water retention and soil structure including providing plant-available water that leads to healthier root growth and water uptake (Huang et al. 2021).

Previous studies have shown that biochar produced through pyrolysis at 300–400 °C was more effective as a biofertilizer and in improving soil fertility than biochar produced at 500 °C. Biochar produced at the lower temperatures showed enhanced electrical conductivity and contained increased levels of organic matter and inorganic salts, such as available nitrogen, phosphorus, potassium, humic and fluvic acids, and micronutrients (Usman et al. 2015; Ippolito 2016). Biochar also improves soil fertility by boosting carbon sequestration, soil nutrient contents, and the proliferation of rhizo-microorganisms. Although C is the major structural component, XRD anal-

ysis also revealed $CaCO_3$ and KCl as other major contributors to soil nutrient availability for plant growth, in relation to K levels in dried banana pseudo-stems. As mentioned above, biochar enhances the proliferation of rhizo-microorganisms; yet, this experiment aimed to reduce the use of chemical fertilizers by combining PGPR with biochar to convert insoluble nutrients into soluble forms.

Generally, chemical fertilizers are added to plantations to increase the availability of macronutrients for plant growth. However, chemical residues such as phosphorus and potassium become fixed in the soil in insoluble forms, rendering them inaccessible to plants. This accumulation can lead to major global challenges, including reduced crop production and environment risks. In the soil, 70% of the soluble phosphate fertilizer is bound to cations such as Al^{3+}, Ca^{2+}, Fe^{3+}, and Mg^{2+}, subsequently converting into insoluble forms that plants cannot absorb (Wu et al. 2019). Similarly, 90–98% of potassium is retained in the soil as a non-exchangeable form (Sood et al. 2023). Potassium is essential for improving plant immunity and metabolism and contributes to increased yields, while phosphorus is crucial for cell division and especially in nucleic acid synthesis, photosynthesis, energy production, homeostasis, and cell signaling (Panda et al. 2021; Pan and Cai 2023). One characteristic of PGPR is their ability to convert insoluble phosphorus and potassium to soluble forms that plant roots can use to promote growth. Previous studies have shown that potassium soluble bacteria (KSB) and phosphorus soluble bacteria (PSB) such as *Acinetobacter*, *Bacillus*, *Pseudomonas*, and *Priestia* species increase P accumulation in plant tissues, resulting in enhanced shoot and root growth (Wu et al. 2019; Phat and Diep 2021; Phringpaen et al. 2023). As shown in Table 3, Table 4, and Figure 3, marigold plants in biopot-4 exhibited the highest values acros in all parameters.

We screened *Bacillus* sp. for their capabilities as PSB and KSB, as well as for their ability to produce IAA production and siderophores. IAA is a phytohormone that promotes plant growth and development, more specifically cell division and elongation, as well as tissue differentiation. Plant roots use IAA to enhance the length and size of the primary root, as well as for lateral root formation and adventitious root development (Overvoorde et al. 2010). Our results showed that marigold plants in biopot-4 produced more lateral roots than those in other treatment groups. Moreover, the marigold stems from the biopot-4 group wer also larger than those from other groups due to the presence of IAA. IAA also stimulated stem elongation, resulting in longer and thicker stems, while inhibiting the growth of lateral branches (Yang et al. 1993). Marigold cultivated in biopot-4 exhibited the highest values in plant height, canopy width, stem diameter, and root length (Table 3 and Figure 3). Previous studies have shown that the induction of IAA and other plant phytohormones such as cytokinin, gibberellins (GA1 and GA3), abscisic acid, salicylic acid, and jasmonic acid by PGPR has contributed to improved yields and plant growth dynamics (Bigatton et al. 2024; Flaviano et al. 2025).

Although PGPR directly affect plant growth, they play an indirect role in defending plants against pathogens via systemic resistant induction, the release of antibiotics, iron depletion, lysis enzyme production, as well as antifungal metabolite production and completion (Saraf et al. 2014). Previous studies have shown that various PGPR exhibit antagonistic characteristics. For example, Ningthoujam et al. (2009) found that *Streptomyces* sp. displayed antagonistic activity against the rice fungal pathogens *Pyricularia oryzae*, *Bipolaris oryzae*, and *Fusarium oxysporum*. Furthermore, *Streptomyces* sp., together with *Trichoderma* sp., was found to secrete antibiotics against the bacterial pathogen that causes potato scab (Porto et al. 2022). In the present study, *Bacillus* sp. strain Ba32 secreted siderophores that chelate iron, an important virulence factor for plant pathogens, mainly fungi that cause plant diseases. During the growth stage, plants were infected with fungi and aphids, resulting in significant stem and leaf damage, particularly in the control group, biopot-0. By contrast, bio-pots containing PGPR exhibited less damage and improved plant growth and yield, as shown in Table 4. These findings align with those from previous studies, which showed that *Bacillus* sp. with antagonistic properties suppressed *P. oryzae* by enhancing antioxidant enzyme (Rais et al. 2016, 2017, 2018).

We did not measure decomposing time in this study. After 12 weeks of cultivation, bio-pots were cracked and left to decompose into biofertilizer. Empty biodegradable pots, composed of biomaterials and banana peels were degraded after 60 days. However, decomposing time depended on tensile strength; a higher tensile strength correlated with a reduced decomposition time due to the varying cellulose content in each bio-pot treatment group (Rafee et al. 2019).

4. Conclusions

Plastic pots and bags are major environmental pollutants that promote global warming, due to their prolonged decomposition time and recycling rates of less than 10%. Chemical fertilizers are also environmental pollutants as most of the potassium and phosphorus cannot be absorbed by plants. Bio-pots made from organic waste, banana-pseudo stem, combined with biochar and PGPR significantly promoted plant growth and yield such as plant height, stem diameter and flower production that compared with control since *Bacillus* sp. directly produced IAA production, phosphate and potassium solubilization. Moreover, these bio-pots decompose into biofertilizer, while PGPR also indirectly protects against plant pathogens. Therefore, bio-pots present a viable alternative to reduce the use of plastic in agriculture, decrease chemical fertilizer and pesticide consumption and reduce the amount of irrigation due to the water absorption properties of biochar.

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Authors' contributions

PS and PI were responsible for the project administration. PS conducted the biochar preparation and analysis, soil sample collection, isolate screening for PGPR, molecular analysis, and wrote the initial draft of the manuscript. PI designed the pot experiments, carried out the pot mechanical testing, and performed plant data collection and analysis. JD contributed as a microbial consultant. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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