

Research Article

Evaluating the phosphate solubilizing ability of novel *Bacillus* sp. strains and their effects on corn production

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Abstract

This study investigated the effects of two phosphate solubilizing bacteria (PSB) isolates, *Bacillus* sp. strain E1 and G4, and three fertilizer types on soil properties, corn growth, and yield. The results showed that PSB E1 increased soil organic matter (SOM) and available phosphorus, while G4 enhanced exchangeable potassium and cation exchange capacity (CEC). Organic fertilizers improved soil fertility, while inorganic fertilizers had a different impact. The combination of PSB G4 and organic fertilizer produced the highest yield, while PSB E1 combined with fertilizers boosted crop growth. The interaction between PSB strains and fertilizers played a significant role in determining soil properties, corn growth, and yield.

Keywords: Phosphate solubilizing bacteria, *Bacillus* sp., Fertilizer management, Corn production, Soil properties

Introduction

Phosphorus is an essential macronutrient for plant growth and development, playing a critical role in photosynthesis, cell division, and crop yield formation (Havlin *et al.*, 2014). However, most phosphorus in soil is present in insoluble forms, making it unavailable to plants. Phosphate solubilizing bacteria (PSB) are beneficial microorganisms that can solubilize phosphorus from insoluble sources, increasing its availability to plants (Rodríguez & Fraga, 1999). PSB produces organic acids, such as gluconic acid, citric acid, and succinic acid, which chelate metal ions and release phosphorus from mineral phosphates (Sharma *et al.*, 2013).

PSB have been identified as a key component of integrated nutrient management strategies, promoting sustainable agriculture practices (da Silva *et al.*, 2023). These bacteria can be applied as biofertilizers, reducing the need for chemical fertilizers and minimizing environmental pollution. Fertilizers, on the other hand, provide essential nutrients for crop growth, but their excessive use can lead to environmental degradation and economic losses (Lal, 2005).

The effectiveness of PSB varies depending on the crop and soil type, and certain strains exhibit specificity towards specific crops (Gaur, 1990; Khan *et al.*, 2014). Corn is a major global crop, and phosphorus deficiency is a significant constraint to its production (Ray *et al.*, 2013). PSB has emerged as a potential biological tool to enhance phosphorus availability, offering a sustainable approach for optimized corn crop performance.

This study aimed to evaluate the effects of two PSB strains, isolated from the rhizosphere of Bermuda grasses, on corn production, and their interaction with different fertilizer types. A pot experiment was conducted at Brgy. Anonang, San Fabian, Pangasinan, from April to July 2024,

to investigate the effects of phosphate solubilizing bacteria and fertilizer interactions on corn growth and yield.

The specific objectives of the study were to evaluate the effects of *B. subtilis* strains, fertilizer types, and their interaction on soil chemical properties, to assess the impact of *B. subtilis* strains, fertilizer types, and their interaction on soil microbial population, and to determine the influence of *B. subtilis* strains, fertilizer types, and their interaction on corn growth parameters and yield.

The study highlights the potential of PSB-fertilizer interactions to enhance corn yields, reduce environmental impact, and promote sustainable agricultural practices. By harnessing the power of PSB-fertilizer interactions, farmers and agricultural practitioners can adopt a more environmentally friendly and economically viable approach to corn production, contributing to a more sustainable food system.

Phosphate solubilizing bacteria mechanisms

Phosphate solubilizing bacteria (PSB) are considered one of the most important traits associated with plant phosphate nutrition. PSB plays a crucial role in enhancing crop performance by increasing phosphorus availability in soils. The use of PSB as an inoculant increases P uptake by plants. Microbial solubilization of phosphate in soil is correlated with the ability of microbes to produce selected organic acids and extracellular polysaccharides.

PSB solubilize or mineralize unavailable P forms in soils through various mechanisms, including: a) Rhizosphere acidification: PSB produce organic acids, lowering soil pH and increasing P availability, b) Phosphatases excretion: PSB release enzymes that hydrolyze organic P compounds, making P available for plant uptake, and c) Root morphology modification: PSB inoculation can modify root architecture, increasing root length and hair density, and enhancing P acquisition (Elhaissofi *et al.*, 2022).

The PSB is associated with the release of low molecular weight organic acids, including acetic acid, gluconic acid, and succinic acid (Illmer & Schinner, 1995), as well as formic acid and propionic acid (de Abreu *et al.*, 2017). These acids chelate the cation bound to phosphate, converting it into soluble forms (Ranjan *et al.*, 2013; Kalayu, 2019). Additionally, PSB secretes enzymes like phytase and phosphatase, which hydrolyze organic phosphates (Singh & Satyanarayana, 2011), and produce siderophores, which bind to iron and other metals, increasing phosphate availability (Sharma *et al.*, 2013).

Effect of PSB on soil chemical properties

PSB has been shown to improve various soil chemical properties, including:

1. Phosphorus availability: PSB increases the soluble phosphorus content in soil, making it available to plants (Rodríguez & Fraga, 1999).
2. pH: PSB can decrease soil pH by producing organic acids, increasing phosphorus availability (Illmer & Schinner, 1995).
3. Nutrient cycling: PSB influence nutrient cycling by solubilizing phosphates and releasing other nutrients like nitrogen and potassium (Sharma *et al.*, 2013).
4. Soil enzyme activity: PSB stimulates soil enzyme activity, including phosphatase and phytase, enhancing phosphorus mineralization (Singh & Satyanarayana, 2011).
5. Microbial community: PSB alter the soil microbial community, promoting beneficial microorganisms and improving soil health (Liu *et al.*, 2023).

Effect of PSB on crop growth and yield

PSB application has been shown to improve plant phosphorus uptake, leading to improved growth and yield (Sharma *et al.*, 2013). PSB promote root development, enhancing plant phosphorus acquisition (Sharma *et al.*, 2013), and improve plant drought tolerance by increasing phosphorus availability (Vurukonda *et al.*, 2016). Additionally, PSB promote root development, increasing root length and root hair density, enhancing phosphorus uptake (Elhaissofi *et al.*, 2022).

Specific bacterial species, such as *Lysinibacillus fusiformis*, *Bacillus circulans*, *Pseudomonas* sp., *Bacillus aryabhatai*, and *Pseudomonas auricularis*, have been shown to affect plant growth and yield (Darzi *et al.*, 2012; Rafique *et al.*, 2017; Przemieniecki *et al.*, 2019; Wu *et al.*, 2019).

Efficiency of PSB under organic and inorganic fertilization

The efficiency of PSB can be influenced by fertilization practices, including organic and inorganic amendments. Organic fertilizers, such as compost and manure, can enhance PSB activity and phosphorus solubilization (Sharma *et al.*, 2013; Adnan *et al.*, 2017). Inorganic fertilizers, such as diammonium phosphate (DAP) and urea, can also impact PSB activity, but excessive use can

inhibit PSB growth and phosphorus solubilization (Chen *et al.*, 2023).

Studies have shown that combining PSB with fertilizers can have beneficial effects on crop production. For example, *Pantoea cypripedi* and *Pseudomonas plecoglossicida* combined with rock phosphate fertilization effectively increased total P uptake in maize and wheat crops (Kaur & Reddy, 2014). Similarly, PSB combined with tricalcium phosphate improved P uptake in mungbean crops (Walpolo & Yoon, 2013).

The combined application of bio-organic phosphate and PSB has been shown to improve crop growth, yield, and phosphorus uptake in various crops, including wheat, maize, and rice (Adesemoye & Kloepper, 2009; Tahir *et al.*, 2018).

Methodology

Site selection and soil sampling

The selection of the study site was based on the requirement for a high initial soil pH. A soil pH map obtained from the BSWM guided the identification of Brgy. Sto. Tomas, San Jacinto, Pangasinan as the sampling location. Initial soil sampling was conducted using a soil test kit to verify pH and phosphorus levels. Composite soil samples were submitted to the Soil Science Facilities at Benguet State University for chemical and biological analysis. Subsequently, bulk soil was transported to Brgy. Anonang, San Fabian, Pangasinan, where it was utilized as potting media for the experimental study.

Experimental design and layout

The experiment used a Randomized Complete Block Design (RCBD) with four replications, comprising 480 pots (10 pots/replication). Each pot contained 20 kg of soil. The treatments consisted of different PSB strains and fertilizer types, applied in a randomized complete block design.

Treatments and experimental layout

The treatments included four PSB strains (T1: control, T2: PSB strain E1; T3: PSB strain G4, and T4: combined strains) and three kinds of fertilizers (K1: organic fertilizer, K2: inorganic fertilizer, and K3: combined fertilizers). The organic fertilizer used was vermicompost, while the inorganic fertilizer was a combination of 16-20-0 and 0-0-60.

Seed inoculation and sowing

Seeds were surface-sterilized and inoculated with PSB strains (10^{-6} CFU mL⁻¹) for 30 minutes. Two seeds were planted at a uniform depth of 2.5 cm in each pot. Thinning was performed to maintain a single seedling per pot.

Fertilizer application

Organic fertilizer was applied at a rate of 10 t ha⁻¹, 7 days prior to sowing. Inorganic fertilizer was applied

in two stages: pre-planting (16-20-0) and side-dressing (0-0-60). Combined organic + inorganic fertilizer was applied at half the recommended rate.

Care and management

Plants were irrigated manually twice daily. Weeding was performed manually, and insect-pest management involved mechanical control and a single chemical application. Harvesting was done 68 days post sowing.

Data collection and analysis

Soil chemical analysis included pH, organic matter, nitrogen, phosphorus, potassium, and cation exchange capacity. Soil biological properties included microbial population (CFUs/g soil). Agronomic parameters included plant height, plant height increment, and fresh yield. Data were analyzed using ANOVA, and significant differences were determined using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Soil chemical properties

Soil pH

Effect of PSB strains

The soil inoculated with phosphate-solubilizing bacteria (PSB) strains significantly lowered the soil pH as presented in Table 1. Soils inoculated with PSB strain E1, G4, and the combination of the two strains decreased the soil pH by 0.12 to 0.24 units compared to the control. Higher decrease was registered from soils inoculated with the combination of the two PSB strains which significantly differed from soils inoculated with PSB strain E1 but not from those inoculated with PSB strain G4. This result implies that inoculating the soil with PSB strain E1 resulted in a lower decrease in soil pH while inoculating the soil with PSB strains E1 and G4 resulted in higher decrease in soil pH. This impact is consistent with previous research of Khan *et al.* (2007), indicating that combined strains enhanced soil acidification. This enhanced acidification is attributed to synergistic mechanisms, including increased organic acid production (Khan *et al.*, 2007), and enzyme

Table 1: Soil pH as affected by the application of PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	7.71 ^a
T2: PSB E1	7.59 ^b
T3: PSB G4	7.53 ^{bc}
T4: Combined strain	7.47 ^c
Kinds of fertilizer	
K1: Organic fertilizer	7.64 ^a
K2: Inorganic fertilizer	7.60 ^a
K3: Combined fertilizer	7.48 ^b
T x K	Highly significant
Initial pH	8.06

activity (Glick, 2012). These organic acids include formic acids, acetic acid and propionic acids which acidify the soil through hydrogen ion (H⁺) release from dissociation and cation exchange, thereby increasing soil acidity (Brady & Weil, 2008). Dissociation of organic acids yields conjugate bases and additional H⁺ ions, further reduced soil pH (Tan, 2010). Additionally, organic acids compete with hydroxide ions for cation exchange sites, contributing to acidification (Adeleke *et al.*, 2017). Thus, these interactions demonstrate the superiority of combined strains over individual strains in reducing soil pH.

The results further show that soils without application of PSB strains decreased in soil pH by 0.35 units compared to the initial pH value. The observed decrease in soil pH can be attributed to the application of N-containing fertilizers, specifically ammonium sulfate. This fertilizer lowers soil pH through two primary mechanisms: ammonium oxidation and sulfate dissociation. Microorganisms convert ammonium to nitrate, releasing H⁺ ions and acidifying the soil (Brady & Weil, 2008). Furthermore, sulfate dissociation forms sulfuric acid, releasing additional H⁺ ions and contributing to soil acidification (Nambu *et al.*, 1994). The addition of vermicast may have also contributed to the pH decrease, as its decomposition stimulates microbial activity, releasing carbon dioxide and forming carbonic acids, further acidifying the soil (Atiyeh *et al.*, 2002; Lazcano *et al.*, 2008).

Effect of fertilizer

Table 1 also showed that the application of the different kinds of fertilizer significantly decreased the soil pH compared to the initial pH value of 8.06. Soils applied with the combination of inorganic and organic fertilizer exhibited the greatest pH reduction by 0.58 units (7.2% reduction) which differed from soils applied with organic fertilizer and inorganic fertilizer. This result can be attributed to the synergistic effects of organic and inorganic components in mixed fertilizer, enhancing microbial activity and nutrient release (Al-Maamori *et al.*, 2018). In addition, nitrification and ammonium oxidation, producing H⁺ ions (Assefa & Tadesse, 2019), and imbalanced cation-anion exchange, increasing acidic nutrient availability, contributes to the greater pH reduction.

Interaction effects

Figure 1 shows that the interaction between the two factors significantly decreased soil pH. The most pronounced decrease occurred in soils treated with combined PSB strains E1 and G4 and mixed organic-inorganic fertilizer. This suggests that simultaneous application of combined PSB strains and mixed fertilizer promotes soil acidification. This effect is attributed to PSB releasing low-molecular-weight organic acids, carboxyl, and hydroxyl ions. These ions chelate cations, forming stable complexes, which exchange cations for H⁺ ions (Alori *et al.*, 2017). This chelation process increases H⁺ ion concentration, reducing soil pH. Furthermore, fertilization enhances soil acidification through nitrification processes,

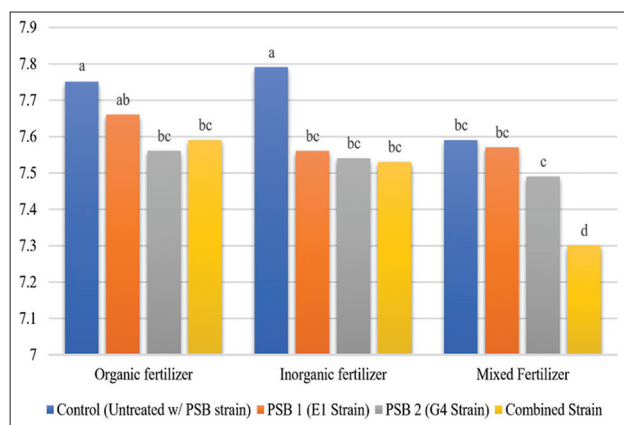


Figure 1: Soil pH as affected by the presence of PSB strain and application of different kinds of fertilizer

converting ammonium to nitrate, releasing additional H⁺ ions, and organic matter decomposition involving ammonium oxidation to nitrate (Assefa & Tadesse, 2019). Thus, for maximum soil pH reduction, combine E1-G4 strains with mixed organic-inorganic fertilizers.

Soil organic matter (SOM, %)

Effect of PSB strains

Soil with the different PSB strains significantly enhanced soil organic matter (SOM) content (Table 2). The soil inoculated with PSB strain E1 resulted in the highest increase in soil OM by 1.67% compared to the control. The lowest increase was registered from soils inoculated with the combination of PSB strains E1 and G4. The results indicate that PSB strain E1 inoculation is more effective in improving soil OM content than PSB strain G4 or their combination. These suggest that PSB strain E1's superior efficacy in improving soil OM content was attributed to its unique metabolic pathways, enzyme activities and nutrient cycling mechanisms (Khan *et al.*, 2007; Alori *et al.*, 2017), which may hinder when combined with PSB strain G4.

Further, the soil untreated with PSB also showed a 0.37% increase in soil OM content compared to the control. This increase can be due to the organic and inorganic fertilizers applied to the soil, which added organic carbon, nutrients and microorganisms (Zhang *et al.*, 2022). Additionally, ammonium sulfate stimulated microbial decomposition, converting organic residues into humic substances (Geisseler & Scow, 2014), while potash promoted plant growth, leading to increased root residues and soil OM accumulation (Havlin *et al.*, 2014).

Effect of fertilizers

The soil showed significant SOM content increased after the application of the organic, inorganic, and organic with inorganic fertilizer with 1.26%, 1.57% and 1.54%, respectively (Table 2). The results showed that the soil treated with inorganic fertilizers had the highest SOM which significantly differed from those applied with organic fertilizer but comparable with those applied with organic

Table 2: Organic matter content (%) of the soil as affected by the presence of PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	0.57 ^c
T2: PSB E1	2.24 ^a
T3: PSB G4	2.01 ^{ab}
T4: Combined strain	1.81 ^b
Kinds of fertilizer	
K1: Organic fertilizer	1.46 ^b
K2: Inorganic fertilizer	1.77 ^a
K3: Combined fertilizer	1.74 ^a
T x K	Highly significant
Initial OM	0.20

+ inorganic fertilizer. These findings align with previous research, including Ladha *et al.* (2011), who found an increase in SOM with inorganic fertilizers compared to organic fertilizers, and Li *et al.* (2022), who reported an enhancement in SOM with integrated organic-inorganic fertilizers. This can be attributed to inorganic fertilizers stimulating enzyme activity and influencing soil pH (Bo *et al.*, 2024) facilitating SOM decomposition and turnover. The comparable SOM levels in organic + inorganic treatments suggest synergistic effects, optimizing nutrient availability and microbial decomposition (Wang *et al.*, 2024). Furthermore, soils applied with organic fertilizer alone registered the lowest OM. This low organic matter can be attributed to rapid decomposition of organic matter, driven by increased microbial activity, enzyme production, and nutrient competition (Kuzyakov *et al.*, 2000; Fontaine *et al.*, 2007).

Interaction effect

The interaction between different PSB strains and kinds of fertilizers significantly improved soil OM content as illustrated in Figure 2. Inoculation of soil with PSB strain E1 combined with mixed organic-inorganic fertilizer yielded the highest soil OM content, which significantly differed among all the treatment combinations. The inoculation of combined strain and organic fertilizer exhibited the lowest soil OM. Additionally, mixed organic-inorganic fertilizers provide a balanced nutrient supply, promoting soil biota (Bender *et al.*, 2016) which eventually turn over to the soil to increase organic matter content of the soil.

The positive impact of PSB strains on soil OM content was not limited to specific combinations, as both individual (E1 and G4 strains) and its combination complemented different kinds of fertilizers, boosting soil OM levels. This consistent improvement highlights the versatility of PSB strains in enhancing soil organic matter. Though, the results indicate that the highest increase in SOM was achieved through inoculating E1 strains combined with mixed organic-inorganic fertilizer application.

Soil available phosphorus (ppm)

Effect of PSB strains

The soil inoculated with PSB strains significantly enhanced soil available phosphorus content as presented in

Table 3. Soils inoculated with PSB strains E1, G4 and the combination of the two strains shows a significant increase of available phosphorus from 10.16-19.91 ppm as compared from control with 22.67 ppm. The soils inoculated with PSB strain E1 exhibited the highest available phosphorus which significantly outperformed PSB strain G4 and the combination of PSB strains E1-G4, suggesting the E1's superior phosphorus solubilization rate. Interestingly, PSB E1 strain's outstanding phosphorus enhancement outweighs its inferior soil pH reduction performance (Table 1). E1's enhanced P-accumulation may be attributed to efficient organic acid production and targeted acidification processes (Alori *et al.*, 2017) which optimized phosphorus release. PSB strain E1's superiority stems from its enzymes effectively breaking down organic phosphorus compounds, optimizing nutrient uptake, promoting nutrient recycling, facilitating microbial cooperation, and adapting to soil conditions (Rodríguez *et al.*, 2006). These factors collectively contribute to PSB strain E1's enhanced phosphorus accumulation.

The control treatment, despite having the lowest available phosphorus, showed a notable increase from the initial phosphorus level. This enhancement can be attributed to fertilizer application, which supplied essential nutrients (Havlin *et al.*, 2014). The fertilizer's phosphorus content likely contributed to this increase, demonstrating the fertilizer's efficacy in improving soil phosphorus availability (Brady & Weil, 2008).

Effect of fertilizers

The application of different fertilizers significantly increased available phosphorus in soil as shown in Table 3.

Table 3: Available phosphorus content of soil as affected by PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	22.67 ^c
T2: PSB E1	42.58 ^a
T3: PSB G4	34.00 ^b
T4: Combined strain	32.83 ^b
Kinds of fertilizer	
K1: Organic fertilizer	33.75 ^a
K2: Inorganic fertilizer	29.69 ^b
K3: Combined fertilizer	35.63 ^a
T x K	Highly significant
Initial P	19.00

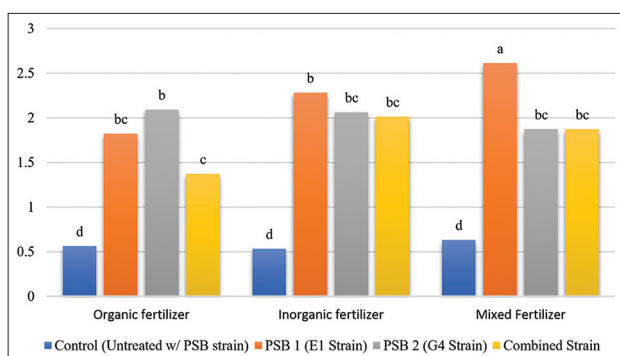


Figure 2: SOM as affected by the presence of PSB strain and application of different kinds of fertilizer

Soils treated with organic and mixed organic-inorganic fertilizers exhibited comparable soil available phosphorus content which was significantly higher than those treated with inorganic fertilizers. This was attributed to organic fertilizers' slow nutrient release mechanisms and pH buffering capacity which reduced phosphorus fixation and increased its availability (Nobile *et al.*, 2019). Additionally, gradual nutrient release synchronized with plant demand, optimizing phosphorus uptake (Richardson *et al.*, 2009) and avoid losses of available phosphorus.

In contrast, inorganic fertilizers showed lower available phosphorus (Table 3) despite elevated soil organic matter levels (Table 2). This could be due to leaching losses especially that the soil pH was moderately alkaline (BSWM, 2022) that enhanced the pH dependent charge which favors negative charges. Negative charge of the soil results to repulsion of available phosphorus especially that available phosphorus is negative charge and allow repulsion leading to leaching. Moreover, organic matter of the soil was low so it contributed to low retention of available phosphorus in the soil.

Interaction effects

The interaction between applied PSB strains and different kinds of fertilizers significantly impacted available phosphorus (Figure 3). The highest available phosphorus content was observed in soil treated with the PSB strain E1 applied with organic fertilizer. The PSB strain E1 complements inorganic and mixed organic-inorganic fertilizers equally well, showing no significant difference when combined with these fertilizers. This demonstrates the versatility of the E1 strain in increasing available phosphorus across various fertilizer types. This can be attributed to synergistic effect of E1 strain to organic fertilizer which likely enhances microbial immobilization of phosphorus that increases its availability in the soil (Oehl *et al.*, 2004). Thus, this indicates that to obtained the highest increase in available phosphorus, the interaction between E1 strain and organic fertilizer was suggested.

Soil exchangeable potassium (ppm)

Effect of PSB strains

Table 4 demonstrated that the soil inoculated with PSB strains significantly enhanced exchangeable potassium

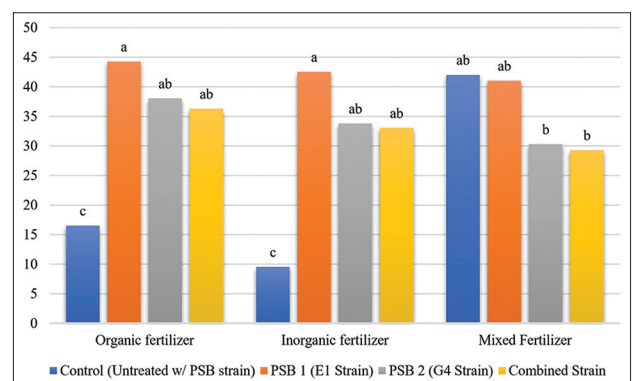


Figure 3: Available phosphorus content as affected by the presence of PSB strain and application of different kinds of fertilizer

Table 4: Exchangeable potassium content of soil as affected by PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	15.08 ^d
T2: PSB E1	21.25 ^c
T3: PSB G4	35.67 ^a
T4: Combined strain	27.83 ^b
Kinds of fertilizer	
K1: Organic fertilizer	26.00 ^a
K2: Inorganic fertilizer	23.50 ^b
K3: Combined fertilizer	25.38 ^a
T x K	Highly significant
Initial K	16.00

level. Specifically, soils treated with PSB strains E1, G4, and their combination, soils exhibited substantial increases of 6.17 to 20.59 ppm, compared to the soil without PSB strain. The PSB strain G4-treated soils displayed the highest exchangeable potassium levels, showing a significant difference compared to PSB strain E1 treated soil and the combination of these two strains. This finding suggests that PSB strain G4 was more effective in enhancing exchangeable potassium than PSB strain E1 and combined strains. Interestingly, PSB strain G4 doesn't excel in rapidly increasing available phosphorus (Table 3) but prioritizes an alternative enhancing potassium. Its outstanding potassium-solubilizing performance may be attributed to specialized enzymes like KSE (Meena *et al.*, 2015), and organic acid production (Rodríguez & Fraga, 1999; Zhang *et al.*, 2024). Specifically, gluconic, citric and oxalic acids chelate metal ions, protonate potassium-bearing minerals and form soluble complexes, enhancing potassium availability (Lodi *et al.*, 2022). Thus, PSB strain G4's unique combination of mechanisms outweighs its phosphate-solubilizing potential, prioritizing potassium solubilization (Nath *et al.*, 2017).

Effect of fertilizer

Soils treated with different kinds of fertilizer exhibited significant increase in the exchangeable potassium as shown in Table 4. Organic and mixed organic-inorganic fertilizers demonstrated equivalent enhancements, differing significantly from soils receiving solely of inorganic fertilizers. These differences were associated from organic fertilizers' ability to release potassium slowly, stimulate beneficial microbes, and produce organic acids (citric, gluconic) that chelate metal ions which released potassium from minerals (Zhang *et al.*, 2024). Mixed fertilizers, on the other hand, offer balanced nutrient supply which enhances the exchangeable potassium (Han *et al.*, 2016).

Interaction effects

Figure 4 illustrates the significant effects of interacting different PSB strains with various kinds of fertilizers. Unlike available phosphorus, where PSB strain E1 combined with various fertilizers surpasses other interactions, exchangeable potassium was enhanced when PSB strain G4 interacted with organic fertilizer. Notable, both PSB strain G4 and organic fertilizer individual effects also showed significant

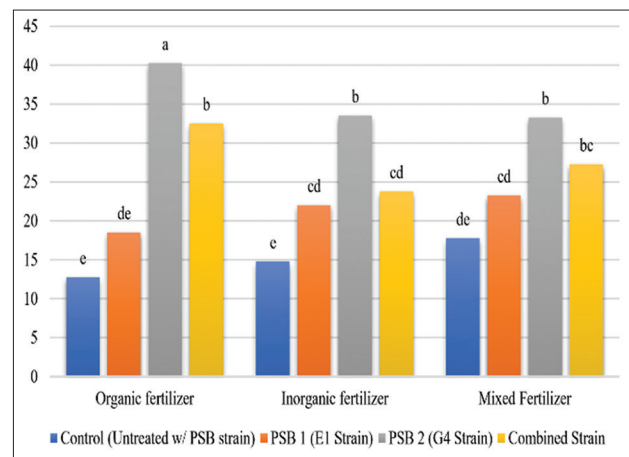


Figure 4: Exchangeable potassium content as affected by the presence of PSB strain and application of different kinds of fertilizer

enhancements in exchangeable potassium (Table 4). The observed synergy between PSB strain G4 and organic fertilizer supports previous research demonstrating that PSB strains can increase potassium solubilization and availability in soil (Han *et al.*, 2006). Moreover, organic fertilizers provide a slow release of nutrients, promoting microbial activity and potassium cycling (Maeder *et al.*, 2002). The combined effect of PSB strain G4 and organic fertilizer likely enhances microbial immobilization of potassium, increasing its availability in the soil (Oehl *et al.*, 2004). This suggests that soil exchangeable potassium benefits most from the interaction between inoculated PSB strain G4 and applied organic fertilizer.

Cation exchange capacity (meq/100 g soil)

Effect of PSB strains

Table 5 demonstrates that soil inoculated with PSB strains significantly impacted cation exchange capacity (CEC). Specifically, PSB strain G4 exhibited the highest CEC content, differing significantly from strains E1 and the PSB strains G4+E1 combination, yet comparable to PSB-free soil. The PSB strain G4's limited impact on CEC may attributed to low phosphatase activity (da Silva *et al.*, 2023) and reduces phytase activity (Mahmud *et al.*, 2021), and may likely contributed to these constraints. These limitations hindered the PSB strain G4's ability to enhance CEC beyond levels observed in soil without PSB strain. These indicated that specific PSB strains, such as PSB strain G4, positively impact CEC.

Effects of fertilizer

Soil cation exchange capacity (CEC) significantly increased following application of various fertilizers. Organic fertilizer treatment differed substantially from inorganic and mixed organic-inorganic treatments. Despite exhibiting the smallest increase in soil organic matter content (Table 2), organic fertilizer's CEC enhancement was driven by stimulated microbial activity (Brady & Weil, 2017), which contributed to enzyme production, nutrient

release, and soil biota stimulation, rather than humus formation.

Interaction effects

There was significant interaction effect of PSB strains and kinds of fertilizer on the CEC of the soil as illustrated in Figure 5. The soil applied with organic fertilizer and inoculated with PSB strain G4 had the highest soil CEC due to increase of organic matter of the soil (Table 2). The observed synergy between PSB strain G4 and organic fertilizer supports previous research demonstrating the PSB strains can improve soil CEC by increasing organic matter content and promoting microbial activity (Goswami *et al.*, 2017). Organic fertilizers, rich in humus, contribute to the formation of stable aggregates, enhancing soil CEC (Haynes & Naidu, 1998). This signified that the interaction between organic fertilizer and PSB strain G4 positively affects and increased the CEC of soil as compared to other interactions of PSB strains and kinds of fertilizers.

Soil biological properties

Soil Microbial Abundance

Figure 6 illustrates substantial variations in bacterial colony formation among Phosphate Solubilizing Bacteria (PSB) strains and kinds of fertilizers. Specifically, soil treated with PSB strain G4 and organic-inorganic fertilizer combinations exhibited the highest bacterial colony

Table 5: Cation exchange capacity of the soil as affected by the presence of PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	29.21 ^{ab}
T2: PSB E1	21.08 ^c
T3: PSB G4	30.67 ^a
T4: Combined strain	25.21 ^b
Kinds of fertilizer	
K1: Organic fertilizer	30.67 ^a
K2: Inorganic fertilizer	25.99 ^b
K3: Combined fertilizer	22.96 ^b
T x K	Highly significant
Initial CEC	14.88

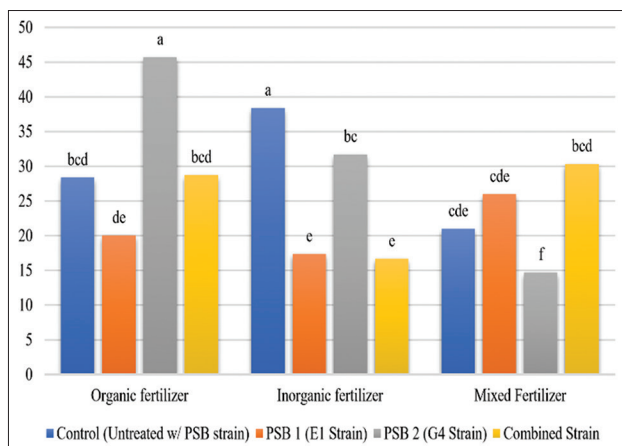


Figure 5: CEC as affected by the presence of PSB strain and application of different kinds of fertilizer

formation rates. Furthermore, the combination of PSB strains E1-G4 applied with inorganic fertilizers also yielded considerable colony formation.

These results suggested a distinct colonogenic capabilities among PSB strains, particularly strain E1 and G4. Specific, interactions between these strains and kinds of fertilizers influenced bacterial colony formation, indicating strain specific responsiveness to nutrient amendments.

PSB Population

Figure 7 presents the total population of PSB isolates, revealing varying responses among strains and fertilizer types. Notable, PSB strain G4 demonstrated exceptional adaptability, thriving under organic + inorganic fertilizer combinations, which yielded the highest colony formation rates. Additionally, PSB strain G4 exhibited robust growth under inorganic fertilizers alone, indicating remarkable flexibility in exploiting diverse nutrient sources.

In contrast, PSB strain E1 displayed consistent growth patterns across organic, inorganic, and its combinations treatments, showcasing resilience to fluctuations in nutrient availability. These distinct responses suggest strain-specific adaptations to different fertilizer types. Overall, these results indicated the differential responsiveness of PSB strains to varied nutrient regimes emphasizing the importance of strain-fertilizer compatibility in optimizing phosphorus solubilization.

Further, an individual PSB strain G4 and E1 demonstrated higher PSB growth rates as showed in Figure 8. The soil inoculated with PSB strain G4 exhibited

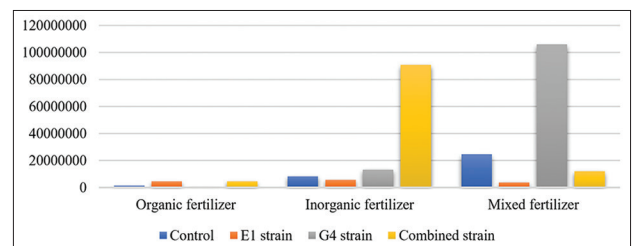


Figure 6: Microbial population

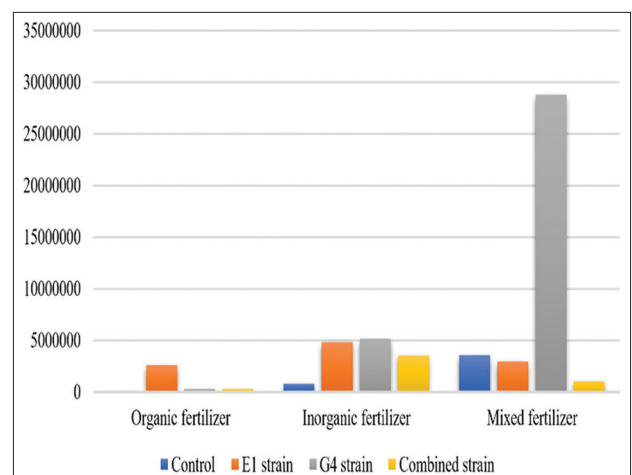


Figure 7: PSB population

PSB growth rates ranging from 27.12% to 72%, while the soil inoculated with PSB strain E1 showed a rate of 59% to 85.69%. In contrast with soil inoculated with combined PSB strain E1-G4 and the untreated soil with PSB showed a lower PSB growth rate. This must be attributed to the potential competition for resources, such as nutrients, space, air, and water between the PSB strain E1 and G4. Thus, separate inoculation of the strains can be more effective for their normal growth.

Agronomic Parameters

Initial Plant Height

Effect of PSB strains

The PSB strains showed no significant effect on initial plant height of corn crop (Table 6). The mean values obtained for the control group and the treatment groups were remarkably consistent, indicating no substantial differences among PSB strains. Specifically, the control group exhibited a mean of 17.18 cm, while the treatment groups showed similar mean values: PSB strain E1 at 16.71 cm, PSB strain G4 at 17.93 cm, and its combination at 17.68 cm. These results suggested that the application of PSB strains did not have a significant effect on the variable under investigation.

Effect of fertilizers

On contrary to the effect of PSB strains, the different kinds of fertilizers showed a significant effect on the initial

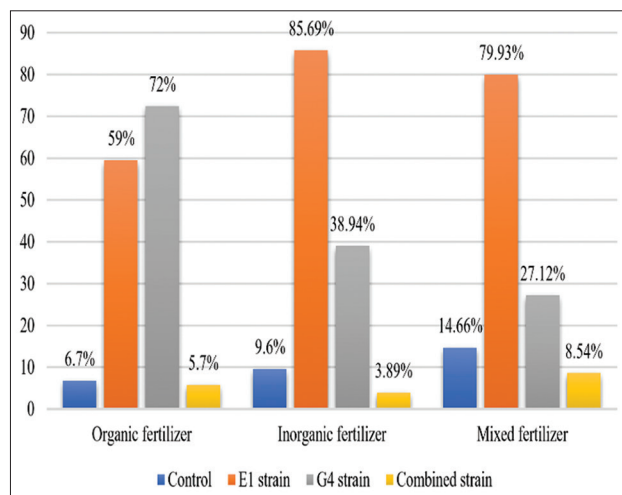


Figure 8: Percentage of PSB grown in an isolates

Table 6: Initial height (cm) as affected by the presence of PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	17.18 ^a
T2: PSB E1	16.71 ^a
T3: PSB G4	17.93 ^a
T4: Combined strain	17.68 ^a
Kinds of fertilizer	
K1: Organic fertilizer	17.27 ^b
K2: Inorganic fertilizer	15.18 ^c
K3: Combined fertilizer	19.66 ^a
T x K	Not significant

lant of corn crops as shown in Table 6. The mixed fertilizer had the highest plant height and was comparable to those received with organic fertilizer and inorganic fertilizer. This finding supports previous research demonstrating mixed fertilizers' superiority in promoting plant growth and yield (Sawan, 2018; Wan *et al.*, 2021). The balanced nutrient profile provided by mixed fertilizers enhances soil fertility and supports optimal plant development (Wang *et al.*, 2021). The soils applied with inorganic fertilizers though its nutrient was concentrated may also lead to soil degradation and nutrient imbalances (Abrol *et al.*, 2024) which causes its low effect in plant height, while organic fertilizers, although beneficial for soil health, may have lower nutrient availability, resulting in reduced plant growth (Diacono & Montemurro, 2010).

Interaction effects

The analysis of the interaction between phosphate solubilizing bacteria (PSB) strains and fertilizer types revealed no significant effects. Despite this, notable trends emerged from the data. The application of organic + inorganic fertilizer consistently yielded higher mean values across all PSB strains, including the PSB-free soil. This suggests that organic + inorganic fertilizer has a positive impact on the variable measured, regardless of the PSB strain used. In contrast, the inorganic fertilizer resulted in lower mean values across all PSB strains, indicating its relatively lower effectiveness. The performance of the PSB strain G4 and the combination PSB strain E1-G4 were remarkably similar across soil applied with organic fertilizer and those applied with inorganic fertilizers. These implied that these strains are robust and adaptable to different fertilizer conditions. Interestingly, the PSB strain E1 showed a weaker response to organic + inorganic fertilizer compared to PSB strain G4 and its combination which warranted a further investigation into its specific characteristics and potential limitations. Overall, the absence of significant interaction between PSB strains and types of fertilizer suggests that these factors influence the variable independently.

Final Plant Height

Effect of PSB strains

The results presented in Table 7 indicate that inoculating corn seeds with phosphate-solubilizing bacterial (PSB) strains does not have a statistically significant impact on the

Table 7: Final height (cm) as affected by the presence of PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	59.40 ^a
T2: PSB E1	57.31 ^a
T3: PSB G4	58.34 ^a
T4: Combined strain	56.15 ^a
Kinds of fertilizer	
K1: Organic fertilizer	55.45 ^b
K2: Inorganic fertilizer	56.99 ^b
K3: Combined fertilizer	60.95 ^a
T x K	Not significant

final height of corn plants. However, a notable trend emerges when examining the mean heights across treatments. The combined PSB strains E1-G4 treatment yielded the highest mean height of 59.49 cm, followed closely by PSB strain G4 and PSB strain E1 treatments, with mean heights of 58.34 cm and 57.31 cm, respectively. In contrast, the untreated PSB strain had the lowest mean height of 56.15 cm. This trend suggests that PSB strains may still contribute to improved corn plant growth, even if the differences are not statistically significant. The combined PSB strains treatment appears to have a synergistic effect, leading to enhanced plant height.

Effect of fertilizers

The different kinds of fertilizer applied on corn crop significantly impacted the final plant height, whereas PSB inoculation does not (Table 7). Notably, the corn plants treated with mixed organic-inorganic fertilizer exhibited comparable heights to those receiving organic and inorganic fertilizer alone. This finding aligns with Islam *et al.* (2017), who demonstrated that combining organic and inorganic fertilizers substantially enhances crop growth, particularly plant height. The observed effect of fertilizer on corn plant height can be attributed to the provision of essential nutrients, enabling optimal growth and development (Wang *et al.*, 2023). Mixed fertilizers, in particular, offer a balanced nutrient supply, promoting healthy growth and maximizing yields (Song *et al.*, 2024). The synergy between organic and inorganic components in mixed fertilizers may stimulate microbial activity, improve soil fertility, and enhance nutrient uptake (Bo *et al.*, 2024).

Interaction effects

The results indicate that the interaction between different PSB strains and types of fertilizers does not significantly impact the final height of corn plants. This suggests that the effects of PSB strains and fertilizers on plant height may be independent of each other. Notably, the combination of PSB free treatment and organic + inorganic fertilizer yielded the highest plant height of 65.48 cm. This implicates that organic + inorganic fertilizers, which provide a balanced nutrient supply, can be more effective in promoting plant growth than organic or inorganic fertilizers alone. In contrast, the interaction between PSB strain G4 and organic fertilizer resulted in the lowest plant height of 52.48 cm. This can indicate that the PSB strain G4, despite its potential to solubilize phosphates, cannot be compatible with organic fertilizers or can require additional nutrients to effectively promote plant growth.

Crop Growth Increment

Effect of PSB strains

The application of PSB strains significantly enhanced the growth increment of corn plants as evident in Table 8. The corn inoculated PSB strains E1, G4 and its combination got a difference increase of 2.01 cm, 0.9 cm, and 1.61 cm, respectively as compared to corn without PSB strain. The corn plant inoculated with PSB strain E1 had the highest increase in plant height, while PSB strain G4 got the

Table 8: Crop growth increment (cm) as affected by the presence of PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate Solubilizing Bacterial Strain	
T1: Control	8.96 ^c
T2: PSB E1	10.97 ^a
T3: PSB G4	9.86 ^{bc}
T4: Combined strain	10.57 ^{ab}
Kinds of Fertilizer	
K1: Organic fertilizer	9.87 ^a
K2: Inorganic fertilizer	9.97 ^a
K3: Combined fertilizer	10.42 ^a
T x K	Not significant

lowest increase. This suggests that PSB strain E1 has a positive impact on corn plant height. The beneficial effects of PSB strains, particularly PSB strain E1, were attributed to their metabolic activities which produce various growth promoting substances (Wu *et al.*, 2019) that positively affect the growth of a crop. These metabolic products include plant hormones, such as auxins, cytokinins, and gibberellins, that regulate cell elongation, cell division, and differentiation (Bhattacharyya & Jha, 2012). Additionally, it also produces organic acids which solubilize phosphates that make it available to corn plants (Sharma *et al.*, 2013).

Effect of fertilizers

The data presented in Table 8 reveals a surprising trend, where the type of fertilizer applied does not significantly impact the growth increment of corn plants. The difference between the different kinds of fertilizers treatment were statistically insignificant and did not reach the threshold for meaningful distinction. The corn treated with organic + inorganic fertilizer treatment yielded the highest growth increment, while the corn treated with organic fertilizer alone got the lowest growth increment.

Interaction effects

The results revealed that the interaction between PSB strains and kinds of fertilizer does not significantly impact the growth increment of corn plants. This suggests that the effects of PSB strains and fertilizers on plant growth are independent of each other. However, a notable trend emerges when examining the combined effects of specific PSB strains and fertilizer types. The highest mean growth increment (11.99 cm) was observed in corn plants treated with PSB strain E1 and organic + inorganic fertilizer combination which indicated a potential synergistic effect in corn plant. In contrast, the combination of PSB strain G4 and organic fertilizer resulted in the lowest mean growth increment (8.93 cm) which highlighted the importance of optimal PSB strain and fertilizer pairing.

Fresh Yield

Effect of PSB strains

Table 9 demonstrates that phosphate-solubilizing bacteria (PSB) strains significantly enhance corn plant yield. Corn plants inoculated with PSB strains outperformed

Table 9: Yield of corn (kg) as affected by the presence of PSB strain and kinds of fertilizer

Treatments	Mean
Phosphate solubilizing bacterial strain	
T1: Control	2.66 ^b
T2: PSB E1	3.97 ^{ab}
T3: PSB G4	4.40 ^a
T4: Combined strain	3.84 ^{ab}
Kinds of fertilizer	
K1: Organic fertilizer	4.38 ^a
K2: Inorganic fertilizer	3.89 ^{ab}
K3: Combined fertilizer	2.88 ^b
T x K	Not significant

non-inoculated ones. Specifically, plants treated with PSB strain G4 exhibited the highest yield, while non-inoculated PSB strain plants yielded the lowest. This aligns with studies showing strain-specific differences in phosphate solubilization and plant growth promotion (Vessey, 2003). The superiority of PSB strain G4 over PSB strain E1 and its combinations can be attributed to variations in colonization, nutrient solubilization and plant hormone production. Wherein, strain-specific differences in PSB are influenced by factors such as phosphate solubilization mechanisms, colonization and root adherence, production of plant growth-promoting substances and soil adaptation (Richardson, 2001; Compant *et al.*, 2010).

Effect of fertilizers

The application of different kinds of fertilizer significantly impacted corn yields as presented in Table 9. Organic fertilizers yielded the highest results, significantly outperformed mixed organic-inorganic fertilizers but not differed from inorganic fertilizers. These findings indicated that organic fertilization enhances yields compared to organic with inorganic fertilizer. This result aligned with previous studies demonstrating the benefits of organic fertilizers on crop yields. For instance, a study by Xing *et al.* (2024) found that organic fertilizer application significantly increased maize yield compared to inorganic fertilizers. Similarly, a review by Assefa and Tadesse (2019) highlighted the potential of organic fertilizers to enhance soil fertility and promote sustainable agriculture. The superior performance of organic fertilizer in this study can be attributed to its ability to increase nutrient availability, and support beneficial microbial activity (Liu *et al.*, 2024). In contrast, organic + inorganic fertilizer has resulted in an over-application of nutrients, leading to decreased yield due to nutrient imbalances (Wang *et al.*, 2021).

Interaction effects

Although the individual effects of Phosphate solubilizing bacteria (PSB) strains and fertilizer types on corn plant yield were notable, the interaction between these factors did not reach statistical significance. However, a close examination of the data reveals intriguing trends. The highest mean yield (5.09 kg/treatment) was observed in corn plants inoculated with PSB strain G4 and applied with organic fertilizer. These implied the potential synergies between these factors. In contrast, the lowest mean yield

(2.23 kg/treatment) occurred in plants without PSB strain and received organic + inorganic fertilizer.

Summary

This study explored the potential benefits of phosphate-solubilizing bacteria (PSB) isolates from Bermuda grass rhizosphere and kinds of fertilizer on corn growth and yield. Specifically, it aimed to 1) evaluate the effects of different PSB isolates, fertilizer types and its interaction effects on soil chemical properties, 2) evaluate the effects of different PSB isolates, fertilizer types and its interaction effects on soil biological properties, and 3) determine the influence of PSB isolates, fertilizer types and its interaction effects on corn growth parameters and yield. The research was conducted in Brgy. Anonang, San Fabian, Pangasinan, utilizing soil samples collected from neighboring Brgy. Santo Tomas, San Jacinto, Pangasinan.

PSB strains E1 elevated soil organic matter (SOM) and available phosphorus, while PSB strain G4 increased exchangeable potassium and cation exchange capacity (CEC). Their combination reduced soil pH. Organic fertilizers increased exchangeable potassium and CEC, whereas inorganic fertilizers enhanced SOM. Combined organic-inorganic fertilizers lowered soil pH, elevated available phosphorus and optimized potassium and CEC.

Soil inoculation with PSB strain G4 yielded the highest bacterial colony counts, especially with organic + inorganic fertilizers. PSB strain E1 supported 59%-85.69% PSB colony growth, whereas PSB strain G4 supported 27.12%-72%. Co-inoculation showed limited growth of 3%-8%. PSB strain E1 improved crop growth index (CGI), while PSB strain G4 influenced initial plant height and yield. Organic + inorganic fertilizers enhanced plant heights and growth increment, with organic fertilizer alone boosting yield.

The interaction between PSB strain G4 and organic fertilizer enhanced potassium and CEC, whereas PSB strain E1 combined with organic + inorganic fertilizers boosted SOM and CGI. Additionally, PSB strain G4 applied with organic fertilizers optimized yield.

Conclusions

The introduction of PSB strains E1 and G4 profoundly impacts soil chemical and biological properties, and crop productivity. PSB strain G4 enhances exchangeable K, CEC and microbial populations, boosting plant height and yield. PSB strain E1 elevates SOM, available P and CGI. Their combination reduces soil pH. Synergistic interactions between PSB strains and kinds of fertilizers optimize soil productivity and crop yields, offering a promising sustainable agricultural approach.

Recommendations

Based on the findings, the following are recommended.

1. Use E1 strain with organic fertilizer to enhance available phosphorus, soil organic matter and microbial population.

2. Apply G4 strain with organic fertilizer to boost exchangeable potassium and cation exchange capacity.
3. Combine E1 and G4 strains with mixed fertilizer for enhanced crop growth and reduced soil pH.
4. A subsequent field-scale or plot-level investigation is recommended to validate the findings of this study and assess the practical applicability of the results under real-world conditions.

Author contributions

Seprida A. Halog: Handled all aspects of the research and writing, including conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, validation, visualization, writing – original draft, and writing – review & editing.

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