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Phosphate Solubilization and Plant Growth-Promoting Potential of *Penicillium oxalicum* (Bt9) in Eppawala Rock Phosphate-Enriched Compost

W. M. B. B. Sathsarini[1], T. G. I. Sandamali[2], M. M. Pathmalal[1] and F. S. Idroos[1]†
[1]Center for Water Quality and Algae Research, Department of Zoology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250, Sri Lanka
[2]Regional Rice Research and Development Center, Bombuwala, Sri Lanka
†Corresponding author: F. Sumaiya Idroos; sumaiyaidroos@sci.sjp.ac.lk

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ABSTRACT

The frequent application of chemical phosphate (P) fertilizers is costly and has emerged as a major concern in the agricultural sector of Sri Lanka. Therefore, the present study evaluated the impact of the fungal inoculum *Penicillium oxalicum* (Bt9) on the bio-solubilization of Eppawala Rock Phosphate (ERP)-enriched compost. A Completely Randomized Design (CRD) was employed, comprising four compost treatments, each replicated four times. Treatment 1 (T1) was amended with *P. oxalicum* (Bt9) and sugar; Treatment 2 (T2) was amended solely with *P. oxalicum* (Bt9); Treatment 3 (T3) was amended solely with sugar, and Treatment 4 (T4) served as the control, lacking both *P. oxalicum* (Bt9) and sugar. The released bioavailable phosphorus (P) content of each treatment was determined using the molybdenum blue method. A pot experiment was conducted using red cowpea (*Vigna unguiculata*), grown in a 1:1 mixture of phospho-compost and soil, to evaluate the effect of ERP bio-solubilization by *P. oxalicum* (Bt9) on plant growth. The results showed significantly higher ($p \leq 0.05$) values for plant growth parameters, including shoot length, root length, and total plant length, in Treatments 1 and 2 compared with Treatments 3 and 4. The highest shoot length, root length, and total plant length were recorded in Treatment 1, with values of 26.58 ± 2.44 cm, 11.36 ± 1.48 cm, and 54.24 ± 2.64 cm, respectively. The study concluded that *P. oxalicum* (Bt9) significantly enhanced phosphate solubilization and promoted the growth of red cowpea (*Vigna unguiculata*). Therefore, ERP-enriched compost inoculated with *P. oxalicum* (Bt9) may be considered a sustainable alternative to imported chemical phosphate fertilizers.

1. INTRODUCTION

Phosphorus is one of the minor elements on Earth, accounting for approximately 0.1% of the total elemental composition (Li et al. 2016), and is one of the most essential macronutrients required for plant growth and development (Khan et al. 2009). Compared with other macronutrients, phosphorus exhibits the lowest mobility in both soil and plants (Anand et al. 2016). It is considered the second most limiting nutrient for crop production after nitrogen in most soils (Biswas & Narayanasamy 2006). The two principal forms of phosphorus in soil are organic and inorganic phosphorus (Wang et al. 2021). Plant-available phosphorus, present in the soil solution, consists mainly of inorganic phosphorus in the form of primary orthophosphate ions ($H_2PO_4^-$) and secondary orthophosphate ions (HPO_4^{2-}), which dissolve in the soil solution and are directly available for plant uptake (Anand et al. 2016). However, only about 0.1% of the total phosphorus content in soil is available for plant utilization (Khan et al. 2009). The concentration of phosphorus in the soil solution typically ranges from 0.001 to 1 $mg.L^{-1}$, which is considerably lower than that of many other essential nutrients (Khan et al. 2014). Soluble phosphorus is rapidly precipitated as metal–cation complexes by highly reactive Al^{3+} and Fe^{3+} ions in acidic soils and by Ca^{2+} ions in calcareous or neutral soils (Anand et al. 2016; Wang et al. 2021).

A substantial proportion of phosphorus exists in insoluble forms and, therefore, cannot be absorbed by plants (Khan et al. 2009). Consequently, chemical phosphorus fertilizers containing soluble inorganic phosphorus are frequently applied to agricultural soils. However, the repeated application of high doses of chemical phosphorus fertilizers is costly and can cause several environmental problems, including soil acidification or alkalization, water pollution through runoff, groundwater contamination via phosphate leaching, suppression of beneficial microorganisms and insects, and nutrient fixation (Billah et al. 2020). Furthermore, only 5–25% of applied chemical phosphorus fertilizer is effectively utilized by plants (Sandamali et al. 2021). Therefore, the development of sustainable and environmentally friendly alternatives has become increasingly important.

Rock phosphate (RP) has traditionally been used as a raw material in the production of phosphorus fertilizers (Vassilev & Vassileva 2003). Eppawala Rock Phosphate (ERP) is an inexpensive phosphorus source in Sri Lanka and contains approximately 28–42% P_2O_5 (Ratnayake et al. 2018). However, due to its limited solubility, directly applied RP tends to persist in soils for extended periods (Khasawneh & Doll 1979). Composting is a natural decomposition process that plays a vital role in agriculture and organic waste management. Consequently, composting is regarded as an environmentally friendly and effective component of waste management within the circular bioeconomy framework (Wijerathna et al. 2024b, 2026).

Soil microorganisms capable of converting insoluble phosphate compounds into plant-available forms are known as phosphate-solubilizing microorganisms (PSMs) (Sandamali et al. 2021). The microbial solubilization of rock phosphate represents an environmentally friendly and mild alternative to chemical treatment methods (Vassilev & Vassileva 2003). The incorporation of rock phosphate with organic fertilizers and phosphate-solubilizing microorganisms during composting has been shown to increase the availability of phosphorus and enhance phosphorus uptake by plants under soil conditions (Ditta et al. 2018).

Several studies have investigated phosphate solubilization by PSMs and demonstrated their ability to convert insoluble phosphate compounds into bioavailable forms. Shrivastava et al. (2011) reported that manure enriched with low-grade Indian rock phosphate and inoculated with two phosphate-solubilizing fungi (PSF), *Aspergillus niger* TMPS1 and *Penicillium oxalicum* TMPS3, exhibited higher water-soluble phosphorus content and greater phosphatase activity than uninoculated treatments. Among the tested fungi, *P. oxalicum* TMPS3 recorded the highest water-soluble phosphorus content. Similarly, Wang et al. (2021) demonstrated the phosphate-solubilizing ability of *Penicillium oxalicum* (Y2) using both in vitro experiments and soil inoculation studies. Singh and Reddy (2011) further reported that the growth and yield parameters of wheat and maize were significantly improved in alkaline soils treated with rock phosphate and inoculated with *Penicillium oxalicum* compared with uninoculated control soils under field conditions.

Penicillium oxalicum (Bt9) has demonstrated enhanced phosphate-solubilizing ability in ERP-amended compost compared with previously reported strains such as TMPS3 and Y2. In addition, *P. oxalicum* (Bt9) exhibits distinctive plant growth-promoting characteristics, highlighting its potential to improve soil fertility and support legume growth.

Sandamali et al. (2021) reported that *P. oxalicum* (Bt9) is an efficient phosphate-solubilizing fungus and demonstrated its effectiveness as a phosphorus biofertilizer under field conditions using the Bw 367 rice variety (*Oryza sativa*). Molecular identification of *P. oxalicum* (Bt9) was performed through DNA sequencing followed by sequence similarity analysis using BioEdit software (version 7.2.5) (Sandamali et al. 2021). Despite previous studies on phosphate-solubilizing *P. oxalicum* strains, the phosphate-solubilizing potential of *P. oxalicum* (Bt9) in ERP-amended compost and its effects on the growth of red cowpea have not yet been investigated. Therefore, the present study aimed to evaluate the phosphate-solubilizing ability of *P. oxalicum* (Bt9) in ERP-enriched compost and to assess its plant growth-promoting effects on red cowpea (*Vigna unguiculata*).

2. MATERIALS AND METHODS

2.1. Fungal Culture Preparation

The phosphate-solubilizing fungal isolate, designated as the Bentota isolate, was recovered from the rice rhizosphere by Sandamali et al. (2017). Pure cultures were prepared and taxonomically confirmed as *Penicillium oxalicum* (Bt9) through DNA sequencing, as reported by Sandamali et al. (2021).

2.2. In Vitro Evaluation of the Phosphate Solubilizing Capability of Fungal Inoculum *P. oxalicum* (Bt9)

The phosphate solubilization index (PSI) was determined to assess the phosphate-solubilizing capability of pure cultures of *P. oxalicum* (Bt9). Pure cultures were inoculated onto Pikovskaya's (PKV) agar medium and incubated at approximately 32°C for three days. The diameters of the halo zone and fungal colony were measured using a vernier caliper with a resolution of 0.05 mm and recorded as described by Mayadunna et al. (2023).

2.3. In Vitro Evaluation of the Phosphate Solubilizing Efficiency of Fungal Inoculum *P.oxalicum* (Bt9)

Quantification of P solubilization of fungal inoculum *P. oxalicum* (Bt9) was measured using Pikovskaya's Broth Medium supplemented with tricalcium phosphate (TCP) at 5 $mg.L^{-1}$ (pH 7.2). The sterilized medium was inoculated with a 5.00 mm mycelial disc from the actively growing colony periphery of a 7-day-old *P. oxalicum* (Bt 9) culture, and an uninoculated medium was maintained as the control. The inoculated growth medium was incubated on a rotary shaker set to 160 rpm at 30^oC for 7 days. Samples of 5 mL were taken from the culture at 3, 5, and 7 days after incubation under sterile conditions, and the amount of released soluble phosphate in the samples was measured via the Molybdenum blue assay at 420 nm, as described by Shrivastava et al. (2011).

2.4. Inoculum Preparation for Composting Studies

P. oxalicum (Bt9) was cultured in a cost-effective growth medium consisting primarily of rice husks and rice seeds. Pure cultures of *P. oxalicum* (Bt9) were used in sterilized inoculum bags for mass multiplication, as described by Sandamali et al. (2021)

2.5. Implementation of Compost Units

Four treatments were arranged in a Completely Randomized Design (CRD). Each treatment consisted of four replicates, resulting in a total of 16 compost piles as experimental units. Compost units were prepared as heaps following the windrow composting approach.

Each compost heap measured 1 m in length, 1 m in width, and 0.5 m in height. The raw materials used included approximately 12 kg of rice straw (an agro-industrial by-product collected from the Regional Rice Research and Development Center, Bombuwala), 8 kg of fresh leaves of *Gliricidia sepium* (green manure), and 10 kg of cow dung (animal manure).

A polythene sheet measuring approximately 1.5 m × 1.5 m was placed on the ground to prevent direct contact between the compost heaps and the soil surface. Rice straw, *G. sepium* leaves, and cow dung were arranged in alternating layers on the polythene sheet. Approximately 1.5 kg of ERP, equivalent to 50 kg per 1000 kg of raw materials, and 600 g of previously prepared compost, equivalent to 20 kg per 1000 kg of raw materials, were incorporated between the layers of each compost unit.

Each compost unit was moistened with approximately 5 L of water to provide adequate moisture for microbial activity and accelerate organic matter decomposition. The heaps were then covered with black polythene and secured using ropes and cleats made from *G. sepium* branches, which allowed airflow and facilitated aerobic decomposition.

Treatment 1 (T1) received approximately 60 g of sugar (equivalent to 400 g per 200 kg of raw materials) and 37.5 g of *P. oxalicum* (Bt9) inoculum (equivalent to 250 g per 200 kg of raw materials) to assess their combined effect on composting. This treatment was replicated four times (T1R1, T1R2, T1R3, and T1R4).

Treatment 2 (T2) received 37.5 g of *P. oxalicum* (Bt9) inoculum (equivalent to 250 g per 200 kg of raw materials) without sugar. This treatment was replicated four times (T2R1, T2R2, T2R3, and T2R4).

Treatment 3 (T3) received approximately 60 g of sugar (equivalent to 400 g per 200 kg of raw materials) without fungal inoculum. This treatment was replicated four times (T3R1, T3R2, T3R3, and T3R4).

Treatment 4 (T4) served as the control and represented standard composting conditions without fungal inoculum or sugar. This treatment was replicated four times (T4R1, T4R2, T4R3, and T4R4).

Compost heaps were turned during the 3rd, 6th, 9th, and 12th weeks of composting to ensure uniform mixing and aeration, thereby promoting microbial activity. The composting process was completed at the end of the 12th week.

2.6. Sampling of Compost Units

Compost samples were collected during the 6th, 9th, and 12th weeks of the composting process and again during the 15th week following compost maturation. Sampling was conducted using a simple random sampling technique.

2.7. Determination of Physicochemical Parameters of Compost Units

The temperature of each compost unit was measured during the 6th, 9th, and 12th weeks of composting. Bioavailable phosphorus (P), pH, and electrical conductivity (EC) were measured during the 6th, 9th, 12th, and 15th weeks.

Moisture content was determined at the end of the composting period (12th week) using the gravimetric method. Compost samples were weighed before and after oven drying at 105°C until a constant weight was achieved. Moisture content was then calculated based on the weight loss resulting from water evaporation.

Bioavailable phosphorus content was determined using the molybdenum blue assay at 882 nm according to Murphy and Riley (1962). Prior to analysis, the spectrophotometer was calibrated using phosphate standard solutions prepared from analytical-grade KH_2PO_4 to generate a standard curve. The bioavailable phosphorus concentrations (ppm) of the treatments were subsequently calculated using this calibration curve.

2.8. Implementation of the Pot Experiment

A soil–compost mixture was prepared at a 1:1 ratio using approximately 100 g of soil and 100 g of phospho-compost obtained from each compost replicate as the potting medium (Jayathilake et al. 2024).

The prepared potting medium was transferred into separate pots. Each treatment (T1, T2, T3, and T4) consisted of four independent compost replicates, with one pot prepared from each replicate, resulting in a total of 16 experimental pots. In addition, two control pots containing 200 g of soil without phospho-compost were included.

Red cowpea (*Vigna unguiculata*) seeds were soaked in water overnight before sowing. Four seeds were planted in each pot, representing a compost replicate. After germination, four healthy red cowpea plants were maintained in each pot under optimal growth conditions for approximately 30 days.

Each pot received approximately 25 mL of water daily, and routine maintenance was carried out to ensure optimal plant growth. After 30 days, all plants were carefully uprooted from their respective pots, and growth parameters, including shoot length, root length, and total plant length, were measured.

2.9. Statistical Analysis

Data was analyzed using two-way analysis of variance (ANOVA) in Minitab software. Significant differences among treatments were determined using Tukey's pairwise comparison test at a significance level of 0.05.

The effects of treatment and sampling time (week) were evaluated for physicochemical parameters, whereas the effects of treatment on plant growth parameters (shoot length, root length, and total plant length) were assessed in the pot experiment.

Table 1: PSI of *P. oxalicum* (Bt9) cultures in PKV medium.

No. of culture	PSI
1	3.01 ± 0.14
2	3.06 ± 0.21
3	2.70 ± 0.34
4	2.38 ± 0.74
5	2.25 ± 0. 32

The assumptions of ANOVA, including normality and homogeneity of variance, were verified prior to statistical interpretation.

3. RESULTS AND DISCUSSION

3.1. Qualitative Phosphate Solubilizing Ability of *P. oxalicum* (Bt9)

Table 1 presents the Phosphate-solubilizing index (PSI) of *P. oxalicum* (Bt9) cultured on Pikovskaya's (PKV) medium, derived from measurements of both colony and halo zone diameters.

All the colonies in the cultures exhibited PSI of greater than 1, as depicted in Table 1, indicating that *P.oxalicum* (Bt9) is capable of phosphate solubilization.

The colony morphology of *Penicillium oxalicum* (Bt9) was characterized by a dark, dull-green appearance with a thin white peripheral margin, as shown in Fig. 1. A slight, clear halo zone was observed around each colony on Pikovskaya's (PKV) medium, indicating the phosphate-solubilizing ability of *P. oxalicum* (Bt9). Similar observations were reported by Sandamali et al. (2021), who documented the formation of clear halo zones around colonies of *P. oxalicum* (Bt9) grown on PKV medium. Furthermore, those cultures retained their phosphate-solubilizing ability on PKV medium for up to 36 months, highlighting the consistent efficacy and long-term applicability of this fungal inoculum for integration into phosphate biofertilizer technology.

3.2. Quantitative Phosphate Solubilizing Ability of *P. oxalicum* (Bt9)

Fig. 1: The colonies of *P. oxalicum* (Bt9) culture grown on PKV medium.

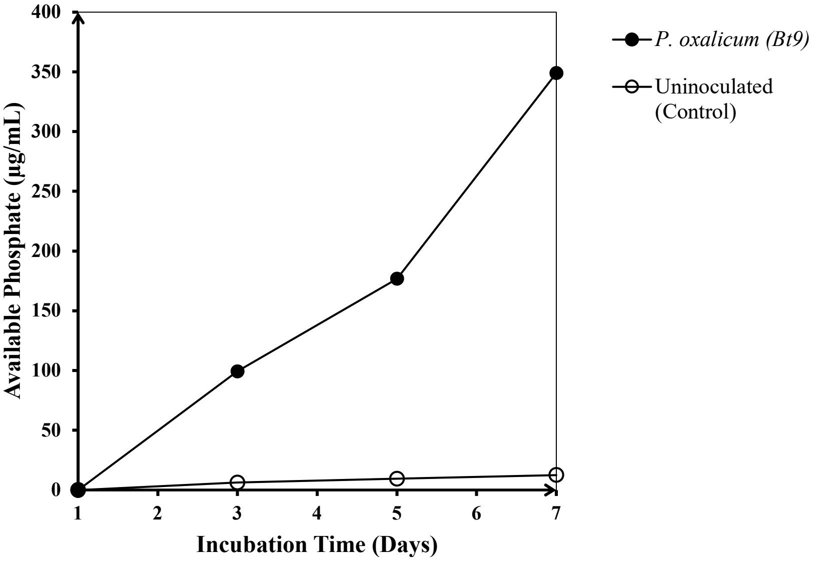

Fig. 2: Solubilized P concentrations after 1st, 3rd, 5th and 7th days of incubation in TCP containing PKV broth inoculated with *P. oxalicum* (Bt9) isolate.

The concentration of solubilized phosphorus released by *P. oxalicum* (Bt9) increased progressively over time compared with the control, as shown in Fig. 2, indicating the strong phosphate-solubilizing capability of this fungal species. Similar findings were reported by Sandamali et al. (2021), who evaluated the quantitative phosphate-solubilizing efficiency of *P. oxalicum* (Bt9) using an *Aspergillus niger*-inoculated medium as the positive control and an uninoculated medium as the negative control.

These results are also consistent with the findings of Yin et al. (2015), who reported a significantly higher concentration of soluble phosphorus (1392.0 ± 233.2 µg.mL^{-1}) in tricalcium phosphate (TCP)-amended Pikovskaya's (PVK) medium inoculated with the *P. oxalicum* P4 isolate on the 7th day of incubation compared with the other treatments. Similarly, Wang et al. (2020) reported enhanced phosphorus solubilization by *P. oxalicum* FJG21 and *P. oxalicum* FJQ5 in PKV broth medium containing insoluble $Ca_3(PO_4)_2$, with soluble phosphorus concentrations of 343.2 µg.mL^{-1} and 339.2 µg.mL^{-1}, respectively, on the 7th day. These values were significantly higher than those recorded for *Bacillus subtilis* BPM12 and the uninoculated control.

Table 2: Mean moisture percentage of compost treatments at the 12th week of the composting process.

The compost treatment No.	Mean moisture percentage at 12th week of composting process [%]
T_1	54.06 ± 3.06
T_2	56.74 ± 3.82
T_3	56. 79 ± 4.64
T_4	57.10 ± 5.66

Overall, the observed increase in soluble phosphorus concentration confirms the effectiveness of *P. oxalicum* (Bt9) as a phosphate-solubilizing fungal inoculum and supports its potential application in phosphate biofertilizer development.

3.3 Impact of Moisture on Composting Process in Compost Units

Table 2 presents the mean moisture percentage of compost treatments at the 12th week of the composting process.

The moisture percentage of all compost treatments ranged between 50% and 60%, corresponding to the ideal range for efficient composting as presented in Table 2.

Table 3: Mean temperature measurements in compost treatments at the 3rd, 6th, 9th and 12th weeks during the composting process.

The compost treatment no.	Mean temperature [°C] at the 3rd week of the composting process	Mean temperature [°C] at the 6th week of the composting process	Mean temperature [°C] at the 9th week of the composting process	Mean temperature [°C] at the 12th week of the composting process
T_1	31.0 ± 0.7	48.3 ± 0.4	47.7 ± 0.9	39.7 ± 0.6
T_2	31.5 ± 0.6	48.6 ± 1.0	48.8 ± 1.1	40.8 ± 0.7
T_3	29.2 ± 0.5	40.0 ± 0.3	38.1 ± 1.0	36.8 ± 1.0
T_4	28.9 ± 0.6	37.6 ± 0.6	39.3 ± 0.4	36.0 ± 1.2

3.4. Effect of Temperature of the Compost Units

Table 3 presents the mean temperatures of compost units at the 3rd, 6th, 9th and 12th weeks during the composting process.

The maximum temperature of the experimental compost units in this study remained below 50°C during the 6th and 9th weeks of the composting process in Treatments 1 and 2, as presented in Table 3. The temperature of compost systems is influenced by the quantity of bulk material being processed; lower substrate volumes generally generate limited heat due to reduced microbial activity and energy release (Quadar et al. 2023). Therefore, the moderate temperatures observed in this study may be attributed to the relatively small-scale nature of the experiment and the limited quantity of raw materials used in compost preparation.

In addition, the compost units were prepared as aerated heaps with proper ventilation, which likely facilitated heat dissipation and prevented excessive temperature build-up. Several studies have reported that optimal thermophilic composting activity typically occurs within the temperature range of 50°C to 60°C, while overall efficient composting is generally observed between 40°C and 65°C (Wijerathna et al. 2024a). In some cases, successful composting has also been reported under mesophilic conditions below 45°C (Tang et al. 2007).

Temperature is a critical factor governing the rate of composting reactions, as it directly influences microbial metabolic activity and community structure. It also plays an important role in pathogen elimination and seed inactivation, thereby ensuring compost sanitation (Wijerathna et al. 2024a, Tang et al. 2007). Tang et al. (2007) reported that the highest cumulative oxygen (O_2) uptake occurred at 43°C across composting systems operating within a temperature range of 22°C to 57°C. Mesophilic conditions support steady organic matter degradation, whereas higher temperatures are more effective for pathogen and weed seed destruction during composting (Tang et al. 2007). Accordingly, the temperature profile observed in this study suggests that the composting process predominantly remained within the mesophilic phase.

The heat generated during composting is primarily a result of microbial metabolic activity and serves as an indicator of microbial activity and organic matter decomposition. In this study, the average temperature recorded in Treatments 1 and 2 was higher than that in Treatments 3 and 4. This trend may indicate enhanced microbial activity in Treatments 1 and 2, which were inoculated with *P. oxalicum* (Bt9), compared with Treatments 3 and 4, which lacked fungal inoculation.

Similarly, Wijerathna et al. (2024a) reported that a consortium-inoculated treatment (C5), containing *Bacillus*

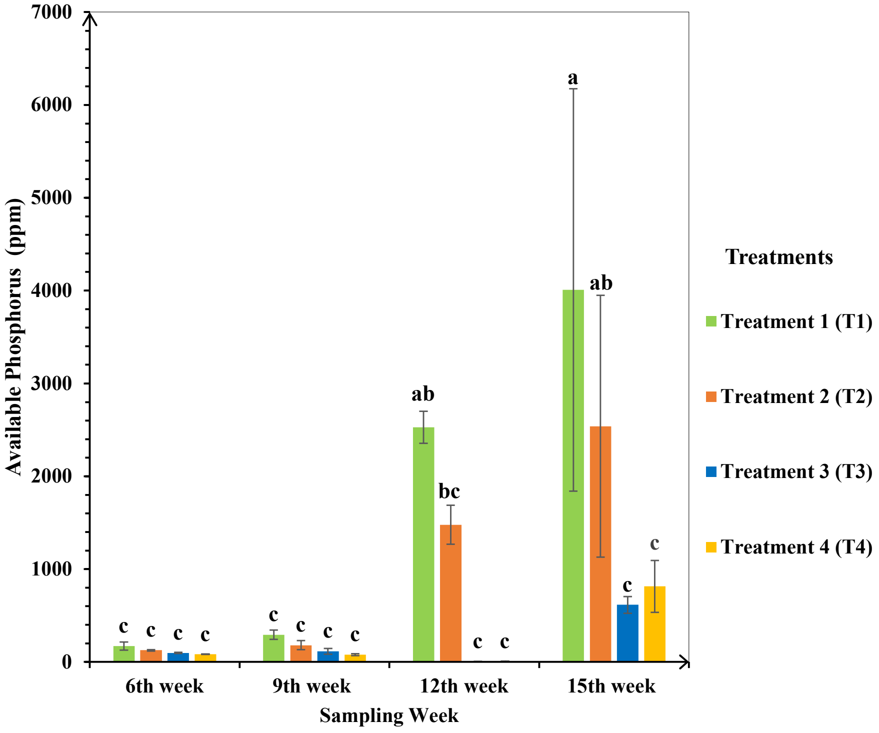

Fig. 3: Distribution of the mean values (± SE, $n = 4$) of available Phosphorus content in the compost treatments throughout the sampling weeks. Bars represent mean ± standard error. The Values labeled with distinct letters indicate significant differences ($P < 0.05$) according to Tukey's pairwise comparison.

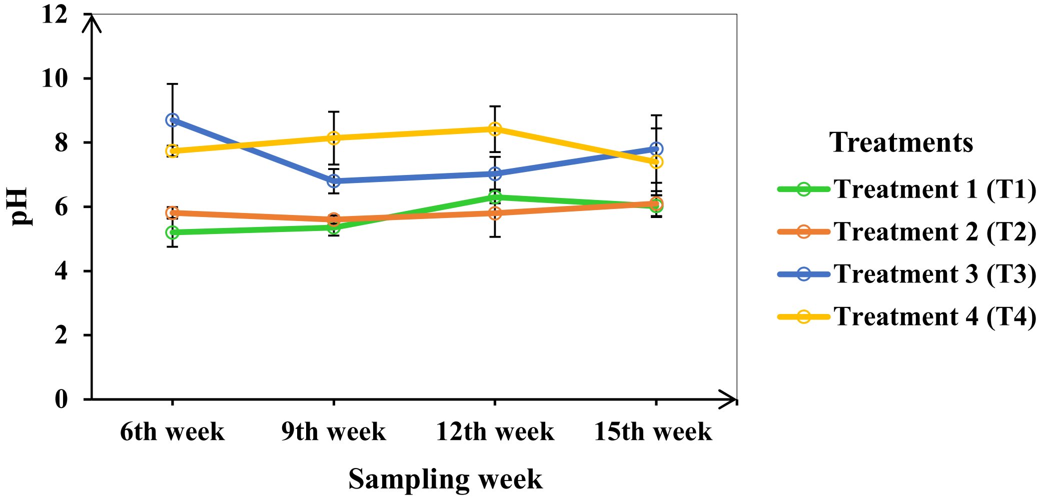

Fig. 4: Distribution of the mean pH values (± SE, $n = 4$) of the compost treatments throughout the sampling weeks. Bars represent mean ± standard error.

haynesii, *Bacillus amyloliquefaciens*, and *Bacillus safensis*, exhibited the highest composting temperature along with elevated enzymatic activities, including cellulase, amylase, protease, and lipase. This enhanced microbial activity contributed to accelerated decomposition and a reduced composting period compared with the control and other treatments.

3.5. Effect of Treatments on Bioavailable Phosphorus Content

According to the data depicted in Fig. 3, the highest recorded available phosphorus (P) content in Treatment 1 (*P. oxalicum* Bt9 with sugar) during the 15th week was 4008 ± 2005.83 ppm. The available P content in Treatment 2 (*P. oxalicum* Bt9 without sugar) at the 15th week and in Treatment 1 at the 12th week showed no significant difference. The lowest available P content was observed in Treatment 3 (sugar without *P. oxalicum* Bt9) at the 15th week, with a value of 614.5 ± 83.66 ppm, but it was not significantly different from Treatment 4 (neither *P. oxalicum* Bt9 nor sugar). Throughout the experiment, Treatment 1 consistently exhibited the highest available P content weekly, with some differences reaching statistical significance. Treatment 2 consistently ranked second. In contrast, Treatments 3 and 4 had significantly lower available P contents each week. This variation can be attributed to the phosphate-solubilizing activity of *P. oxalicum* Bt9 present in Treatments 1 and 2, which was absent in Treatments 3 and 4. Additionally, the negligible differences between Treatments 1 and 2, and between Treatments 3 and 4, suggest that the presence of sugar had little to no effect on ERP solubilization or on the growth of *P. oxalicum* Bt9.

3.6. pH Variation in Compost Treatments During the Sampling Period

Treatment 1 and Treatment 2 consistently exhibited slightly acidic pH values ranging from 5 to 6 throughout the composting period, in contrast to the other treatments, as shown in Fig. 4. Wang et al. (2020) reported a similar reduction in pH associated with enhanced solubilization of insoluble phosphate from various phosphorus sources by *P. oxalicum* FJG21 and *P. oxalicum* FJQ5. These strains exhibited the highest soluble phosphorus concentrations, accompanied by the lowest pH values. Furthermore, HPLC analysis detected malic acid and oxalic acid in cultures of both *P. oxalicum* FJG21 and *P. oxalicum* FJQ5. The dissolution of phosphate was attributed to the combined effects of pH reduction and organic acid secretion.

The primary mechanism responsible for mineral phosphate dissolution in soil involves a reduction in soil pH caused by microbial organic acid production, together with the mineralization of organic phosphorus through the action of acid phosphatases (Anand et al. 2016). The secretion of organic acids is widely recognized as the principal mechanism of phosphate solubilization in phosphate-solubilizing fungi (PSF). These fungi solubilize insoluble phosphate compounds by acidifying the surrounding environment through the release of organic acids (Rawat et al. 2021). In addition, organic acids can chelate calcium, iron, and aluminum ions, thereby facilitating the conversion of insoluble phosphate into plant-available forms (Gong et al. 2014).

Yin et al. (2015) reported that tartaric acid and citric acid were the predominant organic acids secreted by

Penicillium oxalicum P4 during the solubilization of tricalcium phosphate. Similarly, Gong et al. (2014) found that *Penicillium oxalicum* I1 reduced the pH of the culture medium from 6.9 to 1.65 within 26 hours. During this period, oxalic acid production increased progressively, reaching a maximum concentration of approximately 593.9 $\mu g.mL^{-1}$. A statistically significant inverse relationship was observed between organic acid concentration and pH. Although excessive soil acidification can adversely affect crop growth when soil pH falls below 6.5, the pH reduction caused by *P. oxalicum* I1 in soil was only from 7.0 to 6.56, indicating its ability to enhance phosphate solubilization without negatively affecting plant growth. Similarly, Singh & Reddy (2011) reported that *P. oxalicum* increases the concentration of bioavailable phosphorus in soil by lowering pH through mechanisms such as chelation and ion-exchange reactions.

The genus *Penicillium* is one of the predominant fungal groups involved in phosphatase production. Phosphatases, including phytase and acid phosphatase, play a vital role in the mineralization of organic phosphorus compounds into bioavailable forms (Singh & Reddy 2011). Acevedo et al. (2014) reported that *Aspergillus* spp. and *Penicillium* spp. reduced the pH of liquid culture media during the dissolution of recalcitrant phosphate sources, thereby promoting phosphate solubilization. A decrease in pH is generally associated with increased acidity and enhanced phosphate availability (Wang et al. 2020).

Therefore, the decline in pH observed in Treatments 1 and 2 may be attributed to the production of organic acids and other acidic metabolites by *P. oxalicum* (Bt9). The absence of this response in the other treatments further supports the role of *P. oxalicum* (Bt9) in enhancing ERP solubilization through acidification and phosphate-mobilizing mechanisms.

3.7. Effect of EC on Compost Treatments During the Sampling Period

Treatment 1 and Treatment 2 exhibited higher mean EC values throughout the composting period compared to the other treatments, as shown in Fig. 5. Electrical conductivity (EC) reflects the soluble salt content of the final compost product and serves as an indicator of the availability of essential nutrients required for plant growth and development. Therefore, EC is commonly used as a measure of the total ion concentration present in compost.

During the composting process, EC values generally increase due to the mineralization of organic matter, resulting in the formation of inorganic compounds and the release of soluble ions (Wijerathna et al. 2024a). The higher EC values observed in T1 and T2 may be attributed to increased concentrations of soluble orthophosphate ions resulting from enhanced bio-solubilization of ERP by *P. oxalicum* (Bt9). This finding suggests that inoculation with *P. oxalicum* (Bt9) improved phosphorus release and nutrient availability within the compost.

Similarly, Wijerathna et al. (2024a) reported that a consortium-inoculated treatment (C5) containing *Bacillus amyloliquefaciens*, *Bacillus haynesii*, and *Bacillus safensis* exhibited the highest EC values, indicating accelerated decomposition of organic waste and enhanced nutrient mineralization. The findings of the present study further

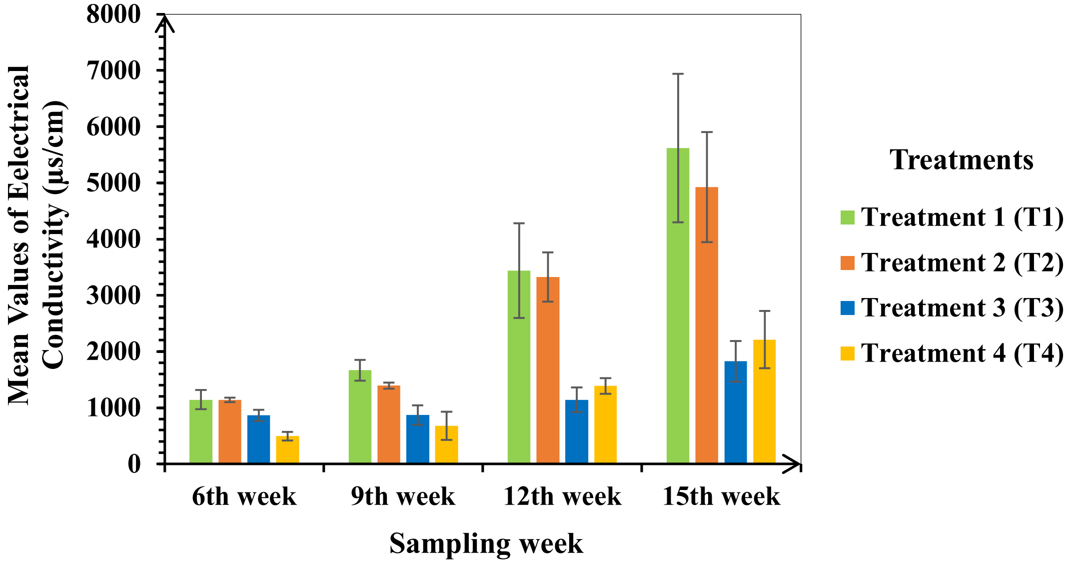

Fig. 5: Distribution of the mean electrical conductivity (EC) values (± SE, *n* = 4) in the compost treatments during the sampling weeks. Bars represent mean ± standard error.

support the role of microbial inoculants in improving compost quality through increased nutrient availability and enhanced decomposition processes.

3.8. Impact of Plant Growth Promotion by *P. oxalicum* (Bt 9) in Red-Cowpea (*V. unguiculata*) Plants

According to the data presented in Figs. 6, 7, and 8, T1 (soil amended with phospho-compost containing *P. oxalicum* (Bt9) and sugar) exhibited the highest values for plant growth parameters, including shoot length (26.58 ± 2.44 cm), root length (11.36 ± 1.48 cm), and total plant length (54.24 ± 2.64 cm). No significant differences were observed between the plant growth parameters of T1 and T2 (soil amended with phospho-compost containing *P. oxalicum* (Bt9) without sugar). Similarly, no significant differences were detected between T3 (soil amended with phospho-compost containing sugar without *P. oxalicum* (Bt9)) and T4 (soil amended with phospho-compost lacking both sugar and *P. oxalicum* (Bt9)).

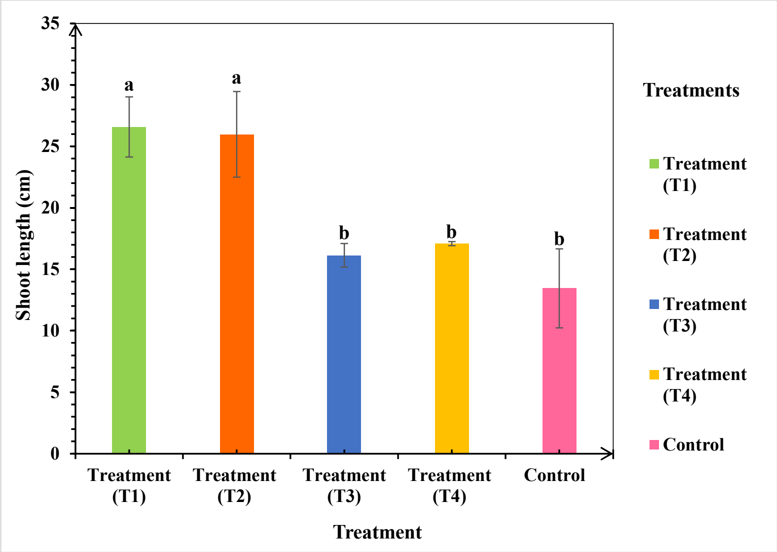

Fig. 6: The effect of different treatments on the mean values of shoot length (± SE, *n* = 4) of red cowpea (*V. unguiculata*) plants. Bars represent mean ± standard error. The values annotated with different letters indicate significant differences at P < 0.05 according to Tukey's pairwise comparison.

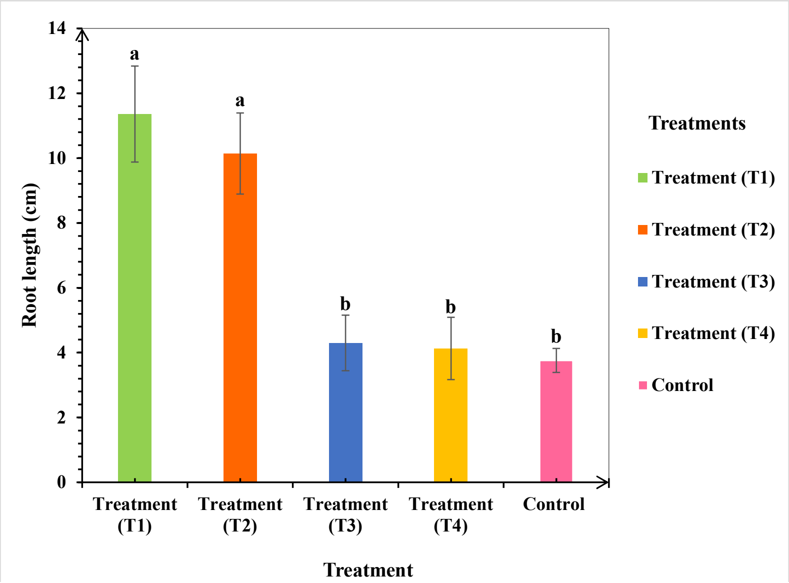

Fig. 7: The effect of different treatments on the mean values of root length (± SE, *n* = 4) of red cowpea (*V. unguiculata*) plants. Bars represent mean ± standard error. The values annotated with different letters indicate significant differences at P < 0.05 according to Tukey's pairwise comparison.

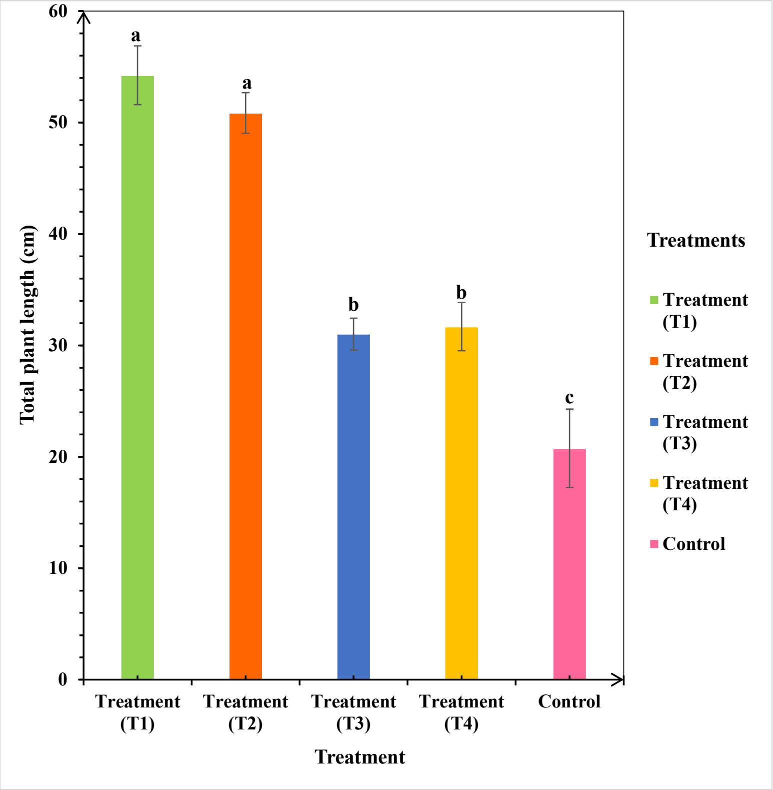

Fig. 8: The effect of different treatments on the mean values of total plant length (± SE, *n* = 4) of red cowpea (*V. unguiculata*) plants. Bars represent mean ± standard error. The values marked with different letters indicate statistically significant differences ($P < 0.05$) according to Tukey's pairwise comparison.

The lowest values for plant growth parameters, including shoot length (13.45 ± 3.22 cm), root length (3.76 ± 0.37 cm), and total plant length (20.76 ± 3.52 cm), were recorded in the control treatment, which consisted solely of soil without phospho-compost, *P. oxalicum* (Bt9), or sugar. These findings indicate that phospho-compost inoculated with *P. oxalicum* (Bt9) promotes the growth of red cowpea (*Vigna unguiculata*). Furthermore, the results suggest that the addition of sugar had no significant effect on microbial activity or on enhancing the bio-solubilization of ERP during phospho-compost preparation.

The present findings are consistent with those of Yin et al. (2015), who demonstrated that inoculation of rock phosphate with *P. oxalicum* P4 significantly increased both bioavailable phosphorus and total plant phosphorus contents. Moreover, isolate P4 significantly enhanced maize fresh biomass, thereby promoting plant growth in rock phosphate-enriched soil compared with the other treatments. In addition, soil amended with rock phosphate and inoculated with isolate P4 showed significantly higher concentrations of organic acids, including malic, formic, succinic, citric, acetic, and lactic acids, compared with the control treatment.

Similarly, Jayathilake et al. (2025) reported that *Bacillus siamensis* strain KCTC 13613 exhibited the highest phosphate-solubilizing activity, accompanied by a reduction in growth medium pH, indicating elevated organic acid production. In a pot experiment using mung bean plants, the application of phosphate-solubilizing bacterial strains significantly improved growth parameters, including root length, shoot length, and biomass. Phosphorus availability in the potting medium was significantly enhanced by *B. siamensis* strain KCTC 13613, resulting in superior plant growth performance compared with the other strains and the control.

Comparable findings were reported by Biswas & Narayanasamy (2006) for the incorporation of *Aspergillus awamori*, a phosphate-solubilizing fungus, into low-grade rock phosphate-enriched compost. Their study demonstrated that inoculation with *A. awamori* increased both total phosphorus and available phosphorus contents compared with compost lacking fungal inoculation. In the pot experiment, compost enriched with low-grade rock phosphate and inoculated with *A. awamori* enhanced both yield and phosphorus uptake in mung bean (*Vigna radiata*) compared with the control. Furthermore, yield and phosphorus uptake were 13.0% and 21.5% higher, respectively, than in the treatment without *A. awamori*. The authors also reported that phosphate-solubilizing microorganisms produce various organic acids, such as tartaric, malic, and citric acids, as well as enzymes, including alkaline and acid phosphatases, which facilitate the conversion of insoluble phosphate into plant-available forms.

Singh & Reddy (2011) reported similar results, demonstrating high phosphate solubilization accompanied by a reduction in the pH of PKV broth medium amended with rock phosphate and inoculated with *P. oxalicum*. In their study, phosphate solubilization was associated with the secretion of organic acids into the culture medium. Malic acid (9435 nmoL.mL^{-1}) was the predominant acid, followed by gluconic acid (4306 nmoL.mL^{-1}), oxalic acid (411 nmoL.mL^{-1}), and acetic acid (92 nmoL.mL^{-1}). Furthermore, field experiments showed that *P. oxalicum* inoculation in rock phosphate-amended alkaline soil significantly improved wheat and maize growth and yield compared with the control.

Shrivastava et al. (2011) investigated the potential of two phosphate-solubilizing fungi, *Aspergillus niger* TMPS1 and *Penicillium oxalicum* TMPS3, for producing phosphate-enriched manure (PEM) using low-grade Indian rock phosphate. Using a ^{32}P radiotracer technique in a pot experiment, they reported phosphorus uptake by mung bean (*V. radiata*) plants as relative agronomic efficiency (RAE). The PEM product inoculated with *P. oxalicum* TMPS3 exhibited the highest RAE among all phosphorus sources. Moreover, phosphorus uptake increased significantly in PSM-inoculated treatments compared with the uninoculated control. These findings suggest that the increased phosphorus uptake resulted from elevated phosphorus concentrations in the rhizosphere soil solution.

In the present study, *P. oxalicum* (Bt9) produced a higher bioavailable phosphorus content than *P. oxalicum* P4 under their respective experimental conditions, as reported by Yin et al. (2015). However, a direct comparison with *P. oxalicum* TMPS3 was not possible because Shrivastava et al. (2011) reported only total phosphorus content rather than bioavailable phosphorus.

Li et al. (2021) identified *Penicillium oxalicum* HZ06 as a plant growth-promoting fungus because of its ability to significantly increase eggplant biomass. Inoculation with HZ06 increased seedling length, dry weight, and fresh weight by 22.35%, 39.26%, and 47.69%, respectively, compared with the control. Root length, dry weight, and fresh weight also increased by 0.83%, 41.30%, and 28.72%, respectively. These findings highlight the potential of *P. oxalicum* as a microbial inoculant for improving crop growth in nutrient-deficient soils. Although direct comparisons between studies are limited due to differences in plant species and experimental conditions, the collective evidence supports the role of *P. oxalicum* (Bt9) as an effective phosphate-solubilizing fungus with plant growth-promoting properties.

Gong et al. (2014) reported the high phosphate-solubilizing ability of *Penicillium oxalicum* I1 (P-I1). This strain effectively solubilized various insoluble phosphate compounds, including $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, $AlPO_4$, $FePO_4$, and $Ca_{10}(PO_4)_6(OH)_2$, converting them into soluble $CaHPO_4$. Furthermore, P-I1 inhibited the reconversion of soluble $CaHPO_4$ into insoluble phosphate forms. The transformation of insoluble Ca_8-P, Al-P, and Fe-P into soluble Ca_2-P was attributed to oxalic acid secretion. Correlation analysis revealed that Ca_2-P content was negatively associated with soil pH and positively associated with P-I1 abundance. In field experiments, maize yield increased by 14.47% in the treatment receiving P-I1 and TCP at 45 kg.ha^{-1} compared with the control. Similar to P-I1, *P. oxalicum* (Bt9) efficiently converted insoluble phosphate into plant-available forms, thereby supporting the growth of red cowpea.

The majority of identified phosphate-solubilizing fungi belong to the genus *Penicillium* (Li et al. 2016). Phosphate solubilization is a well-documented characteristic of this genus (Qiao et al. 2019). Several species, including *P. albidum*, *P. bilaii*, *P. oxalicum*, *P. frequentans*, *P. italicum*, *P. citrinum*, *P. simplicissimum*, *P. expansum*, and *P. rubrum*, are used as commercial biofertilizers because of their ability to mobilize essential nutrients such as Cu, Co, Fe, Mn, Mo, P, and Zn, thereby enhancing plant resilience to both biotic and abiotic stresses (Odoh et al. 2020). Among these, *Penicillium bilaii* has been widely commercialized by Novozymes as a soil inoculant, with numerous field trials confirming its effectiveness and compatibility with modern agricultural practices (Leggett et al. 2007).

Phosphorus is an essential macronutrient required for plant growth and development (Anand et al. 2016, Jayathilake et al. 2024). It plays a central role in photosynthesis, carbon metabolism, membrane synthesis, cell division, and cell enlargement (Singh & Reddy 2011). Phosphorus is also critical for root development and biological nitrogen fixation in legumes (Anand et al. 2016), thereby contributing significantly to both root and shoot growth (Fathi & Afra 2023).

As a legume, red cowpea (*V. unguiculata*) forms symbiotic associations with *Rhizobium* bacteria and arbuscular mycorrhizal fungi (AMF). These microorganisms can interact synergistically with phosphate-solubilizing fungi such as *P. oxalicum* (Bt9) to improve nutrient acquisition. While *P. oxalicum* (Bt9) enhances phosphorus availability through the secretion of organic acids, AMF extend their hyphal networks into the soil and facilitate phosphorus uptake by plants. Therefore, the enhanced plant growth observed in Treatments 1 and 2 may be attributed to increased ERP solubilization by *P. oxalicum* (Bt9), coupled with improved phosphorus uptake and utilization by the plants. Overall, these findings demonstrate that *P. oxalicum* (Bt9) effectively solubilizes ERP and promotes the growth of red cowpea (*V. unguiculata*).

For commercial biofertilizer production, maintaining microbial viability during large-scale propagation and storage is essential. Sandamali et al. (2021) reported that *P. oxalicum* (Bt9) grown on a rice husk- and rice seed-based substrate maintained populations of 22.7×10^8 CFU.g^{-1} at room temperature and 33.5×10^8 CFU.g^{-1} at 8°C for up to four months while retaining its phosphate-solubilizing ability. These findings highlight the potential of *P. oxalicum* (Bt9) for development as a commercial phosphorus biofertilizer.

This study was conducted as a pilot-scale composting experiment to evaluate physicochemical changes during composting and their effects on plant growth. Only a single fungal species, *P. oxalicum* (Bt9), was investigated, and all experiments were performed under controlled pot conditions. Plant growth was assessed only during the first 30 days after planting. Future studies should evaluate the complete crop growth cycle, including flowering, yield, and harvest parameters, to comprehensively assess the agronomic performance of *P. oxalicum* (Bt9). Field-scale validation is also required to confirm its effectiveness as a phosphate-solubilizing and plant growth-promoting inoculant. The present findings indicate that ERP-enriched compost inoculated with *P. oxalicum* (Bt9) can increase bioavailable phosphorus levels in soil and promote legume growth under pot conditions, suggesting its potential as a partial substitute for chemical phosphorus fertilizers. Sandamali et al. (2021) reported that direct application of *P. oxalicum* (Bt9) in rice cultivation reduced chemical phosphorus fertilizer requirements by 50% without affecting yield. However, ERP-enriched compost inoculated with *P. oxalicum* (Bt9) was not evaluated in that study. Therefore, further field-based investigations are needed to determine the extent to which ERP-enriched compost inoculated with *P. oxalicum* (Bt9) can replace chemical phosphorus fertilizers under local agricultural conditions.

4. CONCLUSIONS

This study demonstrated that P. oxalicum (Bt9) is an efficient phosphate-solubilizing fungal inoculum. Therefore, incorporating it into the composting process, enriched with Eppawala Rock Phosphate (ERP), enhances the solubilization of insoluble phosphate in ERP into plant-available forms of phosphorus. Consequently, the increased availability of bioavailable phosphorus promotes the growth of red cowpea (Vigna unguiculata).

The findings suggest that ERP-enriched compost inoculated with *P. oxalicum* (Bt9) has considerable potential for the production of organic phosphorus fertilizer. Such a product could serve as a sustainable and viable alternative to imported chemical phosphorus fertilizers, thereby helping to address a critical challenge facing the agricultural sector of Sri Lanka.

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