



Effects of Biofertilizer Application and Organic Matter Incubation on Soil Chemical Properties

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Abstract: Soil organic matter is essential for maintaining soil fertility and supporting sustainable agricultural production. However, raw organic matter does not directly enhance soil chemical properties without a sufficient decomposition period. This study evaluated the effects of organic matter incubation and biofertilizer concentrations on soil chemical characteristics. The experiment was conducted from June to August 2024 in the Greenhouse Laboratory of Gunadarma Technopark University, Jamali Village, West Java, using a completely randomized factorial design. Treatments consisted of four biofertilizer concentrations (0, 10, 15, and 20 mL/L) and five incubation periods of cow manure (0, 1, 2, 3, and 4 weeks), each replicated four times, resulting in 80 experimental units. The results showed a significant interaction between incubation duration and biofertilizer concentration on soil pH, organic carbon, total nitrogen, C/N ratio, available phosphorus (P_2O_5), and available potassium (K_2O). The four-week incubation combined with 10–20 mL/L of biofertilizer produced the most notable improvements, increasing pH to neutral levels, raising organic carbon and nitrogen contents, achieving an optimal C/N ratio, and enhancing P availability, although K remained low. These findings indicate that combining biofertilizer application with an adequate incubation period effectively improves soil fertility and offers a viable strategy for long-term soil management and agricultural productivity.

Keywords: Biofertilizer; Incubation period; Organic matter; Soil fertility; Sustainable agriculture

Introduction

Soil is fundamental to sustainable agriculture and global food production, yet intensive farming has led to declining soil quality, threatening long-term productivity (Hossain, 2021). Organic matter enhances soil quality by supplying nutrients, improving structure, and regulating nutrient cycling through microbial decomposition (Ma et al., 2023; Tong, 2024). It also increases water and nutrient retention while stabilizing soil aggregates, preventing erosion (X. Li et al., 2023). However, decomposition requires time and optimal conditions, delaying the immediate benefits of organic

fertilization (Arfarita et al., 2020). Biofertilizers significantly enhance microbial diversity and activity, particularly dehydrogenase enzymes, which drive organic matter oxidation and overall soil biological processes (Da Cunha Leme Filho et al., 2024; Guan, 2023; Shi et al., 2023). However, excessive organic inputs may reduce microbial diversity and enzyme function, compromising soil health (Ye et al., 2020). Long-term organic fertilizer application improves soil nutrient concentrations and microbial activity, supporting sustained soil health and plant growth (R. Wang et al., 2021; Wu et al., 2020).

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Organic matter plays a crucial role in soil fertility and the sustainability of agricultural production. Soils with high organic matter content exhibit enhanced structure, aeration, water retention capacity, and nutrient storage, all of which are essential for optimal plant growth (J. Li et al., 2015). Humus, the final product of organic matter decomposition, significantly improves the soil's ability to retain water and nutrients, particularly in regions with erratic rainfall patterns (B. Wang et al., 2020). The decomposition process involves fragmentation, mineralization, and humification, enabling organic matter to maximize soil benefits, enhance crop yields, and reduce reliance on chemical fertilizers (Wan et al., 2021; Wen et al., 2020). However, despite these advantages, organic matter application is often suboptimal due to insufficient microbial activity and inadequate incubation periods.

To maximize the benefits of organic matter, an incubation process is necessary, allowing microorganisms to proliferate and metabolize organic compounds into C-organic fractions, facilitating mineralization and the release of essential nutrients such as nitrogen, phosphorus, and sulfur, along with trace micronutrients (Cui et al., 2018; Salma et al., 2018). This process enhances nutrient availability for plant uptake, improving soil fertility and productivity (Siregar et al., 2017). The duration of the incubation period determines the maturity of organic matter, while biofertilizer application accelerates the decomposition process, ensuring that nutrients become readily available for plant absorption. Moreover, the incubation process involves integrating organic matter into the soil to modify its pH and structure, necessitating a specific waiting period before land use, which varies based on soil type and environmental conditions (Aisyah et al., 2018).

Soil quality is profoundly affected by climate change and unsustainable agricultural practices, necessitating improved organic matter management to enhance sustainability and food security. The incubation of organic matter is a key method because it helps microbes grow and breaks down organic matter into C-organic compounds through mineralization. This releases nutrients like nitrogen, phosphorus, sulfur, and micronutrients (Patriani et al., 2022; Qaswar et al., 2020). Research indicates that combining organic materials with chemical fertilizers is more effective in increasing soil nutrient concentrations than using chemical fertilizers alone, as it enhances nutrient availability and soil fertility (Chen et al., 2022; He et al., 2024). Longer incubation times affect how well organic matter improves the chemical properties of soil because they control how much organic matter breaks down and nutrients are released (J. Li et al., 2015). However,

limited research has explored the specific effects of the incubation period of organic materials combined with biofertilizers on soil chemical properties, highlighting a knowledge gap in sustainable soil management (He et al., 2024).

The application of organic fertilizers has been shown to enhance microbial diversity and activity, which play a vital role in nutrient cycling and soil health improvement (Kuziemska et al., 2020; Xu et al., 2022). Additionally, integrating organic materials with biofertilizers improves the capacity of soil to exchange cations and retain moisture, further supporting plant growth and overall soil productivity (Guo et al., 2023). Understanding the interactions between organic matter incubation and biofertilizer application is critical for optimizing strategies for managing soil fertility. So, the point of this study is to look into how the incubation period of organic materials at different biofertilizer concentrations changes the chemical properties of the soil. Specifically, it examines key soil parameters, including pH, organic carbon, total nitrogen, the C/N ratio, available phosphorus, and available potassium, to provide insights into sustainable soil fertility management.

Method

Soil Sampling and Experimental Design

Soil sampling was conducted at a depth of 0–15 cm in the Universitas Gunadarma Technopark, which had remained fallow for a decade. The samples were placed in 10 kg polybags for experimental treatments. To improve soil fertility, farmyard manure was applied at 10 tons per hectare. The experiment followed a completely randomized factorial design with two factors: biofertilizer application rate and incubation period.

Biofertilizer Treatments and Incubation Procedure

Four biofertilizer treatments were applied: (1) control (H0, no biofertilizer), (2) 10 mL/L (H1), (3) 15 mL/L (H2), and (4) 20 mL/L (H3). The biofertilizer was uniformly mixed with soil and cattle manure. Soil moisture was maintained at field capacity using distilled water to sustain microbial activity. The experiment involved 80 polybags arranged in a randomized factorial design ($4 \times 5 \times 4 = 80$). Incubation periods were set at 0 (M0), 1 (M1), 2 (M2), 3 (M3), and 4 weeks (M4), conducted under ambient laboratory conditions at $\sim 30^{\circ}\text{C}$.

Soil Sample Collection and Analytical Procedures

At each incubation period, soil samples were systematically collected from all treatments. Undisturbed samples were analyzed for aggregate

stability, while disturbed samples were used for physicochemical analysis. Composite samples were air-dried, sieved, and subjected to chemical characterization. Measured parameters included pH (using a calibrated pH meter), moisture content, C/N ratio, available phosphorus (P_2O_5) via Bray I extraction, and available potassium (K_2O) via ammonium acetate extraction. All analyses followed standardized soil testing protocols to ensure accuracy and reliability.

Result and Discussion

Soil pH

Soil pH regulates nutrient solubility and microbial activity, directly impacting plant growth. Optimal pH enhances nutrient uptake and supports beneficial microbes. Higher pH improves macronutrient availability but reduces micronutrient solubility.

ANOVA results confirmed that biofertilizer application with 1–4 weeks of incubation significantly influenced soil pH, while non-treated soils showed no significant changes (Table 1).

A significant interaction was seen between applying biofertilizer (10 mL/L for two weeks) and the changes in soil pH. After four weeks, the highest pH (7.36, neutral) was found at 10–20 mL/L. Biofertilizers stimulate decomposer bacteria, accelerating organic matter mineralization. The rise in pH is linked to organic acid production, which chelates aluminum (Al), reducing acidity. These findings align with Hamed et al. (2014) on organic matter-mineralization correlations and Tan (2010) on soil amelioration. Biofertilizer management that works well improves soil fertility, microbial activity, and long-term use of nutrients (Daniel, 2022; Fitriatin et al., 2021; Kaur, 2024).

Table 1. Interaction between biofertilizer application and incubation period on the average soil pH (H_2O) of the planting medium.

Incubation Period (weeks)	Biofertilizer (ml/L)			
	0 ml/L (H0)	10 ml/L (H1)	15 ml/L (H2)	20 ml/L (H3)
Without Incubation (M0)	5.01 a	5.03 b	5.03 b	5.03 b
Incubation 1 week (M1)	5.02 a	5.06 b	5.06 c	5.06 c
Incubation 2 week (M2)	5.02 a	5.06 c	5.06 c	5.06 c
Incubation 3 week (M3)	5.02 a b	5.40 d	5.40 d	5.41 d
Incubation 4 week (M4)	5.02 a b	7.36 e	7.36 e	7.36 e

Note: Numbers followed by different letters in rows and columns indicate significant differences based on the DMRT test at the 5% significance level.

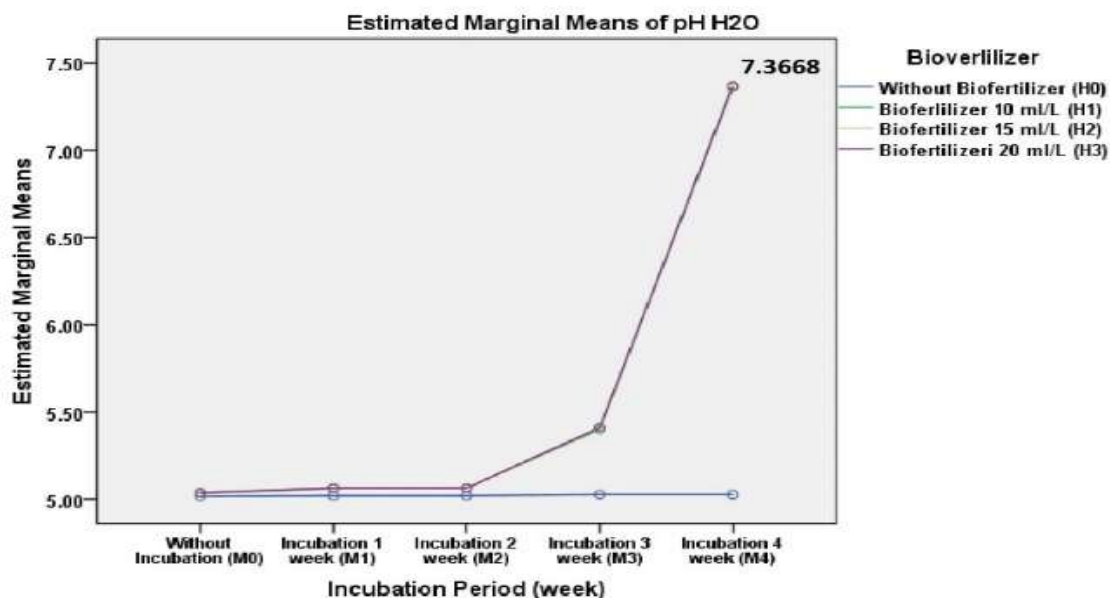


Figure 1. Interaction between biofertilizer treatment and incubation period on the average pH of the planting medium

Figure 1 illustrates the interaction between biofertilizer treatment and incubation period on soil pH. In the absence of biofertilizer, soil pH remained stable with no significant changes. Conversely, biofertilizer

application led to a notable pH increase from the second week onward, with higher concentrations producing a more pronounced effect. After four weeks, the highest pH value (7.3668, neutral range) was seen at a

biofertilizer concentration of 20 mL/L (H3). This shows that biofertilizer works to raise soil pH over time.

Organic Carbon (C-organic)

The incubation of organic matter accelerates decomposition, transforming complex compounds into stable forms, with biofertilizers playing a crucial role in this process. Biofertilizers add beneficial microorganisms that boost microbial activity and raise

the amount of C-organic matter in the planting medium (Debska, 2016). The amount of biofertilizer applied and incubation duration significantly affect organic carbon content, influencing soil fertility and quality. Table 2 illustrates these effects, demonstrating how biofertilizer treatments and incubation periods modify soil organic matter composition. Understanding these interactions is vital for optimizing soil management and promoting sustainable agriculture.

Table 2. Shows how adding biofertilizer and letting it sit for a while affects the average amount of C-organic matter in the planting medium.

Incubation Period (weeks)	Biofertilizer (ml/L)			
	0 ml/L (H0)	10 ml/L (H1)	15 ml/L (H2)	20 ml/L (H3)
Without Incubation (M0)	0.20 a	0.21 a	0.21 a	0.21 a
Incubation 1 week (M1)	0.20 a	0.22 a	0.22 a	0.22 a
Incubation 2 week (M2)	0.21 a	0.22 a	0.30 b	0.22 a
Incubation 3 week (M3)	0.21 a	2.99 b	3.00 c	3.00 b
Incubation 4 week (M4)	0.21 a	4.66 c	4.96 d	5.00 d

Note: Numbers followed by different letters in rows and columns indicate significant differences based on the DMRT test at the 5% significance level.

The findings of this study demonstrate that the interaction between biofertilizer concentration and incubation duration exerts a significant influence on the accumulation of organic carbon (C-organic) in the planting medium. In the absence of an incubation period, the application of biofertilizer did not result in a substantial increase in C-organic content. However, a notable enhancement was observed from the third to the

fourth week of incubation, particularly at a biofertilizer concentration of 10 mL/L, which yielded the highest average C-organic content of 4.6667. This pattern is consistent with the stabilization of soil pH within the neutral range, indicating that an optimal pH environment facilitates the retention and accumulation of soil organic carbon.

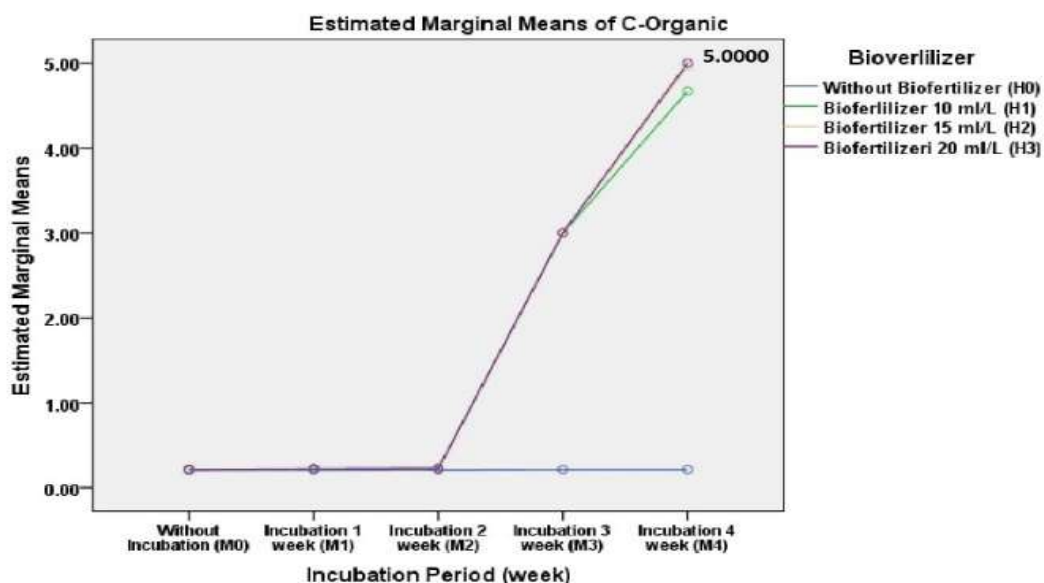


Figure 2. Interaction of biofertilizer treatment and incubation period on the average C-Organic content of the planting medium

Figure 2 presents the interaction between biofertilizer concentration and incubation duration in relation to C-organic content, emphasizing the role of microbial activity in accelerating the decomposition and

mineralization of organic matter over time. Overall, these findings indicate that the incubation period has a significant effect on soil organic carbon content. A longer incubation duration allows for greater decomposition of

organic matter, leading to an increased accumulation of organic carbon. This decomposition process is driven by enhanced microbial activity over time, resulting in more stable and plant-available organic carbon. Extending the incubation period can improve the quality of the growing medium and support better plant growth (Harsani et al., 2023; Husni et al., 2022).

Total Nitrogen (N Total)

Total nitrogen (N total) is crucial for plant growth, particularly in protein and chlorophyll synthesis, and is influenced by microbial decomposition during organic

matter incubation (Saputri et al., 2021). Cattle manure decomposition accelerates nitrogen release, enhancing plant uptake (Indriana et al., 2021). Experimental results showed significant nitrogen variations across biofertilizer treatments and incubation periods. The highest levels were recorded at 20 mL/L without incubation (H3M0, 0.1166%) and after three weeks without biofertilizer (H0M3, 0.1067%). Notably, the nitrogen content rose sharply after three weeks of using 10 mL/L biofertilizer (H1M3, 0.8132%), reaching 0.8400% after four weeks. This shows that the microbes were better at mineralizing nitrogen.

Table 3. Shows how adding biofertilizer and letting it sit for a while affects the total nitrogen content of the planting medium on average.

Incubation Period (weeks)	Biofertilizer (ml/L)			
	0 ml/L (H0)	10 ml/L (H1)	15 ml/L (H2)	20 ml/L (H3)
Without Incubation (M0)	0.08 a	0.08 a	0.08 a	0.11 b
Incubation 1 week (M1)	0.09 a	0.11 b	0.11 b	0.13 a
Incubation 2 week (M2)	0.10 a	0.80 c	0.80 c	0.80 c
Incubation 3 week (M3)	0.10 b	0.81 c	0.81 c	0.81 c
Incubation 4 week (M4)	0.10 b	0.84 d	0.84 d	0.84 d

Note: Numbers followed by different letters in rows and columns indicate significant differences based on the DMRT test at the 5% significance level.

Figure 3 illustrates the interaction effects of biofertilizer concentration and incubation period on the average total nitrogen (N) content in the planting medium. The significant interaction between treatments is reflected in the highest total N values observed with biofertilizer applications at concentrations of 10 to 20 mL/L over the incubation period. This interaction plays

a crucial role in enhancing the effectiveness of biofertilizer application. These findings align with the organic matter mineralization process, during which nitrogen, phosphorus, and sulfur are released in substantial quantities, along with smaller amounts of micronutrients (Dikinya & Mufwanzala, 2010).

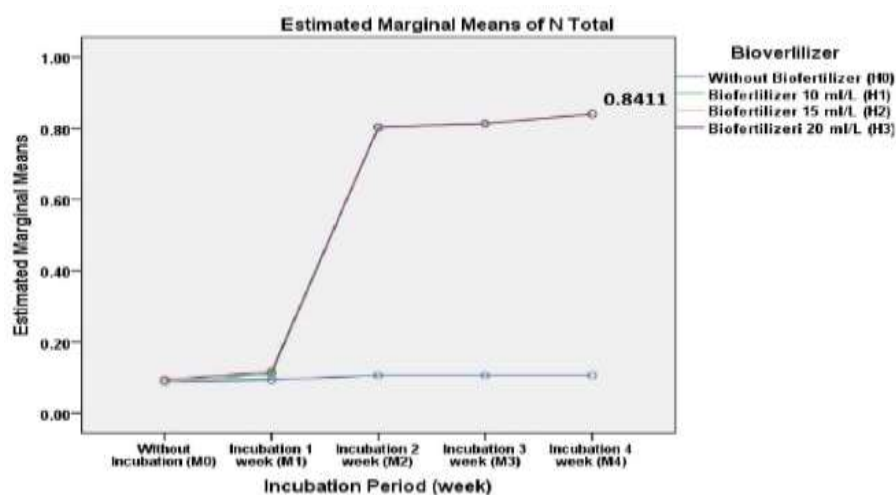


Figure 3. Interaction graph of biofertilizer treatment and incubation period on total N of planting media

C/N ratio

The C/N ratio is a key determinant of organic matter decomposition, influencing nutrient release and soil fertility. It regulates microbial activity, affecting nitrogen mineralization and humus formation, making

it a critical indicator of soil quality (Haibin et al., 2001). The study findings (Table 4) reveal a significant interaction between biofertilizer application and incubation duration, impacting the C/N ratio. This interaction enhances microbial activity, accelerates

nitrogen mineralization, and optimizes nutrient availability for plant growth.

Table 4. Interaction between biofertilizer application and incubation period on the average C-organic content in the planting media.

Incubation Period (weeks)	Biofertilizer (ml/L)			
	0 ml/L (H0)	10 ml/L (H1)	15 ml/L (H2)	20 ml/L (H3)
Without Incubation (M0)	3.09 a	3.10 a	3.11 a	3.11 a
Incubation 1 week (M1)	3.09 a	3.13 a	3.14 a	3.14 a
Incubation 2 week (M2)	3.09 a	3.19 a	3.20 a	3.20 a
Incubation 3 week (M3)	3.09 a	3.20 a	3.20 a	3.21 a
Incubation 4 week (M4)	3.13 a	11.66 b	11.73 b	12.00 b

Note: Numbers followed by different letters in rows and columns indicate significant differences based on the DMRT test at the 5% significance level.

The C/N ratio is a sensitive indicator of soil quality, where a low ratio ($C/N < 10$) accelerates organic matter decomposition but may lead to nitrogen unavailability for plants, while a high ratio ($C/N > 12$) slows decomposition and reduces microbial activity (Haibin et al., 2001). As shown in Table 4, the average C/N ratio without biofertilizer application was very low (3.097–3.137), indicating an imbalance between carbon and

nitrogen that hinders nitrogen availability. In contrast, the optimal C/N ratio for planting media ranges from 10 to 12 (Sanchez, 2019). The results demonstrate a significant interaction between biofertilizer application (10–20 mL/L) and a 4-week incubation period, leading to an ideal C/N ratio (11–12), which supports nutrient balance and enhances microbial activity.

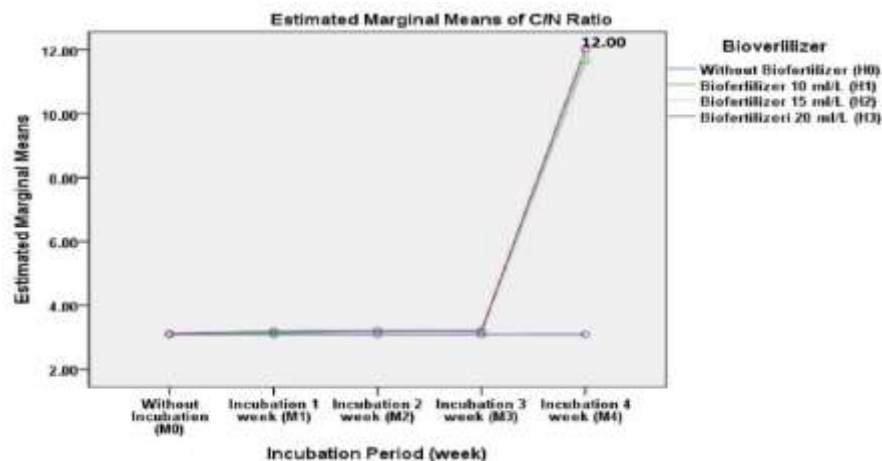


Figure 4. Graph illustrating the interaction between biofertilizer treatment and incubation period on the average C/N ratio of the planting media.

The relationship between the incubation period and the C/N ratio is shown in Figure 4. Treatments with biofertilizer at 10–20 mL/L for 3–4 weeks led to a significant rise in the ratio. When biofertilizer is added to manure, it helps break down organic matter by giving microbes more nitrogen. This speeds up the breakdown of carbon and keeps the C/N ratio normal. A study found that using 10 mL/L of biofertilizer (H1M4) for four weeks raised the average C/N ratio from 3.097 (very low) to 11.667 (moderate).

Available P

Available phosphorus (P) refers to the fraction of phosphorus in the soil that is readily accessible for plant

uptake and utilization. The incorporation of biofertilizers during incubation introduces inoculants that enhance the soil P cycle by solubilizing both organic and mineral-bound phosphorus. Phosphate-solubilizing microorganisms contribute to this process through multiple mechanisms, including the dissolution of P-containing minerals via soil acidification, the release of metal-chelating compounds (primarily organic acid anions), and the enzymatic degradation of organic phosphorus compounds (Jones & Oburger, 2010). Soil pH plays a crucial role in determining P availability (Table 5).

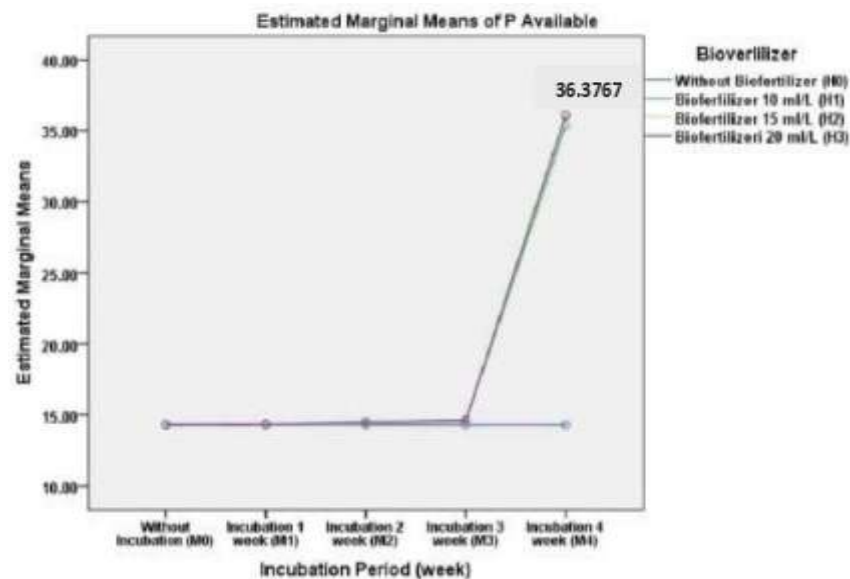
Table 5. Illustrates the interaction between biofertilizer concentration and incubation duration in influencing the average available P content in the planting medium.

Incubation Period (week)	Biofertilizer (ml/L)			
	0 ml/L (H0)	10 ml/L (H1)	15 ml/L (H2)	20 ml/L (H3)
Without Incubation (M0)	14.30 a	14.33 a	14.34 a	14.35 a
Incubation 1 week (M1)	14.32 a	14.35 a	14.36 a	14.36 a
Incubation 2 week (M2)	14.32 a	14.41 a	14.44 a	14.53 a
Incubation 3 week (M3)	14.32 a	14.54 a	14.55 a	14.64 a
Incubation 4 week (M4)	14.32 a	35.06 b	36.36 c	36.37 c

Note: Numbers followed by different letters in rows and columns indicate significant differences based on the DMRT test at the 5% significance level.

Table 5 highlights the significant impact of organic fertilizer and incubation duration on available phosphorus (P) content. Without biofertilizer, available P remains low (14.3033 mg/kg). A slight increase is observed at 10 mL/L, though still in the low range. A substantial rise occurs at 15 mL/L after four weeks,

reaching 36.3667 mg/kg, classified as high. This trend aligns with Mukhtar & Lifia (2020), who reported increasing P availability over 3–7 weeks. The positive correlation between pH and available P ($r = 0.9891$) supports findings by Manoj et al. (2020), who reported $r = 0.6466$.

**Figure 5.** Interaction graph of biofertilizer treatment and incubation period on the available P value of planting media

Available K

Potassium (K) is a vital macronutrient that significantly influences plant growth, physiological processes, and overall crop productivity. Its bioavailability in soil is governed by multiple factors, including fertilizer composition, incubation duration,

and soil physicochemical properties. As shown in Table 6, a significant interaction was observed between biofertilizer concentration and incubation period, with notable increases in available K at biofertilizer concentrations of 10–20 mL/L across all incubation stages.

Table 6. shows how the amount of biofertilizer and the time it is incubated affect the amount of K that is available in planting medium (me/100 g).

Incubation Period (week)	Biofertilizer (ml/L)			
	0 ml/L (H0)	10 ml/L (H1)	15 ml/L (H2)	20 ml/L (H3)
Without Incubation (M0)	1.0333 a	1.0367 a	1.0367 a	1.0367 a
Incubation 1 week (M1)	1.0367 a	6.6733 b	6.6633 b	6.6833 b
Incubation 2 week (M2)	1.0433 a	6.7067 c	6.7167 c	6.7467 c
Incubation 3 week (M3)	1.0467 a	6.7900 d	6.7900 d	6.8033 d
Incubation 4 week (M4)	1.0468 a	6.8633 e	6.8700 e	6.8733 e

Note: Numbers followed by different letters in rows and columns indicate significant differences based on the DMRT test at the 5% significance level.

Table 6 demonstrates a significant effect of all treatments on potassium (K) availability, except for those without biofertilizer application. The availability of K is regulated by multiple factors, including soil mineral composition, organic matter content, pH, and cation exchange capacity (CEC) (Thomas & Hipp, 1968). Although biofertilizer application at concentrations of 10–20 mL/L with incubation periods of 1–4 weeks exhibited a notable interaction, the overall levels of

available K remained low. A slight increase in available K was observed in week 4 with biofertilizer application at 10–20 mL/L, indicating a delayed response to organic matter mineralization and microbial activity. As shown in Figure 6, available K continued to rise up to week 4 under biofertilizer treatments; however, the persistently low K levels suggest that soil properties, particularly CEC and mineral composition, play a more dominant role in K retention and availability (Cui et al., 2018).

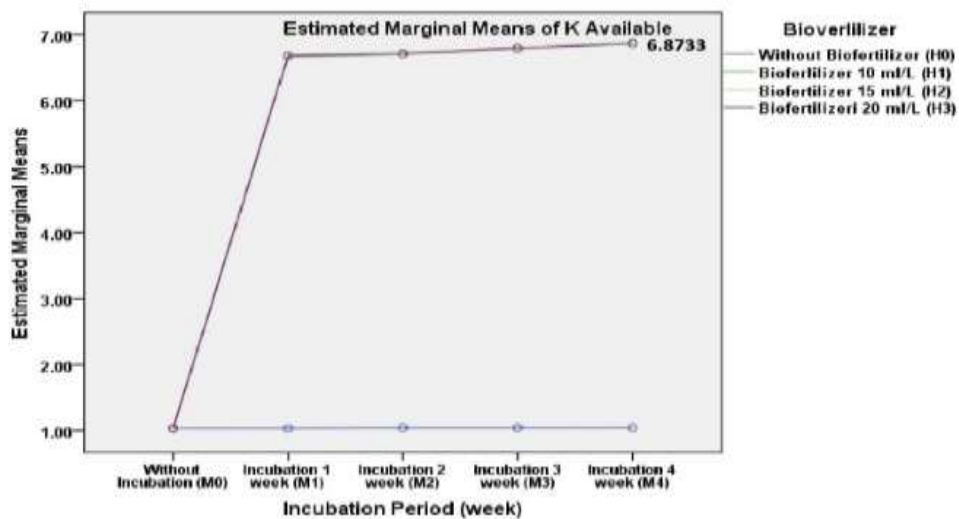


Figure 6. Interaction graph of biofertilizer treatment and incubation period on the average value of available K in planting media

Figure 6 illustrates that the treatment interaction significantly influenced the average available K. The application of biofertilizer at concentrations of 10–20 mL/L across all incubation periods led to an increase in available K; however, the levels remained very low up to the fourth week of incubation (H3M4). Despite this increase, biofertilizer application was insufficient to stabilize K availability at moderate to high levels. This limitation is likely due to the inherently low potential K content in the soil, measured at 1.04 mg/100 g, and a low cation exchange capacity (CEC) of 24.42 cmol(+)/kg, as indicated by the initial routine laboratory analysis of the planting medium.

Correlation Coefficient between pH (H₂O) and P available

The relationship between pH and available phosphorus (P) shows how acidity in the soil affects the amount of P that is available. This relationship is controlled by mineralization rates and soil chemistry (Corbett, 2022). Factors such as soil type, organic matter, and cation presence (Ca, Mg) affect this relationship. Organic amendments, particularly cattle manure and biofertilizers, enhance soil pH during incubation, increasing P availability. In contrast, inorganic P sources have minimal impact and may contribute to acidification (Opala et al., 2012).

Table 7. Correlation coefficient between soil pH (H₂O) and available P.

		pH H ₂ O	P Available
pH H ₂ O	Pearson Correlation	1	0.98
	Sig. (2-tailed)		0.00
	N	60	60
P Available	Pearson Correlation	0.98	1
	Sig. (2-tailed)	0.00	
	N	60	60

Correlation is significant at the 0.01 level (2-tailed).

The correlation between soil pH (H₂O) and available phosphorus (P) in Table 7 is highly significant

and strong, as indicated by a correlation coefficient of 0.989, which is close to +1. The positive sign denotes a

direct relationship, meaning that an increase in soil pH (H_2O) is accompanied by a corresponding rise in available P, with a proportional increase of 98%.

Conclusion

Adding biofertilizer and letting organic matter, like cow manure, sit for a while has been shown to improve soil pH, organic carbon (C-organic), total nitrogen (N), the C/N ratio, available phosphorus (P), and available potassium (K). The results of this study show that adding 10–20 ml/L of biofertilizer to soil for four weeks raises the p Application of biofertilizer at 10–20 ml/L, combined with organic matter like cow manure, significantly improves soil chemical properties. After four weeks, soil pH increased from slightly acidic to neutral (highest at 7.36 for 20 ml/L), organic carbon reached 5%, and total nitrogen was 0.84%, indicating effective organic matter mineralization and nutrient availability. The optimal C/N ratio of 11–12 at 20 ml/L suggests balanced decomposition, while available phosphorus reached a very high category, enhancing nutrient accessibility for plants. However, available potassium did not reach optimal levels, indicating a need for additional management strategies. These results suggest that integrating biofertilizer with organic amendments can sustainably enhance soil fertility and support intensive cultivation in fallow or organically managed land. Practically, this approach provides a cost-effective strategy for improving soil quality and crop productivity, contributing to sustainable agricultural management.

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Author Contributions

Conceptualization, AIS; methodology, AIS; software, PAS; validation, AIS and PAS; formal analysis, AIS; investigation, PAS; resources, FIT; data curation, AIS; writing—original draft preparation, PAS; writing—review and editing, PAS; visualization, PAS; supervision, FIT; project administration, FIT; funding acquisition, FIT; pest management, RIS. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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