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Impact of liquid biofertilizer from cocoa shells on the growth and chlorophyll content of sweet peppers (*Capsicum chinense* L.) in San Martín, Peru

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The growth and yield of sweet peppers are constrained by factors such as fertilization practices and edaphoclimatic conditions, which ultimately threaten global food security in the context of an ever-growing population. This study evaluated the effect of a liquid biofertilizer derived from cocoa husks on the growth and chlorophyll content of sweet pepper (*Capsicum chinense*) cultivated in San Martín, Peru. The experiment was conducted at the experimental station of the National Institute of Agrarian Innovation in Tarapoto, San Martín, Peru (6°35′00″ S, 76°19′46″ W). A completely randomized design was applied, consisting of five treatments (0, 750, 1250, 2250, and 3000 mL) with 20 plants per treatment, totaling 100 experimental units. The biofertilizer was applied eight days after sowing (days). Plant height, stem diameter, and leaf chlorophyll content were measured at 15, 35, and 85 days. After 85 days, the highest plant height was observed with the 3000 mL and 1250 mL treatments, reaching averages of 29.98 and 28.25 cm, respectively. Stem diameter was maximized with 3000 mL (6.25 cm), whereas the highest chlorophyll content was recorded with 1250 mL, averaging 35.37 SPAD units. These results highlight the potential of liquid biofertilizers produced from cocoa shells to enhance nutrient uptake, increase plant biomass, and improve photosynthetic capacity, thereby contributing to sustainable sweet pepper production.

KEYWORDS

plant height, *Capsicum chinense*, cocoa shell, chlorophyll, stem diameter

1 Introduction

The global use of fertilizers has been one of the most critical practices since the systematization of agriculture and the declaration of food security as a global goal in 1974 (Penuelas et al., 2023). Recent geopolitical tensions between Russia and Ukraine have highlighted the heavy reliance of Latin American countries on fertilizer imports from the Black Sea region. Peru was no exception, experiencing sharp increases in food prices that further undermined national food security (FAO et al., 2023). At the same time, continuous population growth demands a sustainable increase in agricultural productivity to ensure global food security for an ever-expanding population (Arora et al., 2020; Singh and Gurjar, 2022).

Modern intensive agricultural practices face multiple challenges that threaten global food security. To meet the nutritional demands of a growing population, mineral fertilizers and pesticides are widely applied to boost agricultural production. However, the indiscriminate use of agrochemicals has resulted in severe environmental pollution and poses serious risks to public health, underscoring the substantial impact of fertilizer use on agricultural development (Li et al., 2024). In addition, agricultural soils are progressively losing their quality and physical properties, along with their chemical balance (nutrient imbalances) and biological health (Kumar et al., 2022). Moreover, the excessive application of mineral fertilizers—despite their high nutrient content and ability to accelerate crop growth—has been shown to endanger both human health and the environment, while also contributing to groundwater contamination and atmospheric pollution in the long term (Mahmud et al., 2021).

Given these challenges and the pressing need to promote biofertilizer use to enhance the production of crops such as sweet pepper (*Capsicum chinense*) in Peru, it is essential to develop strategies that reduce dependence on imported fertilizers and foster the creation of innovative products to strengthen fertilizer supply (Hellegers, 2022). In this context, biofertilizers are emerging as nutrient-rich soil amendments of growing relevance [9]. Moreover, inadequate waste management provides an opportunity for their development, as the bioconversion of organic compounds into biofertilizers can serve as a sustainable alternative to conventional fertilizers. This approach not only mitigates the toxic effects of chemical inputs but also improves crop yields, enhances soil fertility, and contributes to soil protection (Esmaeilian et al., 2022; Nosheen et al., 2021).

Therefore, there is a pressing need for complementary or alternative approaches that enhance agricultural productivity in an environmentally sustainable manner (Hamid et al., 2021; Majeed et al., 2018). Among these, the use of biofertilizers is regarded as one of the most promising tools for achieving sustainable gains in crop production (Kumar et al., 2022). Biofertilizers are gaining momentum as both complements and, in some cases, alternatives to chemical fertilizers. They are widely recognized for their ability to stimulate plant growth by improving nutrient availability and uptake efficiency (Fadiji et al., 2024). Numerous studies have reported that biofertilizers enhance growth, yield, and mineral concentrations in crops such as lettuce, broccoli, chickpea, and date palm (Demir et al., 2023; Nabati et al., 2025; Anli et al., 2020), thereby contributing to food security and sustainable agriculture (Demir et al., 2023; Daniel et al., 2022).

Despite the growing body of research on liquid biofertilizers and their effects on sweet pepper growth and chlorophyll content, few studies have specifically addressed their influence during the early developmental stages of *Capsicum chinense* under local agroecological conditions. This study evaluates the impact of liquid biofertilizer on the growth and chlorophyll content of sweet pepper in San Martín, Peru. The findings contribute to the development of sustainable alternatives to chemical inputs and expand current knowledge on the application of biostimulants in high-value tropical chili crops cultivated across the Peruvian territory.

2 Materials and methods

2.1 Study site

The research was conducted at the El Porvenir Agricultural Experiment Station of the National Institute of Agrarian Innovation (INIA), located in the district of Juan Guerra, province and department of San Martín, Peru (6°35′00″ S, 76°19′46″ W). The study area is characterized by a tropical climate, with average minimum and maximum temperatures ranging from 19 to 34.5 °C. Mean annual precipitation is 1123 mm, with monthly rainfall ranging from 43 mm in July to 151 mm in February. Average monthly relative humidity (RH) varies between 69% and 75%, and the mean wind speed is 2.4 m s^{-1}.

2.2 Soil characteristics and liquid biofertilizer

The analytical characteristics of the soil were as follows: total nitrogen (N) 0.19%, phosphorus (P) 342.12 mg kg^{-1}, organic matter 3.2%, pH 7.2, and electrical conductivity (EC) 0.11 mS/m. The liquid biofertilizer (biol) was produced by fermenting 800 kg of cocoa shells (*Theobroma cacao*) in a plastic tarp with 100 L of water and 5 L of molasses for four months. The mixture was stirred three times per week, and the liquid biofertilizer was obtained at the beginning of the third month, prepared from solid biofertilizer. Production followed the methodology for enriched liquid biofertilizers proposed by the FAO (2013). The properties of the mature biofertilizer were as follows: total N, 0.02%; total P, 0.40%; total K, 5.64%; organic matter, 55.70; Ca, 21050.19 mg kg^{-1}; Mg, 8103.58 mg kg^{-1}; pH, 9.80; and EC, 15.28 mSm^{-1}.

2.3 Experimental design

The experiment comprised five doses of liquid biofertilizer (biol): 0.0, 37.5, 62.5, 112.5, and 150 mL $plant^{-1}$, corresponding to the control, 750, 1250, 2250, and 3000 mL per treatment, respectively. Treatments were arranged in a completely randomized design with 20 plants per treatment (20 replicates), resulting in a total of 100 experimental units. Seeds extracted from ripe *Capsicum chinense* fruits, commonly consumed and marketed locally, were sown. The liquid biofertilizer was applied eight days after sowing (seven days before the first evaluation) via foliar spraying. The trial was conducted under nursery and field conditions from June to December 2024. Plants were irrigated periodically with treated water to maintain 60% of field capacity, without the addition of chemical fertilizers. Growth evaluations were performed at 15, 35, and 85 days after sowing, measuring plant height, stem diameter, leaf number, and chlorophyll content.

2.4 Evaluated parameters

Plant height (cm) was measured from the soil surface to the apex using a metallic measuring tape (precision ±1 mm). Stem diameter (mm) was determined with a digital vernier caliper (± 0.01 mm) at 2–3 cm above the plant collar, avoiding nodes and swellings. The number of leaves was recorded as the count of fully expanded true leaves (≥50% unfolded). These measurements followed the definitions reported in the international descriptors for Capsicum, ensuring comparability across studies (IPGRI, 1995).

Relative chlorophyll content was determined non-destructively using a Konica Minolta SPAD-502Plus. At each evaluation date, the third and fourth fully expanded leaves from the apex of each plant were selected. Three readings were taken at the midpoint of the leaf blade, avoiding the central vein, and averaged to obtain the SPAD value per plant; treatment means were then calculated. The device was calibrated beforehand according to the manufacturer's manual, and homogeneous light conditions were maintained during all measurements (Konica Minolta, 2013).

2.5 Statistical analysis

For statistical analysis, data were first tested for normality and homogeneity of variance using the Shapiro–Wilk and Bartlett tests ($p < 0.05$). Mean comparisons were performed with Tukey's test ($p < 0.05$) using the agricolae package (Mendiburu, 2010). To visualize and better interpret the dynamics of plant height in response to liquid biofertilizer (biol) treatments over time, a three-dimensional graphical approach was applied through interpolated response surfaces. This analysis employed the R packages plot3D and akima. The plot3D package enables the construction of customizable three-dimensional graphics, suitable for representing complex agronomic responses to continuous variables (Soetaert, 2023), while akima was used for bilinear interpolation of the data matrix, generating continuous relief surfaces that realistically reflect growth trends and reduce distortion from isolated points (Akima and Gebhardt, 2022). These "hill" plots facilitated the visual identification of treatment zones with the highest responses, particularly valuable in field experiments where interactions between input dose and evaluation time are critical for decision-making. Complementary analyses were performed using GraphPad Prism to generate descriptive statistics and high-quality graphical outputs suitable for scientific publications. The software is widely recognized for its ease of use, integrated statistical tools, and capacity to produce publication-ready figures, which enhance data interpretation and presentation in biological sciences (Motulsky, 2022). Its user-friendly interface and customization options made it an effective complement to the advanced analyses conducted in R, resulting in a robust and visually consistent graphical–statistical dataset. Overall, these procedures enabled a comprehensive characterization of the treatments and their effects on the evaluated parameters. Statistical analyses were performed in RStudio (R Core Team, 2024) and GraphPad Prism (Motulsky, 2022).

3 Results

3.1 Biol influence on plant diameter and height

Table 1 shows significant differences among treatments for all three variables studied (plant height, stem diameter, and chlorophyll content) at the three evaluation times (15, 35, and 85 days after sowing) in sweet pepper plants treated with liquid biofertilizer derived from cocoa shells.

According to Tukey's test (Figure 1A), at 15 days, plants treated with 3000 mL of biofertilizer exhibited the highest mean growth and were grouped as "a," indicating a statistically significant difference compared to the other treatments. The control treatment showed the lowest mean values, and plant growth increased progressively with higher biol doses. At 35 days, the treatment responses shifted, with growth responses becoming less consistent across doses, showing an inverse pattern relative to the 15-day evaluation. By 85 days, treatment differences were more pronounced: the

TABLE 1 Bidirectional F values and probabilities (P) examining the effects of liquid biofertilizer on height, diameter, and chlorophyll content in chili pepper plants.

Variable	Treatments (15 das)		Treatments (35 das)		Treatments (85 das)	
	F	*p*	F	*p*	F	*p*
Height plant	1464.37	<0.0001	4.84	0.0016	11.98	<0.0001
Stem diameter	129.92	<0.0001	7.1	0.0001	6.43	0.0002
Chlorophyll in plants	5.03	0.0012	5.42	0.0007	4.34	0.0032

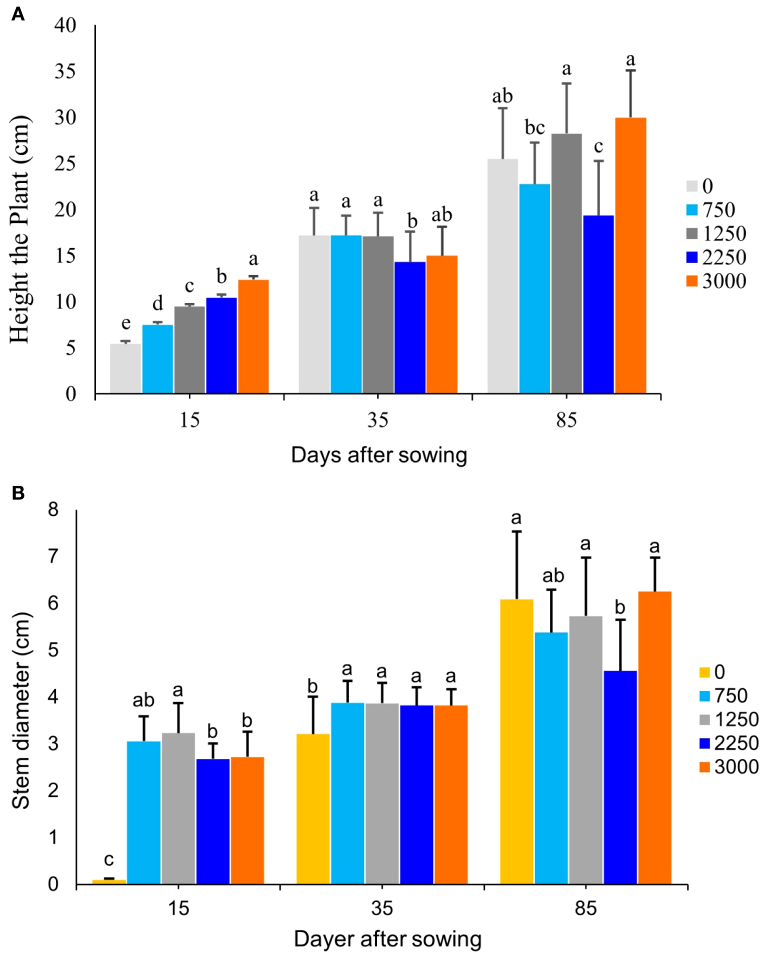

FIGURE 1
(A) State of the height average **(B)** average of plants diameter of *Capsicum chinense* to 15, 35 y 85 das and seven days after biol application. Different letters in bars indicate significant differences between treatments according to the Tukey test (P < 0.05). The vertical bars correspond to the standard error.

application of 3000 mL biol produced the highest values, with significant differences compared to the other treatments (Figure 1A). Among the treatments, the control (without biol application) exhibited the lowest plant height at both 15 and 85 days after sowing, with mean values of 5.44 and 25.48 cm, respectively. According to Tukey's test for stem diameter (Figure 1B), at 15 days after sowing, the 1250 mL biol treatment produced the greatest mean stem diameter (3.23 cm), while the control (without biol application) presented the lowest value, showing significant differences among treatments. At 35 days, two distinct groups were observed: the highest average diameters corresponded to the 750, 1250, 2250, and 3000 mL treatments, all of which outperformed the control. By 85 days, the control, 1250 mL, and 3000 mL treatments formed the first group with the greatest stem diameters (6.22, 5.73, and 6.25 cm, respectively), whereas the lowest diameters were recorded in the second group, ranging from 4.56 to 5.38 cm, with significant differences between the two groups (Figure 1B). The control treatment exhibited the greatest stem diameter in sweet pepper at both 15 and 35 days after planting, with mean values of 0.10 and 3.21 cm, respectively.

Significant differences in mean plant height were observed at 15, 35, and 85 days after biofertilizer application. At 15 days, the most pronounced growth curves corresponded to the 2250 and 3000 mL treatments. At 35 days, the most marked responses were recorded in the control, 750, and 1250 mL treatments. By 85 days, the highest curves were observed with the 1250 and 3000 mL treatments (Figure 2A). Similarly, mean stem diameter showed significant differences at all evaluation times. At 15 days, the 750 and 1250 mL treatments consistently displayed the highest curves. At 35 days, pronounced responses were observed with 750, 1250, 2250, and 3000 mL. By 85 days, the control, 1250, and 3000 mL treatments exhibited the most pronounced curves (Figure 2B).

3.2 Biol influence on chlorophyll content in plant leaves

According to Tukey's test (Figure 3), at 15 days after sowing, the 1250 mL biol treatment exhibited the highest chlorophyll content, averaging 27.1 SPAD units, whereas the control (without biol

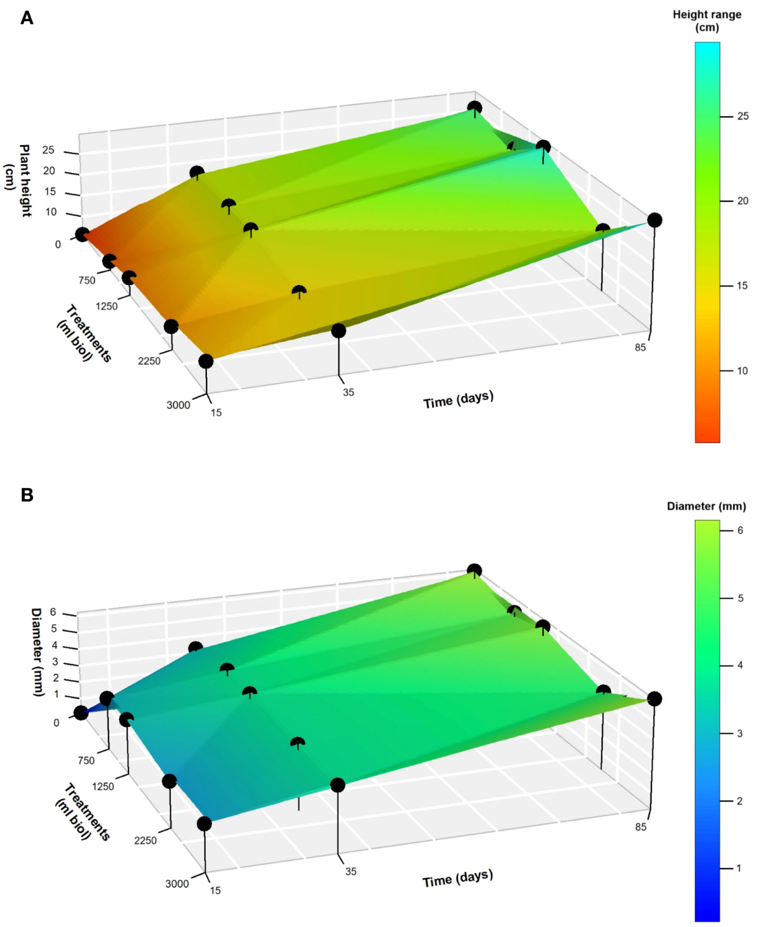

FIGURE 2
(A) Distribution of height average and **(B)** average of diameter in plants of *Capsicum chinense* at 15, 35 and 85 das; seven days after biol application.

application) showed the lowest value (22.2 SPAD units), with significant differences among treatments. At 35 days, the 1250 and 750 mL treatments recorded the highest values (43.0 and 40.3 SPAD units, respectively), while the control presented the lowest value (39.3 SPAD units), again with significant differences between treatments. By 85 days, the 1250 and 3000 mL treatments produced the highest chlorophyll contents (35.4 and 32.9 SPAD units, respectively), which were significantly different from the other treatments (Figure 3). Meanwhile, one of the treatments that showed the lowest chlorophyll content was the control (without biol application), with mean values of 22.95, 39.35, and 28.97 SPAD units at 15, 35, and 85 days after sowing, respectively.

Significant differences in mean values were observed at 15, 35, and 85 days after biofertilizer application. At 15 days, the 750 mL treatment consistently exhibited the highest curve compared to the other treatments. At 35 days, pronounced responses were recorded in the 1250 and 750 mL treatments. By 85 days, the most pronounced curves were observed with the 1250 and 3000 mL treatments (Figure 4).

4 Discussion

4.1 Biol influence on plant diameter and height

Sini et al. (2024) reported an average plant height of 15.62 cm with organic fertilizer and 15.54 cm without fertilizer, measured 145 days after the emergence of *Capsicum annuum* L. seeds. Similarly, Coulibaly et al. (2021) evaluated tomato plant height under compost- and bokashi-based biofertilizer treatments, obtaining values of approximately 14 cm at 30 days, 16 cm at 45 days, 25 cm at 60 days, and 50 cm at 80 days. Biofertilizers are recognized for enhancing nutrient availability and promoting soil and plant health, thereby improving crop yields (Suliasih, 2018; KamLesh and Smritikana, 2019). They represent a modernized form of organic fertilizers enriched with beneficial microorganisms (Kalbani et al., 2016). Nacro (2018) further demonstrated improved plant growth through organic fertilizers that supply additional nutrients and enhance the efficiency of mineral fertilizers by increasing nutrient availability. Likewise, compost-based biofertilizers have been shown

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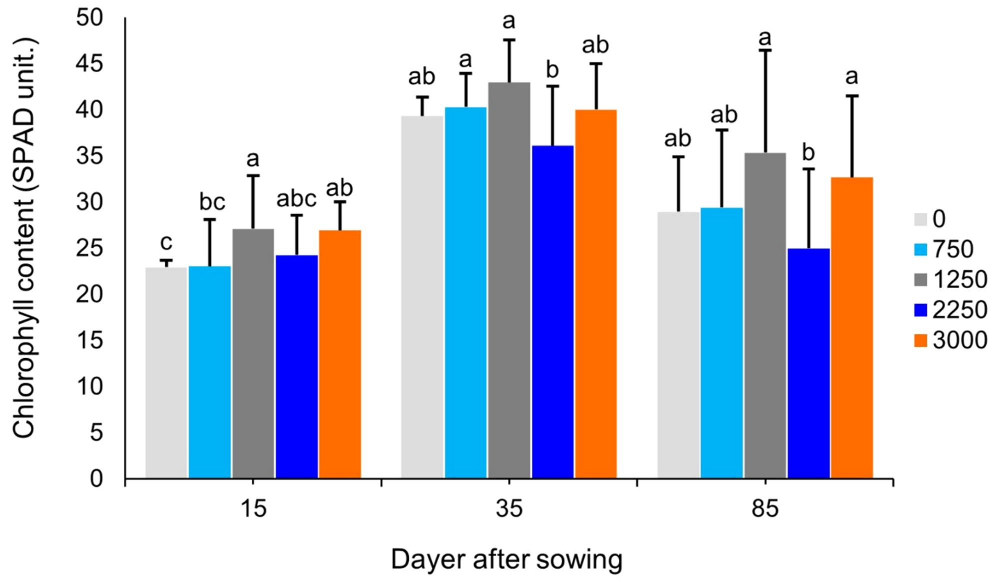

FIGURE 3
Chlorophyll content in plants of *Capsicum chinense* at 15, 35 y 85 das; seven after biol application. Different letters in bars indicate significant differences between treatments according to the Tukey test (P < 0.05). The vertical bars correspond to the standard error.

to improve the height of coffee and cocoa plants (Vallejos-Torres et al., 2019; 2022).

The findings of this study are consistent with Lu et al. (2020), who reported that biofertilizers effectively enhance plant growth and influence soil microbial community diversity. In line with this, Coulibaly et al. (2021) found that tomato stem diameter under compost- and bokashi-based biofertilizers reached approximately 8 cm at 80 days, results comparable to those obtained in sweet pepper at 85 days. Liquid biofertilizers (LBF) have been reported to improve plant growth and soil fertility (Le et al., 2025). Similarly, Lee et al. (2025) demonstrated that LBF treatments significantly enhanced the morphological and physiological traits of Chinese cabbage. Organic fertilizers primarily release nutrients, whereas biofertilizers improve nutrient uptake through beneficial microbial activity, generating a synergistic effect that enhances soil fertility and plant resilience (Elsayed et al., 2020; Koskey et al., 2021). These improvements are consistent with reports showing that biofertilizers increase soil organic matter and facilitate the availability and uptake of essential nutrients, partly through bacterial secretion and pH modulation (Kour et al., 2019).

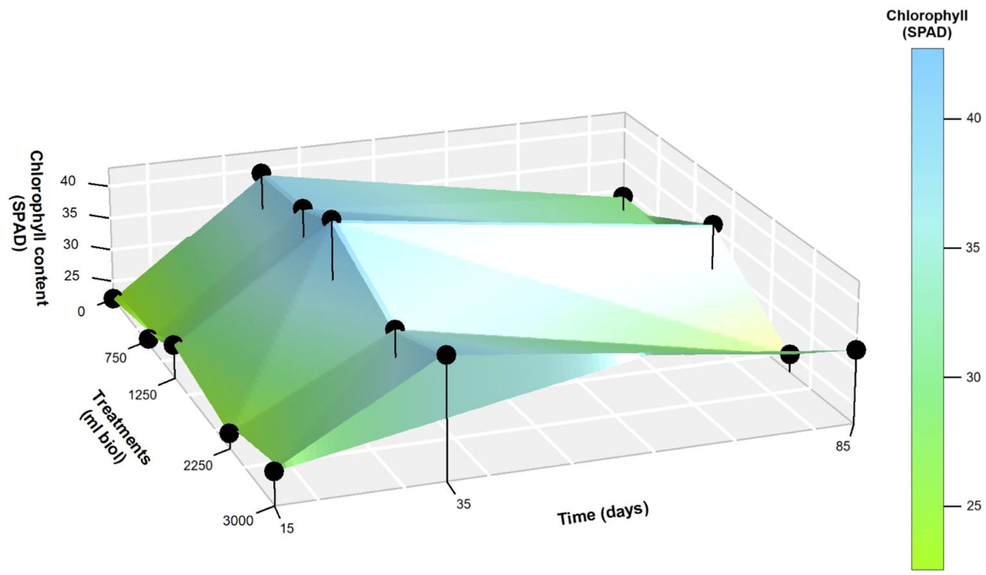

FIGURE 4
Distribution of chlorophyll content in plants of *Capsicum chinense* at 15, 35 y 85 das; seven days after biol applications.

TABLE 2 Effect of amendments obtained from cocoa shell.

Journal	Quartile	Effect of biofertilizer obtained from cocoa shells	Author and year
Journal of Cleaner Production	Q1	Cocoa pod shells are an important agricultural waste that represents a considerable export of nutrients, particularly phosphorus and potassium.	Mwafulirwa et al. (2024)
Plant and Soil	Q1	Organic amendments from cocoa pod shells can replenish soil nutrients, representing an economical and affordable strategy to maintain soil fertility and productivity; particularly phosphorus and potassium.	Hougni et al. (2021)
Carbohydrate Polymers	Q1	Cocoa pod shells represent between 52% and 76% of the dry pods or biomass of the crop.	Muñoz-Almagro et al. (2019)
Archives of Agronomy and Soil Science	Q2	Application of biochar to soil from cocoa husk can increase the total concentration of soil nutrients, as well as mediate their availability by increasing soil pH.	Pouangam et al. (2023)
Scientific African	Q1	Composting cocoa pod shells helps recycle nutrients and restore ecological functions; in turn contribute to the formation and maintenance of soil organic matter	Amponsah-Doku et al. (2022)

4.2 Biol influence on chlorophyll content in plant leaves

At 15 days after sowing, the 1250 mL biol treatment produced the highest chlorophyll content, with a mean of 27.1 SPAD units. At 35 days, the 1250 and 750 mL treatments exhibited the highest values, averaging 43.0 and 40.3 SPAD units, respectively. By 85 days, the highest chlorophyll contents were recorded in the 1250 and 3000 mL treatments, with averages of 35.4 and 32.9 SPAD units, respectively, clearly highlighting the positive effect of liquid biofertilizer on chlorophyll accumulation. These findings are consistent with Le et al. (2025), who reported SPAD values of 42.51 and 43.26 in liquid biofertilizer treatments at 54 and 73 days, compared with 40.15 and 41.86 in the control group at the same time points.

Overall, the results demonstrate that biofertilizer application significantly enhances chlorophyll content and thereby promotes plant growth. Similar results were obtained by Mthiyane et al. (2024), who recorded values of approximately 42 SPAD units in rice plants following biofertilizer application. Other studies have also shown that biofertilizers increase total chlorophyll content and photosynthesis (Khajeeyan et al., 2019), with positive effects on corn grain yield (Dragičević et al., 2024; Janosevic et al., 2017). Since chlorophyll is a key indicator of crop growth and a direct measure of leaf photosynthetic capacity, it provides valuable insights into the exchange of materials and energy between crops and their environment, serving as a reliable marker of crop health and development (Shi et al., 2023).

Moreover, the use of biofertilizers has significant potential for improving soil nutritional quality and enhancing crop growth and development (Table 2). Organic amendments derived from cocoa shell can replenish soil nutrients, providing an economical and accessible strategy to maintain soil fertility and productivity, particularly with respect to phosphorus and potassium (Table 3). Chlorophyll is the primary pigment responsible for light absorption during photosynthesis and plant growth. In domesticated chili crops cultivated under modern high-density systems, excessive investment in chlorophyll production has been reported to reduce both light-use efficiency and nitrogen-use efficiency (Cho et al., 2024; Chu et al., 2024). In this study, the cocoa shell-based biofertilizer significantly increased chlorophyll content, suggesting an enhanced nitrogen supply, as most plant nitrogen is incorporated into leaf chlorophyll (Schlemmer et al., 2013; Lu et al., 2019).

TABLE 3 Effect of liquid biofertilizers.

Journal	Quartile	Effect of liquid biofertilizer	Author and year
Frontiers in Plant Science	Q1	Biofertilizers improve the yield of various crops by 25% and reduce nitrogen requirement by 50% and phosphorus requirement by 25% in agriculture	Aloo et al. (2022)
Field Crops Research	Q1	Biofertilizers increase the yield of crops such as cotton (*Gossypium* sp.) by increasing nutrients and microbial biomass; at the same time, appropriately reduce the use of chemical fertilizers.	Ding et al. (2024)
Applied Microbiology and Biotechnology	Q1	Biofertilizers have the effect of nourishing plants, with greater root production, they are healthier and more resistant to pest attacks, diseases and drought damage.	Santos et al. (2024)
Sustainability	Q1	Biofertilizer application increases plant height by 31% and chlorophyll content increase by 42% in *Oryza sativa* L.	Mthiyane et al. (2024)
Environmental Science and Pollution Research	Q1	Biofertilizers promote sustainable agriculture, due to their higher nutrient content; in turn they improve soil fertility and increase plant productivity.	Ammar et al. (2023)

5 Conclusions

At 15 days after sowing (days) *Capsicum chinense*, the maximum plant height was obtained with the 3000 mL biofertilizer treatment, with an average of 12.4 cm. Stem diameter was greatest at the 1250 mL dose, averaging 3.23 cm. Similarly, the highest chlorophyll content was observed at 3000 mL, with a mean value of 26.96 SPAD units. At 35 days, maximum plant height was recorded in the control and 750 mL treatments, averaging 17.20 cm. Stem diameter was highest at 750 and 1250 mL, with mean values of 3.88 and 3.87 cm, respectively, while chlorophyll content peaked at 1250 mL with an average of 42.99 SPAD units. At 85 days, plant height reached its maximum under the 3000- and 1250-mL treatments, averaging 29.98 and 28.25 cm, respectively. Stem diameter was greatest at 3000 mL (6.25 cm), and the highest chlorophyll content was found at 1250 mL, with a mean of 35.37 SPAD units.

Overall, liquid biofertilizer from cocoa shells improved nutrient uptake and increased plant height, stem diameter, and chlorophyll content by 17.66%, 2.63%, and 22.10%, respectively. These results underscore the potential of biofertilizers to enhance early plant development and physiological performance. Future research should address their long-term agronomic impact under diverse field conditions, particularly in relation to yield and post-harvest quality, to provide a more comprehensive understanding of their role in sustainable agriculture and their applicability in the Amazon region.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RS: Methodology, Investigation, Writing – review & editing, Formal analysis, Validation. JC: Funding acquisition, Software, Formal analysis, Project administration, Writing – review & editing. NG: Writing – original draft, Project administration, Conceptualization. AL: Writing – original draft, Software, Conceptualization. HD: Investigation, Supervision, Writing – original draft. GV: Data curation, Validation, Conceptualization, Writing – review & editing. RS: Investigation, Writing – original draft, Data curation.

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Conflict of interest

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