Research Article

Synergistic Effects of Biostimulant and Liquid Organic Fertilizer (LOF) on Agronomic Traits of Sugarcane Seedling

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Abstract |Improving vegetative growth of sugarcane (*Saccharum officinarum* L.) is essential to enhance productivity and promote sustainable future agriculture. This study investigates effects of different concentrations of liquid organic fertilizer (LOF) application and biostimulants on the growth of sugarcane, approximately 12 weeks after planting (WAP). A split-plot arrangement was employed using a randomized complete block design with liquid organic fertilizer (LOF) treatments assigned to the main plots and biostimulant concentrations to the subplots. The LOF treatments consisted of no LOF (p0), 3 mL L^{-1} (p1), and 6 mL L^{-1} (p2), while the biostimulant treatments included no biostimulant (b0), 1 mL L^{-1} (b1), 2 mL L^{-1} (b2), and 3 mL L^{-1} (b3). This experimental design resulted in a total of 12 treatment combinations, each replicated three times. Data were analyzed using analysis of variance (ANOVA) with least significant difference (LSD) test at 5% probability level ($\alpha = 0.05$) using STAR version 2.0.1. Correlation analysis, principal component analysis (PCA), and scatter plot visualization were performed using RStudio version 4.2.2. Results have shown that the application of biostimulants significantly improved agronomic traits, including plant height, leaf area, root and shoot biomass. The most effective treatment was the combination of 2 mL L^{-1} biostimulant and 3 mL L^{-1} liquid organic fertilizer (LOF), which produced the highest root volume (180 mL) and enhanced shoot traits. In contrast, LOF applied alone had no significant effect on growth parameters such as plant height, number of leaves, or leaf area, indicating that biostimulants play a more critical role in improving seedling vigor. Correlation and PCA results confirmed a strong relationship among growth traits, indicating a coordinated response to the biostimulant. These findings highlight the potential of combining biostimulants and moderate LOF, positively supporting early sugarcane growth, which contributes to sustainable crop management strategies of sugarcane production.

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Introduction

Sugarcane (*Saccharum officinarum* L.) is a major agro-industrial crop grown primarily for sugar production. It plays a critical role in supporting national food and energy security (Musa and Bahrun, 2021; Aguilar-Rivera, 2024). Despite the importance, domestic sugarcane yields remain insufficient to meet local consumption demands. This shortfall is primarily attributed to stagnant productivity, limited land availability, declining soil fertility, and widespread use of suboptimal agronomic practices (Statistics Indonesia, 2022; Sulaiman *et al*., 2019). The expansion of sugarcane cultivation into marginal dryland areas faces significant challenges due to poor soil conditions, low organic matter content, weak soil structure, and long-term degradation resulting from intensive use of chemical fertilizers (Hasan *et al*., 2019a). Ongoing dependence on synthetic inputs has been linked to nutrient imbalances, increased soil acidity, and a decline in beneficial soil microorganisms, all of which reduce the long-term productivity and sustainability of sugarcane farming systems (Hasan *et al*., 2019b; Ramakrishna *et al*., 2021).

In response, organic soil management practices are increasingly promoted to restore soil function and to improve crop performance. Among them, liquid organic fertilizer (LOF) has shown promise due to its balanced nutrient composition and bio-active nature (Utamy *et al*., 2025; Yassi *et al*., 2023). LOFs are derived from fermentation of plant biomass, manure, and microbial substrates, providing both macro- and micro-nutrients. LOF enhances nutrient use efficiency and has been shown to improve morphological traits, such as plant height, leaf development, and stem biomass, in various crops, including sugarcane (Sani *et al*., 2020).

In parallel, plant biostimulants become innovative inputs for sustainable crop production. These biostimulants, comprising compounds such as seaweed extracts, humic and fulvic acid, protein hydrolysates, and beneficial microbial synergism, enhanced plant growth and stress resilience. It also modulates nutrient uptake, hormonal balance, and enzymatic activities (Du Jardin, 2020; Kumari, 2022). Recent studies have demonstrated the effectiveness in improving physiological processes and root system development during the seedling stage, thereby promoting early crop vigor (Saa *et al*., 2020; Reed *et al*., 2022).

Although the individual benefits of LOF and biostimulants are well-established, the study of the combined or synergistic effects of both treatments, especially on sugarcane, remains limited. This study, therefore, investigates the interactive impact of LOF and biostimulants on agro-morphological traits of sugarcane seedlings. The results are expected to inform eco-efficient, low-input management and strategies to enhance early growth and sustainability in sugarcane cultivation.

Materials and Methods

Study site and experimental design

This study was conducted from July to October 2023 at the Teaching Farm of the Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia (5°07'53"S 119°29'04"E). A split-plot design was employed within a randomized complete block design (RCBD), with liquid organic fertilizer (LOF) treatments assigned to the main plots and biostimulant concentrations to the subplots. The LOF treatments included no LOF (p0), 3 mL L^{-1} (p1), and 6 mL L^{-1} (p2), while the biostimulant treatments consisted of no biostimulant (b0), 1 mL L^{-1} (b1), 2 mL L^{-1} (b2), and 3 mL L^{-1} (b3). This arrangement resulted in a total of 12 treatment combinations, each replicated three times. Each experimental unit comprised three sugarcane seedlings, resulting in a total of 108 plants.

Planting material and nursery management

Sugarcane sets of the Bululawang variety (6–8 months old, approximately 10 cm) with a single bud were used as planting material. Sets were treated with a Nordox fungicide solution for 15 minutes to prevent fungal infections, followed by immersion in a commercial plant growth regulator for an additional 15 minutes to stimulate germination. Nursery beds (2m × 1m) were prepared and filled with compost. Setts were sown with the bud facing upward and allowed to sprout for 2–4 weeks. Subsequently, seedlings were transplanted into 25 cm × 30 cm polybags filled with a mixture of 2:1 soil and compost substrates, arranged according to the experimental layout.

Application of treatments

Biostimulants and liquid organic fertilizer (LOF) were applied using a pre-calibrated foliar sprayer, with discharge rates determined on a per-unit-area-per-time basis. Biostimulants were foliar-sprayed 14 days after transplanting (DAT) and applied at 10-day intervals for a total of three applications. In contrast, LOF was applied weekly starting at 15 DAT for four consecutive weeks. All applications were conducted between 07:00–08:00 A.M. under calm weather conditions to minimize drift, using consistent nozzle height, pressure, and spraying speed to ensure even coverage of all aerial plant parts of plants.

Crop management

Basal fertilization was performed by manually applying 5g of compound N-P-K fertilizers (15:15:15) to each polybag and thoroughly incorporating them into the upper soil layer to ensure even nutrient distribution. Irrigation was carried out manually using watering cans, applied twice daily (morning and late afternoon on the day), with water volume standardized by pre-measuring the can's capacity to maintain uniform soil moisture. Abnormal or dead seedlings were identified based on visual symptoms such as wilting, discoloration, or stunted growth, and promptly replaced with healthy seedlings of similar age and size to ensure uniform stand establishment. Weed control was conducted manually, every two weeks, through hand-weeding, coinciding with shallow soil loosening to improve aeration. Pest management was conducted by handpicking visible pests and removing affected plant parts. Fungal diseases were controlled through application of broad-spectrum fungicides at recommended rates using a handheld sprayer, applied in early morning to enhance adherence and reduce evaporation.

Observed parameters

The observed parameters in this study included plant height (cm), number of leaves (n), stem diameter (cm), leaf area (cm^2), root volume (mL), fresh and dry root weight (g), fresh and dry shoot weight (g), and root-to-shoot ratio. Plant height was measured from base to the tip of longest leaf using a ruler. The number of leaves was counted manually per plant. Stem diameter was measured at basal portion of the stem using a digital caliper. Leaf area was measured using a portable leaf area meter (Plant Leaf Area Tester, with a width range of 0–150 mm and a length range of 0–1000 mm, and a resolution of 0.01 cm^2). Root volume was measured using the water displacement method, whereby volume of water displaced by root mass was recorded. Fresh weights of roots and shoots were measured immediately after harvesting using an analytical balance.

In contrast, dry weights were obtained after oven-drying samples at 70°C until a constant weight was achieved. Root-to-shoot ratio was calculated by dividing the dry root weight by the dry shoot weight. These measurements were used to evaluate the early vegetative growth response of sugarcane seedlings under different concentrations of biostimulant and liquid organic fertilizer (LOF).

Statistical analysis

All measured data were subjected to analisis of variance (ANOVA). When significant differences were detected, treatment means were compared using the Least Significant Difference (LSD) test at 5% significance level ($\alpha = 0.05$) using STAR version 2.0.1 (IRRI, Philippines). In addition to univariate analysis, Pearson's correlation coefficients were calculated to assess the relationships among morphological variables. To visualize bivariate associations, scatter plots with regression lines were generated for selected variable pairs. Multivariate analysis was performed using Principal Component Analysis (PCA), which was conducted in RStudio version 4.2.2 (R Core Team, 2022).

Results and Discussion

Growth trait responses and development of sugarcane seedlings

The results of the analysis of variance (ANOVA) for growth traits and early development of sugarcane seedlings, specifically plant height, number of leaves, stem diameter, and leaf area, are summarized in Table 1.

Based on data in Table 1, the analysis of variance (ANOVA) revealed that biostimulant concentration had a significant effect ($p < 0.05$) on plant height and leaf area meter. In contrast, neither the liquid organic fertilizer (LOF) treatment nor the LOF × biostimulant interaction produced any significant effect on number of leaves. Plant height responded significantly to the combined application of LOF and biostimulant. The tallest plant height of seedlings (20.93cm) were observed under the application of 3 mL L^{-1} LOF (p1) and 1 mL L^{-1} biostimulant (b1), closely followed by the p1b3 treatment (20.16 cm) and p1b2 treatment (20.15 cm).

Table 1: *Growth trait responses of sugarcane seedlings to LOF and biostimulant applications.*

		Biostimulant concentration (mL L^{-1})					
	LOF Concentration (mL L^{-1})	0 (b0)	1 (b1)	2 (b2)	3 (b3)	Average	LSD α (0.05)
Plant Height	0 (p0)	18.86	18.86	19.94	18.91	19.14±0.46	-
	3 (p1)	18.30	20.93	20.15	20.16	19.89±0.97	
	6 (p2)	17.95	20	20.19	19.45	9.40±0.88	
	Average	18.37±0.46^{q}	19.93±1.04^{p}	20.09±0.13^{p}	19.51±063^{pq}		
	LSD α (0.05)	1.19					
Number of Leaves	0 (p0)	10	9.56	9.55	9.11	9.55±0.31	-
	3 (p1)	10	10.11	10.44	10.33	10.22±0.17	
	6 (p2)	9.89	9.89	9.89	10.11	9.95±0.10	
	Average	9.96±0.06	9.85±0.28	9.96±0.45	9.85±065		
	LSD α (0.05)	-					
Stem Diameter	0 (p0)	13.55	13.41	13.38	13.21	13.39±0.12^{b}	0.15
	3 (p1)	14.05	14.31	14.23	14.02	14.15±0.1^{2a}	
	6 (p2)	14.02	14.01	14.24	14.29	14.14±0.13^{a}	
	Average	13.87±0.28	13.91±0.46	13.9±50.49	13.84±0.56		
	LSD α (0.05)	-					
Leaf Area Meter	0 (p0)	155.95	166.55	175.86	174.85	168.30	-
	3 (p1)	166.55	170.37	183.87	172.73	173.38	
	6 (p2)	164.8	170.88	185.12	175.77	174.14	
	Average	162.43±5.68^{r}	169.27±2.3^{7q}	181.62± 5.02^{p}	174.44± 1.56^{q}		
	LSD α (0.05)	5.37					

Note: Data are presented as mean ± SD. Means within the same row (p, q, r) or the same column (a, b) followed by the same letter are not significantly different at the 5% probability level, as determined by the least significant difference (LSD) test (α = 0.05).

In contrast, the shortest plants (17.95 cm) were recorded under 6 mL L^{-1} LOF with no biostimulant (p2b0), indicating that LOF alone at higher concentrations was insufficient to enhance seedling elongation. On average, plant height increased with the addition of biostimulant, particularly at 1–2 mL L^{-1} concentrations, suggesting a synergistic interaction between moderate LOF and biostimulant levels in promoting shoot growth. These findings suggest that a moderate application of biostimulant promotes vertical growth during the early vegetative stage, potentially due to its bioactive composition, comprising phytohormones, amino acids, and microbial metabolites, which are known to enhance cell elongation and division (Calvo *et al*., 2014).

The number of leaves showed minimal variation across treatments, with values ranging from 9.11 to 10.44. The highest leaf count was recorded under p1b2 (10.44), while the lowest was observed in the control (p0b3; 9.11). However, the absence of LSD value suggests that these differences were not statistically significant at α = 0.05. Overall, the average number of leaves remained relatively consistent across treatments, indicating that this trait was less responsive to input levels compared to plant height and stem diameter.

For stem diameter, LOF concentration had a highly significant effect ($p < 0.01$), whereas biostimulant concentration and the interaction between the two factors were not significant. The greatest mean stem diameter (14.15 mm) was obtained under 3 mL L^{-1} LOF (p1), which was statistically similar to 6 mL L^{-1} (p2: 14.14 mm) but significantly greater than the control (p0: 13.39 mm). These findings highlight the role of LOF in promoting structural stem development, likely by improving the availability of macro- and micronutrients that stimulate cambial activity and secondary thickening. The leaf area showed a highly significant response ($p < 0.01$) to biostimulant concentration. However, similar to the pattern observed in plant height, neither LOF treatment nor the interaction term significantly affected this parameter. The maximum leaf area

(181.62 cm^2) was achieved at 2 mL L^{-1} biostimulant (b2), significantly higher than at 3 mL L^{-1} (174.44 cm^2), 1 mL L^{-1} (169.27 cm^2), and 0 mL L^{-1} (162.43 cm^2). This finding suggests a dose-dependent stimulation, peaking at 2 mL L^{-1}, which likely reflects enhanced nitrogen assimilation and auxin-mediated cell expansion. The absence of a significant interaction further implies that the biostimulant exerted its effect independently of LOF in driving early leaf expansion.

To illustrate the temporal dynamics of early vegetative growth, the Figures present a bar chart showing the progression of growth traits of sugarcane seedlings from 3 to 12 weeks after planting (WAP). The traits evaluated include plant height, number of leaves, and stem diameter, to assess development and responsiveness of seedlings to the applied treatments.

Figure 1 showed across observation ages (3, 6, 9, and 12 WAP) and treatment combinations. In general, the seedlings exhibited the most rapid increase in height between 3 and 6 WAP, followed by a gradual slowdown. This pattern is typical of early vegetative growth phases in sugarcane, where internode elongation dominates

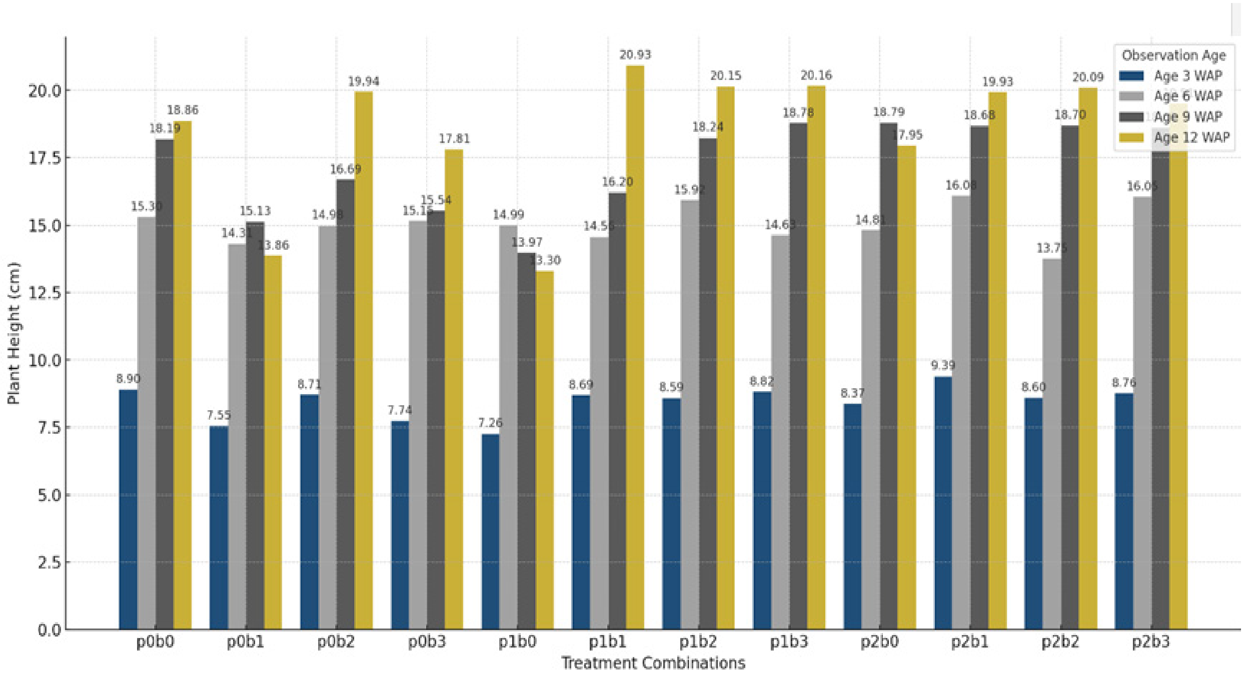

Figure 1: *Plant height in sugarcane seedlings at different weeks after planting (WAP)*

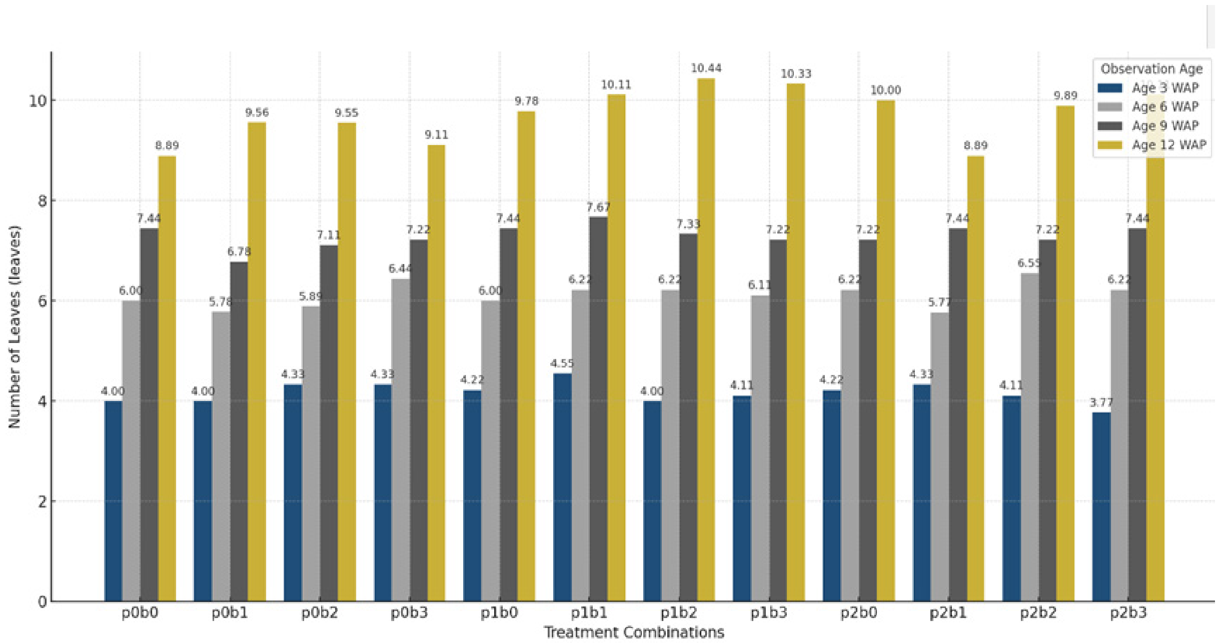

Figure 2: *Number of leaves in sugarcane seedlings at different weeks after planting (WAP)*

early development (Verma *et al.*, 2024). Among the treatment combinations, p1b1 (3 mL L^{-1} LOF and 1 mL L^{-1} biostimulant) showed the highest final height (20.93 cm), whereas p2b0 (6 mL L^{-1} LOF and 0 mL L^{-1} biostimulant) produced one of the lowest results, suggesting the essential role of biostimulant presence regardless of LOF concentration. These findings are consistent with previous research indicating that biostimulants improve plant height and biomass accumulation under both optimal and suboptimal conditions. For instance, Sani *et al.* (2020) found that protein hydrolysate-based biostimulants significantly enhanced shoot length and vigor in crop seedlings. Similarly, Adedayo and Babalola (2023) reported positive height responses in sugarcane varieties treated with microbial-based biostimulants, attributing the effects to improved root-soil nutrient interactions and hormonal balance. On the other hand, the non-significant influence of LOF suggests that nutrient contributions from the liquid organic fertilizer alone may not be sufficient to stimulate early shoot elongation in sugarcane seedlings. This finding aligns with the results of Kumar *et al.* (2024), who observed limited vegetative response in early growth stages when LOF was applied without accompanying hormonal or microbial bioactivators.

Figure 2 showed the progression of number of leaves from 3 to 12 WAP. All seedlings experienced the most significant increase in number of leaves between 9 and 12 WAP, a phase typically associated with intensified shoot development and rapid canopy expansion. This trend corresponds with established sugarcane growth models, indicating that the rate of leaf appearance (phyllochron) tends to increase in the later vegetative stages under favorable conditions (Verma *et al.*, 2024). The non-significant effects observed here align with earlier study reporting variable responses of sugarcane morphology to organic and biostimulant inputs. For example, Kumar *et al.* (2024) found that while organic fertilizer improved soil properties, its short-term effects on leaf emergence were minimal without complementary stimulatory inputs. Similarly, Baltazar *et al.* (2021) highlight that the effect of biostimulants on the number of leaves is often noticeable and more pronounced under stress conditions or over developmental periods. Moreover, the physiological process of leaf initiation is not solely driven by the availability of nutrients or biostimulants, but is also influenced by internal hormonal balances, environmental cues, and genotypic factors (Calvo *et al.*, 2014). Hence, the early vegetative stage may not be the optimal phase for detecting strong treatment responses in leaf number, particularly under non-stress conditions.

Figure 3 illustrates the trend of stem diameter development across time. All treatments demonstrated the highest rate of stem diameter thickening during the 9–12 WAP period. This finding aligns with the

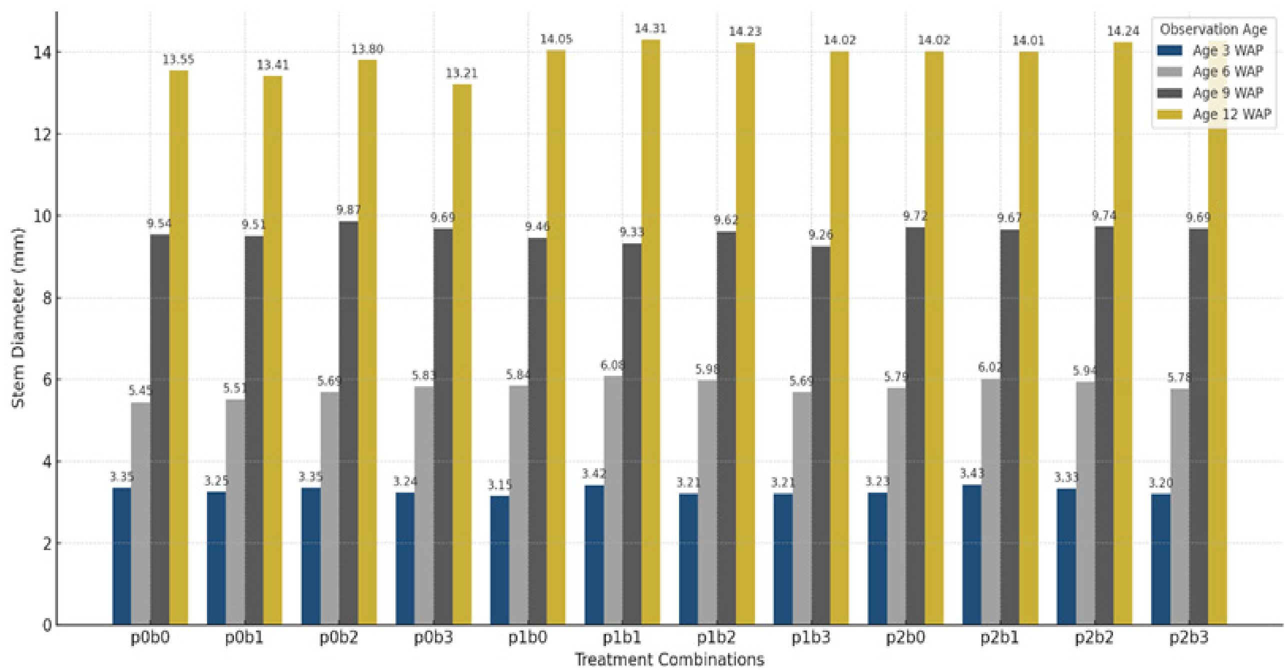

Figure 3. *Stem diameter in sugarcane seedlings at different weeks after planting (WAP)*

findings of Sohel *et al.* (2021), who reported that stem diameter expansion in sugarcane accelerates significantly during the late vegetative phase due to increased photosynthate allocation and lignin deposition. The effect of LOF aligns with previous work by Sinha *et al.* (2024), who showed that organic fertilizers enhance vegetative biomass and stem diameter in sugarcane through improved nutrient cycling and rhizosphere microbial interactions. Similarly, Pandey *et al.* (2021) found that LOF derived from vermicompost and enriched compost stimulated shoot thickening and plant robustness under rainfed conditions. Conversely, the absence of a significant biostimulant effect on stem diameter may indicate that biostimulants exert a more prominent influence on traits such as root initiation or shoot elongation, especially under stress conditions (Nephali *et al.*, 2020).

Since this study was conducted under non-limiting conditions, the hormonal effects of biostimulants

Table 2: *Root and shoot trait responses and biomass of sugarcane seedlings to LOF and biostimulant applications*

Parameter		Biostimulant concentration (mL L^{-1})					
	LOF Concentration (mL L^{-1})	0 (b0)	1 (b1)	2 (b2)	3 (b3)	Average	LSD α (0.05)
Root Volume	0 (p0)	100.00^{a}_{r}	120.00^{a}_{q}	141.67^{b}_{p}	133.33^{b}_{pq}	123.75±15.74	21.60
	3 (p1)	100.00^{a}_{r}	133.33^{a}_{q}	180.00^{a}_{p}	143.33^{ab}_{q}	173.38±6.45	
	6 (p2)	80.00^{a}_{r}	116.67^{a}_{q}	170.00^{a}_{p}	156.67^{a}_{p}	130.84±35.31	
	Average	93.33±11.55	123.33±8.82	163.89±19.88	144.44±11.71		
	LSD α (0.05)	18.10					
Root Fresh Weight	0 (p0)	126.17	117.53	152.57	167.90	141.04±20.18	-
	3 (p1)	117.00	142.37	179.30	180.47	154.78±26.66	
	6 (p2)	114.60	131.77	190.570	150.00	146.74	
	Average	119.26±6.11^{q}	130.56±12.46^{q}	174.14±19.52^{p}	166.12±15.31^{p}		
	LSD α (0.05)	0.73					
Root Dry Weight	0 (p0)	28.96	33.27	37.00	37.47	34.16±3.45	-
	3 (p1)	29.90	30.37	40.10	33.63	33.50±4.07	
	6 (p2)	31.97	36.43	40.60	39.37	37.09±3.32	
	Average	30.26±1.57^{r}	33.36±3.03^{qr}	39.23±1.95^{p}	36.82±2.92^{pq}		
	LSD α (0.05)	3.82					
Shoot Fresh Weight	0 (p0)	56.27	58.43	68.67	69.17	63.14±5.84	-
	3 (p1)	70.23	76.37	85.20	87.53	79.83±6.93	
	6 (p2)	63.17	77.10	90.17	82.63	78.27±9.87	
	Average	63.22±6.98^{r}	70.63±10.57^{q}	81.34±11.26^{p}	79.78±9.51^{p}		
	LSD α (0.05)	5.74					
Shoot Dry Weight	0 (p0)	11.63	19.30	16.57	14.27	15.44±2.83	-
	3 (p1)	16.00	19.57	22.20	21.43	19.80±2.39	
	6 (p2)	14.97	16.63	22.77	23.10	19.37±3.62	
	Average	14.20±2.28^{q}	18.50±1.63^{p}	20.50±3.43^{p}	19.60±4.96^{p}		
	LSD α (0.05)	2.84					
Root-Shoot Ratio	0 (p0)	2.49	1.86	12.23	2.64	4.81±4.30	-
	3 (p1)	1.93	1.78	1.80	1.56	1.77±0.13	
	6 (p2)	2.15	2.27	1.78	1.71	1.98±0.24	
	Average	2.19±0.28	1.97±0.28	1.93±0.26	1.97±0.59		
	LSD α (0.05)	-					

Note: Data are presented as mean ± SD. Means within the same row (p, q, r) or the same column (a, b) followed by the same letter are not significantly different at the 5% probability level, as determined by the least significant difference (LSD) test (α = 0.05).

might not have manifested in a measurable morphological response such as stem diameter.

Root and shoot traits and biomass of sugarcane seedlings

The results of the analysis of variance (ANOVA) for root and shoot traits and biomass of sugarcane seedlings, such as root volume, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, and root-shoot ratio, are summarized in Table 2. These parameters were evaluated to assess the effects of liquid organic fertilizer (LOF) and biostimulant concentrations on 12 weeks after planting (WAP).

The analysis of variance indicated that biostimulant concentration and its interaction with liquid organic fertilizer (LOF) had a highly significant effect ($p < 0.01$) on the root volume of sugarcane seedlings. However, the main effect of LOF concentration alone was not statistically significant ($p > 0.05$). As shown in Table 2, the highest root volume was recorded under the treatment combination of 3 mL L^{-1} LOF and 2 mL L^{-1} biostimulant (p1b2), reaching 180.00 mL. This value was statistically similar to 6 mL L^{-1} LOF + 2 mL L^{-1} biostimulant (p2b2: 170.00 mL), but significantly higher than 0 mL L^{-1} LOF + 2 mL L^{-1} biostimulant (p0b2: 141.67 mL). Furthermore, the p1b2 treatment significantly outperformed other combinations at 3 mL L^{-1} LOF, including b0, b1, and b3, indicating that 2 mL L^{-1} biostimulant is optimal for promoting early-stage root development when applied alongside moderate LOF input. Conversely, the lowest root volume (80.00 mL) was observed in the treatment with 6 mL L^{-1} LOF + 0 mL L^{-1} biostimulant (p2b0). This was statistically similar to other control-level treatments (p1b0 and p0b0), but significantly lower than all treatments containing biostimulant at 2 mL L^{-1}. These findings highlight that LOF alone is insufficient to stimulate root volume. The stimulatory effect is likely due to auxin-mediated root elongation, increased root hair density, and improved rhizosphere nutrient acquisition. Wahyuni *et al*. (2024) reported that biostimulants such as sucrosin, humic acid, and mycorrhiza significantly enhanced sugarcane root growth under dryland conditions. Similarly, Alasvandyari *et al*. (2024) found that seaweed extract-based biostimulants increased sugarcane root volume and drought resilience by hormonal modulation and antioxidant activity.

Biostimulant concentration had a highly significant effect ($p < 0.01$) on root fresh weight, while neither LOF concentration nor the LOF × biostimulant interaction was significant ($p > 0.05$). As shown in Table 2, the highest root fresh weight was observed at 2 mL L^{-1} biostimulant (b2) with an average of 174.14 g, which was significantly greater than b1 (130.56 g) and b0 (119.26 g), but not significantly different from b3 (166.12 g). This result confirms that moderate biostimulant concentrations substantially increase root biomass accumulation. This pattern aligns with research showing that biostimulants containing amino acids, humic substances, or microbial extracts promote root growth by enhancing metabolic activity, nutrient uptake efficiency, and water absorption capacity. Adedayo and Babalola (2023) demonstrated significant gains in root biomass in sugarcane following biostimulant application, particularly at moderate doses. Nephali *et al*. (2020) similarly found that biostimulants enhanced turgor pressure, root elongation, and carbohydrate storage in root tissues. The absence of LOF effect in this context further reinforces that bioactive stimulants, not nutrient supply, are the primary drivers of early root mass increases.

Root dry weight was also significantly affected ($p < 0.01$) by biostimulant concentration, while the LOF treatment and its interaction with biostimulant showed no significant effects ($p > 0.05$). As presented in Table 2, the highest average root dry weight (39.23 g) was recorded under 2 mL L^{-1} biostimulant (b2), which was statistically similar to b3 (36.82 g), but significantly higher than b1 (33.36 g) and b0 (30.26 g). The increased dry root biomass at optimal biostimulant concentrations is likely due to improved water retention, enhanced cellular metabolism, and nutrient uptake efficiency. Jangpromma *et al*. (2012) observed similar improvements in dry matter accumulation in sugarcane roots under both optimal and stress conditions. Rathor *et al*. (2024) reported that humic-based and microbial biostimulants improve enzymatic activity and root architecture, thereby increasing dry biomass. The lack of response to LOF may indicate that organic fertilizers lack the signaling properties necessary for stimulating dry matter in early developmental stages.

Biostimulant application significantly influenced shoot fresh weight ($p < 0.01$), whereas neither LOF nor its interaction with biostimulant showed a significant effect ($p > 0.05$). The highest average value (81.34 g) was achieved at a concentration of 2 mL

L^{-1} biostimulant, followed by 3 mL L^{-1} (79.78 g), both of which were significantly greater than 1 mL L^{-1} (70.63 g) and 0 mL L^{-1} (63.22 g). This suggests that biostimulants play a crucial role in promoting aerial biomass through mechanisms such as enhanced chlorophyll content, improved photosynthetic rate, and better nutrient remobilization. Zuo *et al*. (2025) confirmed that biostimulant treatments significantly enhanced photosynthetic capacity and biomass accumulation. Likewise, Sun *et al*. (2024) reported that protein hydrolysates and seaweed extracts promote shoot growth by facilitating cell division and expansion. The non-significant LOF response indicates that while soil conditions may improve, shoot growth is predominantly governed by physiological stimuli provided by biostimulants.

Biostimulant concentration significantly affected shoot dry weight ($p < 0.01$), whereas the LOF treatment and the interaction between LOF and biostimulant were not significant. The highest dry biomass (20.50 g) was observed at a biostimulant concentration of 2 mL L^{-1} (b2), significantly higher than b0 (14.20 g) and b1 (18.50 g), and comparable to b3 (19.60 g). These findings reaffirm the positive influence of biostimulants on the partitioning of dry matter to above-ground tissues. Jacomassi *et al*. (2022) demonstrated that seaweed-based biostimulants increased dry biomass and stalk yield in sugarcane under drought by stimulating antioxidant activity. Hamid *et al*. (2021) emphasized that biostimulants improve nutrient use efficiency and growth signaling pathways, promoting shoot dry mass even in early growth stages. Furthermore, Li *et al*. (2022) confirmed, through a meta-analysis of over 1,000 field trials, that biostimulants enhance dry biomass, yielding mean increases of ~18%. Nephali *et al*. (2020) also observed that biostimulants improved tillering and shoot mass by stimulating internal nutrient partitioning and root–shoot signaling in sugarcane.

The root-to-shoot ratio was not significantly affected by LOF concentration, biostimulant concentration, or their interaction ($p > 0.05$). Nevertheless, the trend analysis revealed that the highest ratio (2.64) occurred at 0 mL L^{-1} LOF and 3 mL L^{-1} biostimulant (p0b3), suggesting a shift toward below-ground biomass accumulation without exogenous nutrients. The lowest ratio (1.56) was observed at 3 mL L^{-1} LOF + 3 mL L^{-1} biostimulant (p1b3), indicating a relative shift toward shoot development under nutrient-enriched conditions. Although non-significant, these patterns are consistent with literature suggesting that biostimulants may influence carbon partitioning and assimilate allocation based on hormonal cues and environmental inputs. Lamar *et al*. (2024) reported that humic substances enhance root vigor, especially under stress, without drastically altering biomass ratios. Lucini *et al*. (2020) found that protein hydrolysate-based biostimulants affect biomass allocation depending on crop type and developmental stage. Similarly, Han *et al*. (2024) concluded that biostimulant-induced changes in root–shoot dynamics are contingent on plant phenology and environmental context.

Correlation analysis among sugarcane seedling traits

Correlation analysis among sugarcane seedling traits is presented in Figure 4. This analysis was conducted to evaluate the relationships between growth traits and root parameters under varying concentrations of liquid organic fertilizer (LOF) and biostimulants.

The correlation analysis revealed strong interrelationships among agromorphological and physiological traits of sugarcane seedlings, indicating a coordinated developmental pattern under the influence of biostimulant and LOF applications. Plant height demonstrated a strong positive correlation with leaf area ($r = 0.68$), shoot fresh weight ($r = 0.82$), and shoot dry weight ($r = 0.82$), suggesting that taller plants tend to exhibit greater canopy expansion and biomass accumulation. These findings align with observations by Elrys *et al*. (2023), who reported that increases in plant height following biostimulant application were closely associated with enhanced photosynthetic area and assimilate partitioning to shoot organs. Similarly, leaf area exhibited a very strong correlation with shoot fresh weight ($r = 0.98$), reinforcing its role as a primary determinant of biomass production during early vegetative development. This relationship was also observed by Malik *et al*. (2020) and Syaiful *et al*. (2025), who emphasized that leaf expansion under biostimulant treatment contributes directly to shoot mass gains through increased light capture and gas exchange efficiency, in similar trends. Root volume was highly correlated with root fresh weight ($r = 1.00$) and root dry weight ($r = -0.22$), indicating that volumetric expansion of the root system corresponds strongly with total root biomass. These results are in agreement with the findings of Wahyuni *et al*. (2024), who demonstrated that biostimulant-induced increases

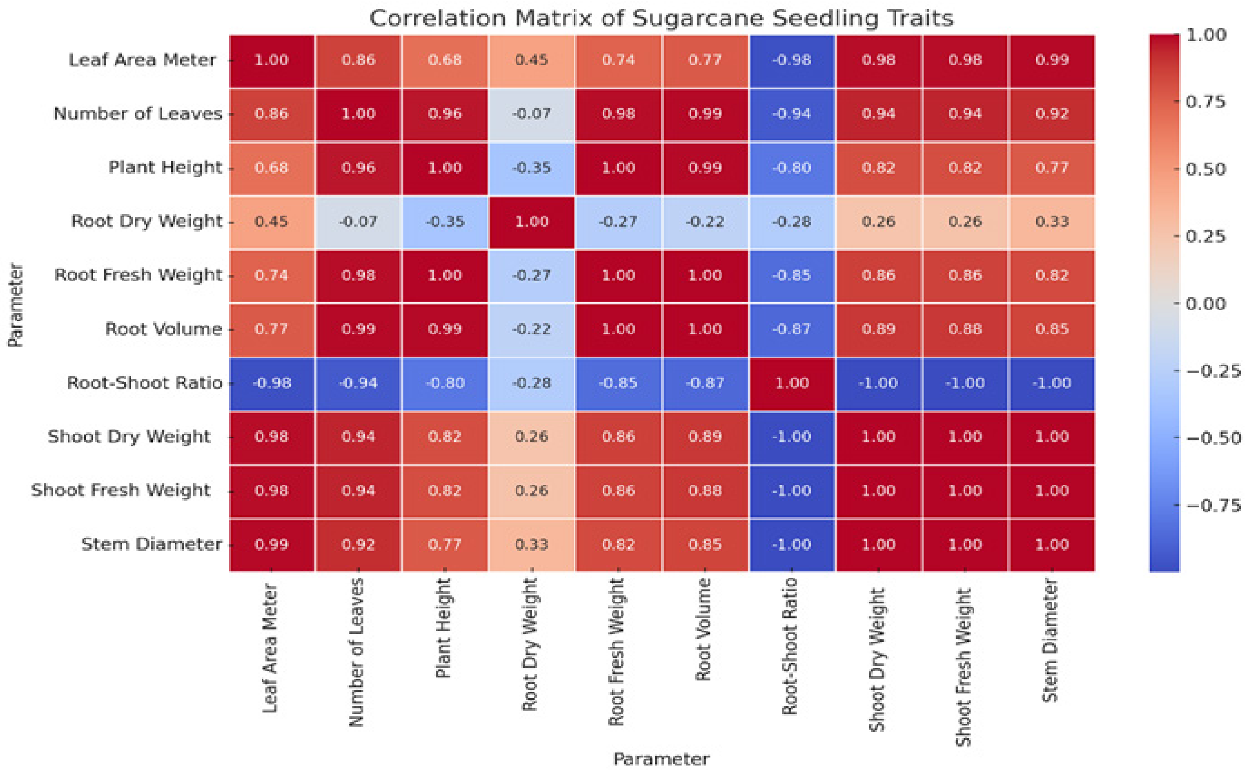

Figure 4: *Pearson correlation coefficients on sugarcane seedling traits. At significance at the 95% confidence level (α = 0.05)*

in root volume were associated with improved biomass and water uptake in sugarcane under dryland conditions. However, the root-to-shoot ratio showed weak or negative correlations with shoot-related traits (e.g., shoot fresh weight r = −1.00), suggesting that higher investment in root biomass may reduce shoot growth under certain input conditions. This trade-off aligns with the framework presented by Han *et al.* (2024), who explained that biostimulants may modulate assimilate allocation depending on resource availability and stress levels.

Interaction between traits and principal component analysis of sugarcane seedling traits

The interaction between traits and Principal component analysis of sugarcane seedlings is presented in Figures 5 and 6. Figures 5 and 6 present scatter plots and principal component analysis (PCA) to examine the relationships among sugarcane seedling traits under different LOF and biostimulant treatments. Scatter plots illustrate pairwise trait correlations, while PCA summarizes multivariate patterns and highlights key treatment effects on growth.

The scatter plot (Figure 5) illustrates a strong positive linear relationship among traits of sugarcane seedlings. A moderate positive relationship was observed between plant height and leaf area. A strong linear correlation between root volume and root fresh weight suggests that larger root systems not only occupy a greater soil volume but also retain more water and biomass, indicating improved nutrient absorption efficiency. Similarly, the positive correlation between stem diameter and shoot fresh weight highlights the role of thicker stems in supporting enhanced above-ground biomass. Furthermore, the strong correlation between leaf area and root dry weight. The strong correlation between root fresh weight and shoot fresh weight showed seedlings with more developed root systems tend to accumulate greater shoot biomass, indicating a coordinated growth response between below- and above-ground organs. The trend aligns with prior findings, such as Sharma *et al.* (2023), who reported that biostimulants improved both root and shoot biomass through enhanced metabolic activity and nutrient uptake. Goni *et al.* (2022) further confirmed that increased root mass contributes directly to shoot biomass by improved water relations and photosynthate partitioning. Collectively, these data emphasize that sugarcane seedlings exhibiting stronger shoot growth traits tend to simultaneously develop more vigorous root systems, supporting the efficacy of biostimulant and liquid organic fertilizer applications during early vegetative stages.

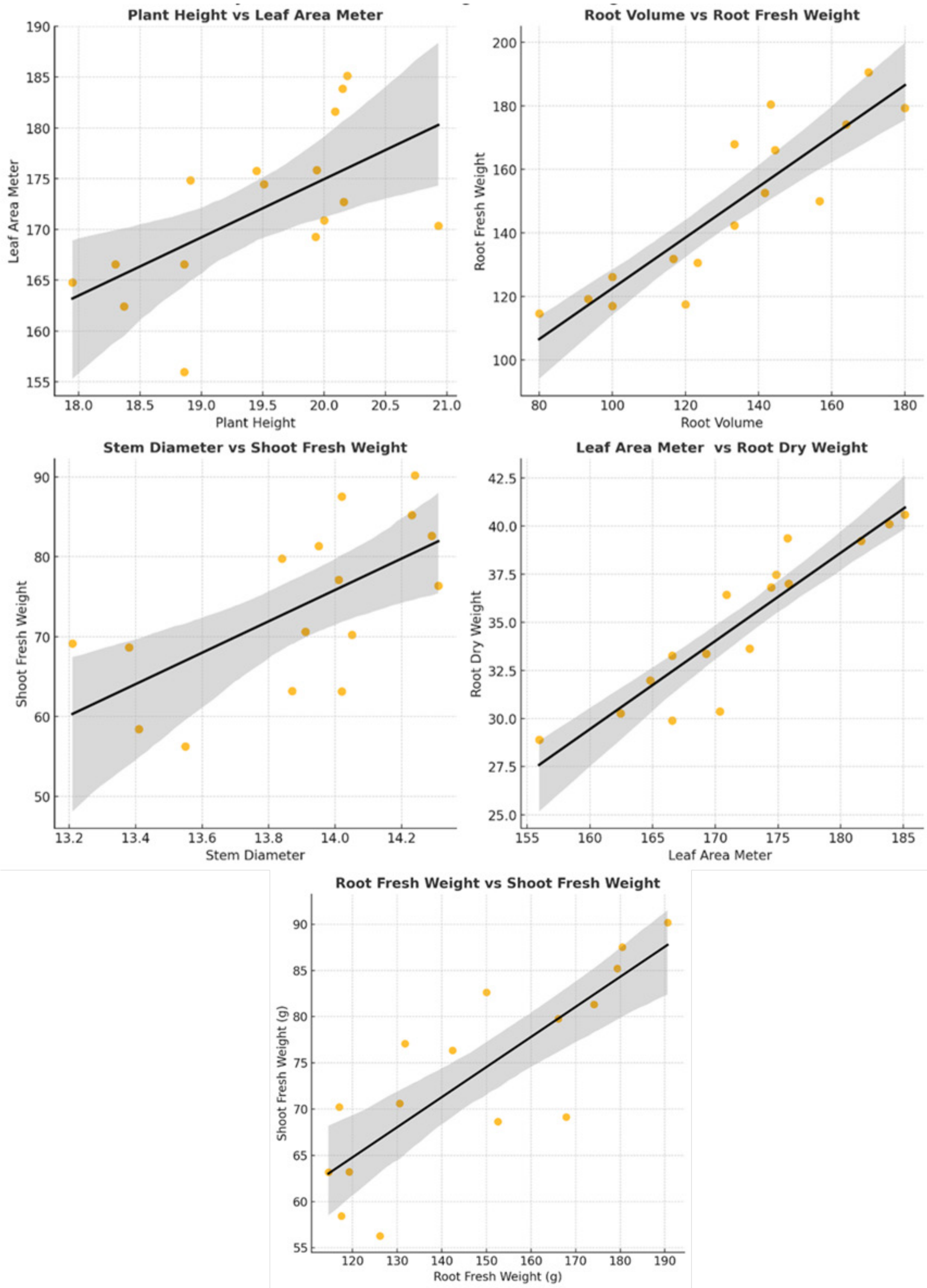

Figure 5: *Interaction between traits of sugarcane seedling*

The Principal Component Analysis (PCA) biplot (Figure 5) provides a multivariate overview of how sugarcane seedling traits respond to combinations of liquid organic fertilizer (LOF) and biostimulant concentrations at 12 weeks after planting (WAP). The PC1 explains 82.85% and PC2 explains 17.15%, indicating that these components comprehensively represent the variability among treatment responses.

Parameters such as shoot fresh weight, shoot dry weight, leaf area meter, and root fresh weight showed a strong and positive correlation along PC1. In contrast, the root-to-shoot ratio was oriented in the opposite direction.

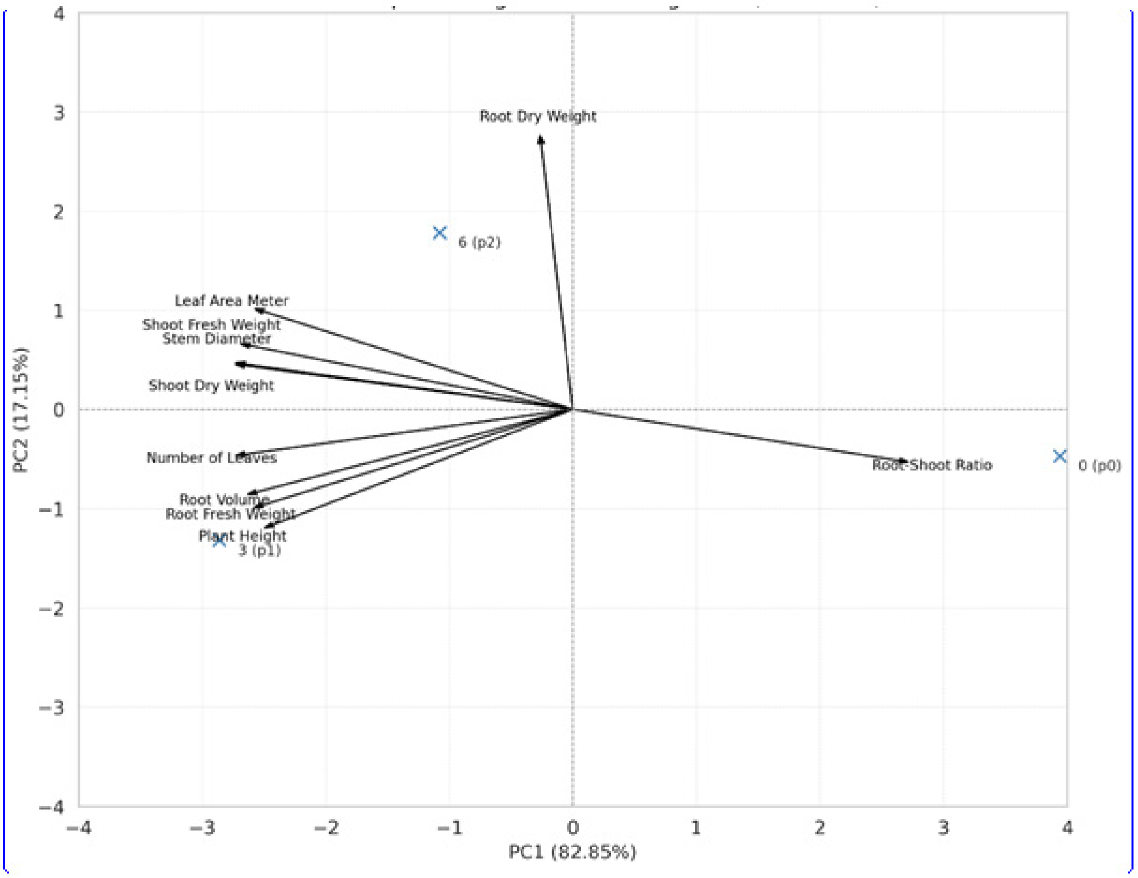

Figure 6: *Component analysis of sugarcane seedling traits*

PC2 primarily captured variability associated with stem diameter, plant height, and number of leaves, which are indicative of vertical growth. These traits contributed less to overall biomass performance but may play complementary roles in structural adaptation to nutrient or biostimulant inputs. Treatment combinations were spatially distinct within the PCA space, reflecting differential multivariate responses. Notably, treatments involving 3 mL L^{-1} LOF combined with 2 mL L^{-1} biostimulant were located in the positive PC1 quadrant, indicating enhanced performance along the growth traits.

Additionally the results of the study indicated that the combination of 6 mL L^{-1} liquid organic fertilizer (LOF) and 0 mL L^{-1} biostimulant resulted in the lowest root volume. This finding is likely attributable to the absence of biostimulant input, which limited nutrient absorption solely to what was provided by the LOF, without the additional improvement typically facilitated by biostimulants. This observation aligns with the findings of Tabacchioni *et al*. (2021), reported that biostimulants contain bioactive compounds and beneficial microorganisms that show positive effects on plant growth. These findings confirm that biostimulant concentration is the primary driver of early-stage sugarcane seedling.. The lack of strong effects with LOF suggests that bioactive compounds in biostimulants may play a more decisive role than basal organic nutrition during early vegetative growth.

Conclusions and Recommendations

The combined application of 3 mL L^{-1} LOF and 2 mL L^{-1} biostimulant significantly improved early growth traits of sugarcane seedlings, including plant height, stem diameter, leaf area, root volume, and biomass. This combination also promoted balanced root-shoot traits for sugarcane seedling. Further field-based studies are required to assess their long-term effects on cane yield, particularly under diverse and challenging agroecological conditions.

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Novelty Statement

This study presents a novel contribution to sugarcane (*Saccharum officinarum* L.) agronomy by elucidating the synergistic effects of liquid organic fertilizer (LOF) and biostimulant combinations on early seedling development. By integrating both univariate (ANOVA) and multivariate (principal component analysis, correlation matrices, and scatter plot visualization) approaches, this research offers a comprehensive evaluation of treatment responses across multiple growth traits. Unlike prior studies that have typically examined LOF or biostimulants in isolation, this work identifies optimal input concentrations that simultaneously enhance both shoot and root development. In particular, the combination of 3 mL L^{-1} LOF and 2 mL L^{-1} biostimulant consistently promoted balanced vegetative growth and efficient resource allocation. These findings provide insights for establishment strategies in sugarcane cultivation.

Author's Contribution

Rafiuddin: Conceptualization, supervision, project administration, writing – original draft
Ambo Ala: Methodology, investigation, resources, validation
Willy Agung: Data curation, visualization, software
Purnama Isti Khaerani: Writing – review and editing, data curation and validation

Generative AI or AI assisted technology statement
Generative AI tools were used only to improve grammar and language clarity. No AI tools were employed for data analysis and drawing scientific conclusions. All authors reviewed the content carefully and take full responsibility for the final version of the manuscript.

Conflict of interest
The authors declare that there is no conflict of interest among the authors of the manuscript.

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