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Comparative Effects of *Polyalthia longifolia* Leaf Extract and Synthetic Growth Hormones on the Nursery Development of Cocoa (*Theobroma cacao* L.) Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. Author OSI was responsible for conceptualization. Authors CEO and OA-D were responsible for experimental design, data collection, and drafting the original manuscript. Author SAJ-O contributed to methodology development, data curation, and formal analysis. Author YMO supervised the study, while Author OU critically reviewed the manuscript and validated its final intellectual content. All authors read and approved the final manuscript.

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Abstract

Background: Quality seedling production is fundamental to sustainable cocoa (*Theobroma cacao* L.) establishment, yet the cost and environmental burden of synthetic hormones have renewed interest in botanical alternatives.
Aim: The study investigated the comparative effects of Masquerade tree (*Polyalthia longifolia*) leaf extract (MLE), indole-3-acetic acid (IAA) and a commercial synthetic auxin on the emergence and early nursery growth of cocoa seedlings.

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Study Design: A 2 × 3 factorial experiment (hormone source × application rate), plus an untreated control, was laid out in a completely randomised design with three replications. The treatment sources were indole-3-acetic acid and a synthetic auxin (each at 100 mg/L and 50 mg/L) and *Polyalthia longifolia* leaf extract (1 kg/3 L and 1 kg/5 L water), giving seven treatments: A100, A50, I100, I50, M5L, M3L and control (Cn).
Place and Duration of Study: The study was conducted at the screen house of the Cocoa Research Institute of Nigeria (CRIN), Ibadan, south-western Nigeria, for three months.
Methodology: Freshly harvested F3 Amazon cocoa beans (18 per treatment) were soaked in the respective hormone or extract solution for 24 hours before sowing; control beans were sown untreated. One bean was sown per bag, with six seedlings per treatment per replicate (42 per replicate; 126 total).
Emergence was recorded at 14, 18, 22, 26 and 30 days after sowing (% of beans sown). Plant height, leaf number, stem diameter and leaf area were measured every four weeks from 4 to 12 weeks after sowing (WAS). At 12 WAS, seedlings were destructively sampled: taproot length and shoot and root dry weights (oven-dried at 70°C to constant weight) were recorded.
Results: Synthetic auxin consistently depressed emergence (as low as 11.1% at 14 days after sowing) and suppressed vegetative growth relative to the control, indicating supra-optimal auxin stress. MLE and IAA produced growth comparable to, and at several stages exceeding, those of the control, with MLE giving the highest leaf area (83.65 cm²) and dry shoot weight (4.07 g) at 12 weeks. Root traits were largely unresponsive to treatment.
Conclusion: These findings indicate that *Polyalthia longifolia* leaf extract is an effective, low-cost organic alternative to synthetic auxin for raising vigorous cocoa nursery seedlings, and its use at 1 kg/3 L is recommended over high-concentration synthetic auxin.

Keywords: Theobroma cacao; Polyalthia longifolia; indole-3-acetic acid; synthetic auxin; seedling emergence; nursery growth; botanical biostimulant.

1. Introduction

Cocoa (*Theobroma cacao* L.) is one of the most economically important tree crops in the tropical world, sustaining millions of smallholder farmers across West Africa, South-East Asia and Latin America and serving as the principal raw material for the global chocolate industry (Otekunrin, 2025). In Nigeria, cocoa remains the leading agricultural export commodity, with national production fluctuating between roughly 195,000 and 290,000 metric tonnes over the 2012/2013 to 2023/2024 period, placing the country among the world's top four producers after Côte d'Ivoire, Ghana and Indonesia (Esan *et al.*, 2025). Despite this standing, the sub-sector continues to face constraints linked to ageing plantations, inconsistent seedling quality and limited uptake of improved nursery technologies (Esan *et al.*, 2025). Since field performance is strongly determined by nursery seedling vigour, improving nursery practice remains a direct route to reversing this decline (Baligar *et al.*, 2021). A recent cacao nursery study also documented differences in growth among varieties under poultry-manure application, indicating that treatment context merits consideration when evaluating early seedling development (Alawode *et al.*, 2026).

Cocoa seedling establishment is frequently constrained by irregular, slow germination, prompting extensive research into pre-sowing treatments intended to hasten emergence and improve early vigour (Chuquibala-Checan *et al.*, 2026). Among available interventions, exogenous application of auxins is most widely practiced, as auxins regulate cell division, elongation and mobilization of stored seed reserves during early seedling development (Zhao *et al.*, 2025). Indole-3-acetic acid (IAA), the principal natural auxin, along with synthetic analogues, has therefore been used to prime seeds of numerous tree and field crops, including cocoa, to encourage faster, more uniform emergence (Baligar *et al.*, 2021; Kusmiati *et al.*, 2026). In spinach, seed priming with *Pongamia pinnata* leaf extract was associated with improved germination and subsequent vegetative growth compared with untreated seeds (Singh *et al.*, 2025).

However, plant response to auxin is markedly dose-dependent. At low to moderate concentrations, auxins promote elongation, root initiation and shoot growth, but at supra-optimal levels they become inhibitory, triggering stress-related ethylene and abscisic acid biosynthesis that suppress germination and biomass accumulation (Bunsangiam *et al.*, 2021; Zhao *et al.*, 2025). This biphasic behaviour is well documented: high IAA concentrations inhibit weed seed germination (Bunsangiam *et al.*, 2021) and synthetic analogues such as naphthaleneacetic acid and 2,4-D suppress wheat seedling growth even at micromolar levels (Lyubushkina *et al.*, 2025). Because commercial hormone formulations are applied at fixed rates that may not match a species' optimal window, treatments meant to promote vigour can instead depress it, a risk compounded by the cost and environmental concerns of synthetic hormones (Mashamaite *et al.*, 2022). A recent study of cacao seedlings treated with a protein hydrolysate-based biostimulant likewise reported dose-dependent growth responses, further supporting the consideration of treatment concentration in nursery studies (Hoste *et al.*, 2026).

Botanical leaf extracts offer a promising alternative, containing natural auxins, cytokinins, gibberellins, amino acids and phenolics that confer a milder, self-limiting biostimulant effect (Mashamaite *et al.*, 2022; Yuniati *et al.*, 2022). *Moringa oleifera* extract is the most studied example, consistently enhancing germination, vigour and stress tolerance across crops, often matching synthetic promoters (Mashamaite *et al.*, 2022; Yadav *et al.*, 2024). In separate seed-priming experiments, *Moringa oleifera* leaf extract was associated with improved early growth in moringa and with improved germination under salinity stress in fenugreek (Soares *et al.*, 2023; Pandey *et al.*, 2025). *Polyalthia longifolia* (Sonn.) Thwaites, widely planted ornamentally in Nigeria, contains alkaloids, flavonoids, tannins and phenolics with documented antioxidant and biostimulant potential (Firdous *et al.*, 2022), yet its agronomic use in cocoa nursery establishment remains undocumented (Firdous *et al.*, 2022). Given the documented risk of auxin-induced phytotoxicity at high concentrations, cocoa seedling responses to synthetic auxins and a botanical alternative require direct comparison under identical nursery conditions to inform evidence-based recommendations. Additionally, as Nigerian cocoa nurseries operate largely at smallholder scale with limited agrochemical access, a low-cost local extract matching synthetic hormone

performance offers direct practical value (Esan *et al.*, 2025). This study was therefore designed to address this gap. The specific objectives were to:

1. Evaluate and compare the effects of *Polyalthia longifolia* leaf extract, indole-3-acetic acid and a synthetic auxin, each applied at two rates, on the emergence and early growth of cocoa seedlings in the nursery.
2. Determine the treatment combination that produces the most vigorous seedlings, as measured by plant height, leaf number, stem diameter, leaf area and dry biomass accumulation.
3. Provide an evidence-based recommendation on whether *Polyalthia longifolia* leaf extract is a viable, lower-risk substitute for synthetic auxin hormones in cocoa nursery practice.

2. Methodology

2.1 Experimental Design, Hormone Treatments and Experimental Setup

The study was conducted at the screen house of the Cocoa Research Institute of Nigeria (CRIN), Ibadan, south-western Nigeria. A 2 × 3 factorial experiment (hormone source × application rate), plus an untreated control, was laid out in a completely randomised design with three replications. Hormone sources were indole-3-acetic acid and a synthetic auxin (each at 100 mg/L and 50 mg/L) and *Polyalthia longifolia* leaf extract (1 kg/3 L and 1 kg/5 L water), giving seven treatments: A100, A50, I100, I50, M5L, M3L and control (Cn). Before the experiment, soil samples were collected at a soil depth of 0–20 cm, air-dried and sieved through a 2 mm sieve. The soil samples were then analysed for physicochemical properties. Air-dried, sieved (2 mm) topsoil (0.8 kg) was placed in perforated polyethylene bags (12.5 × 25 cm), watered to field capacity and arranged on a raised platform. For the leaf extract, 1 kg of harvested *Polyalthia longifolia* leaves and young branches was soaked separately in 3 L and 5 L of water for 21 days, after which the plant material was removed and the extract collected. Freshly harvested F3 Amazon cocoa beans (18 per treatment) were soaked in the respective hormone or extract solution for 24 hours before sowing; control beans were sown untreated. One bean was sown per bag, with six seedlings per treatment per replicate (42 per replicate; 126 total).

2.2 Data Collection

Emergence was recorded at 14, 18, 22, 26 and 30 days after sowing (% of beans sown). Plant height, leaf number, stem diameter and leaf area were measured every four weeks from 4 to 12 weeks after sowing (WAS). At 12 WAS, seedlings were destructively sampled: taproot length and shoot and root dry weights (oven-dried at 70°C to constant weight) were recorded.

2.3 Statistical Analysis

Data were analysed by ANOVA using Minitab (version 17), with treatment means separated by Tukey's test at $p \leq 0.05$.

3. Results and Discussion

Table 1 shows the physicochemical properties of the soil before planting. The soil pH in water (7.13) indicates a neutral to slightly alkaline soil, which is within the acceptable range of 5.6-7.2 in which cocoa can grow. Cocoa is generally adapted to a relatively wide range of soil pH, although nutrient availability and root development are strongly influenced by soil reaction (Amoah-Antwi *et al.,* 2021). The observed pH therefore did not suggest a major acidity-related constraint to seedling establishment in the present study. The soil organic carbon content of 20.9 g/kg (2.09%) and organic matter content of 36.03 g/kg (3.60%), compared with critical values of 2.03% and >3 %, indicate a satisfactory organic matter status. Organic matter is important in maintaining soil aggregation, water retention, microbial activity and nutrient cycling, which are particularly relevant to nursery soils used for cocoa seedling production (Odoemelam *et al.,* 2023).

The total nitrogen (N) content of the soil was 1.63 g/kg (0.163%), which was above the critical threshold of 0.09%, suggesting that the soil had adequate nitrogen status before planting. Available phosphorus content of the soil was 19.30 mg kg^{-1}, which was above the critical value of 10 mg kg^{-1} reported for Nigerian soils, indicating that the soil had adequate available phosphorus before planting (Akinrinde & Obigbesan, 2000).

Exchangeable calcium was 4.65 cmol kg^{-1}, which was above the critical value of approximately 2.0 cmol kg^{-1} reported for Nigerian soils, indicating that calcium was unlikely to be a major nutritional constraint in the soil before planting (Akinrinde & Obigbesan, 2000). Exchangeable magnesium was 1.32 cmol/kg, which was above the stated reference value of 0.4 cmol/kg, indicating adequate magnesium status. The exchangeable sodium concentration was 0.53 cmol/kg; exchangeable potassium was 0.47 cmol kg^{-1}, which was above the critical range of 0.16–0.20 cmol kg^{-1} reported for Nigerian soils, indicating an adequate potassium status before planting (Akinrinde and Obigbesan, 2000). Exchangeable acidity (Al^{3+} + H^{+}) was very low at 0.05 cmol/kg, suggesting that aluminium toxicity was unlikely to be an important limitation under the initial soil conditions. The CEC (6.97 cmol/kg) and ECEC (7.02 cmol/kg) were relatively low considering the critical value of 10–12 cmol/kg, indicating poor nutrient holding capacity. This is consistent with the sandy texture and low clay content of the soil. Nutrient management may become increasingly important as the cocoa plants grow and their nutrient requirements increase. The percentage base saturation (99.29%) was very high, considering the critical value of > 50%, indicating that most of the effective cation exchange sites were occupied by basic cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}), with very little contribution from acidic cations. This is consistent with the very low exchangeable acidity (0.05 cmol/kg) observed in the soil.

The micronutrient status showed that manganese (Mn) concentration was 35.90 mg kg^{-1}, which was above the critical level of approximately 25 mg kg^{-1} reported for sedimentary soils of south-western Nigeria. This indicated an adequate to relatively high Mn status (Adeoye & Agboola, 1985). Iron (Fe) concentration was 17.60 mg kg^{-1}. This value was above the minimum level of approximately 5 mg kg^{-1} reported as adequate for soils in Nigerian studies, suggesting that Fe was unlikely to be deficient in the soil (Sillanpää, 1972). Copper (Cu) concentration was 0.84 mg kg^{-1}, which was below the critical range of 1.2–2.0 mg kg^{-1} reported for soils supporting cereal production in Nigeria (Agboola & Corey, 1973). In contrast, zinc (Zn) concentration was 8.30 mg kg^{-1}, which was above the critical range of approximately 3.0–3.45 mg kg^{-1} reported for Nigerian soils (Sobulo & Osiname, 1981). The result therefore indicated an adequate Zn status prior to planting.

The particle size analysis of the soil showed that the soil contained 892 g/kg (89.2 %) sand, 80 g/kg (8.0%) silt and 28 g/kg (2.8 %) clay. Based on these proportions, the soil was classified as sand. The predominantly sandy texture (89.2% sand) and very low clay content (2.8%) are consistent with the relatively low CEC of 6.97 cmol/kg. Coarse-textured soils generally have fewer surfaces for retaining exchangeable nutrients and may have lower water-holding capacity, making organic matter management particularly important for nursery production (Adegbite *et al.*, 2020; Amoah-Antwi *et al.,* 2021; Odoemelam *et al.,* 2023). The sandy texture has important implications for the establishment and nutrient management of cocoa. Although good drainage is desirable for cocoa because prolonged waterlogging can restrict root development, excessive sand content can reduce the soil's ability to retain water. Such soils generally have low water-holding capacity and may dry rapidly during periods of inadequate rainfall. The relatively good organic matter status may therefore be beneficial, because organic matter can contribute to soil aggregation, moisture retention and nutrient retention in coarse-textured soils.

Table 1. Physio-chemical properties of the soil before planting

Parameter	Values
Depth	(0-20 cm)
PH (1:2 H_2O)	7.13
Organic carbon (g/kg)	20.9
Organic matter (g/kg)	36.03
Nitrogen (g/kg)	1.63
Avail. Phosphorus (mg/kg)	19.30
Exchangeable Bases	
Calcium (cmol/kg)	4.65
Magnesium (cmol/kg)	1.32
Sodium (cmol/kg)	0.53
Potassium (cmol/kg)	0.47
Al^{3+}+H^{3+} (cmol/kg)	0.05
CEC (cmol/kg)	6.97
ECEC	7.02
%BS	99.29
Micro-nutrients status	
Manganese (mg/kg)	35.90
Iron (mg/kg)	17.60
Copper (mg/kg)	0.84
Zinc (mg/kg)	8.31
Particle size	
Sand (g/kg)	892
Silt (g/kg)	80
Clay (g/kg)	28
Textural class	Sand

Seedling Emergence

Seedling emergence was highest and complete (100%) in the untreated control at every assessment date, while synthetic auxin (A) significantly ($p \leq 0.05$) depressed emergence, falling to 11.1% at 14 days after sowing (DAS) and recovering to only 72.2% by 30 DAS. Suppression was concentration-independent, as A100 and A50 produced statistically similar depressed emergence, indicating a supra-optimal hormonal load regardless of rate. This aligns with the biphasic seed response to auxin, where physiological concentrations support reserve mobilization and radicle emergence, but supra-optimal levels trigger stress-induced ethylene and abscisic acid biosynthesis that suppresses germination (Bunsangiam *et al.*, 2021). Bunsangiam *et al.* (2021) similarly reported that crude IAA inhibited *Cyperus rotundus* germination at concentrations as low as 50 mg/L, underscoring the narrow promotive window for exogenous auxin.

Indole-3-acetic acid (I) and *Polyalthia longifolia* extract (M) produced emergence statistically comparable to the control from 22 DAS onward, with M3L matching 100% emergence by this date. This suggests that the natural phytohormone complement in *Polyalthia longifolia* extract, expected to include auxin, cytokinin and gibberellin-like compounds within a matrix of phenolics and micronutrients (Firdous *et al.*, 2022; Mashamaite *et al.*, 2022), supplied a more tolerable hormonal signal than synthetic auxin. Similar buffering has been reported for botanical extracts improving seed reserve mobilization and reducing oxidative stress in cocoa (Adu *et al.*, 2017; Chuquibala-Checan *et al.*, 2026) and other crops (Yadav *et al.*, 2024). These findings caution against undifferentiated synthetic auxin use and support *Polyalthia longifolia* extract, particularly at 1 kg/3 L, as a viable substitute.

Table 2. Effect of hormone source and application rate on percentage seedling emergence of cocoa (Theobroma cacao L.) at 14–30 days after sowing

Treatment	D14	D18	D22	D26	D30
A. Hormone Source					
Cn	100.00a	100.00a	100.00a	100.00a	100.00a
I	61.10a	88.89a	94.44a	94.44a	94.44a
A	11.11b	50.00b	55.56b	66.67b	72.23b
M	77.80a	88.89a	100.00a	100.00a	100.00a
Mean	62.50	81.95	87.50	90.80	91.67
B. Application Rate					
0 (control)	100.00a	100.00a	100.00a	100.00a	100.00a
100 mg/L	27.80b	66.70a	77.80a	83.34ab	83.34a
50 mg/L	44.40ab	72.20a	72.20a	77.78b	83.34a
1 kg/3 L	88.90a	100.00a	100.00a	100.00a	100.00a
1 kg/5 L	66.70ab	77.80a	100.00a	100.00a	100.00a
Mean	65.56	83.34	90.00	92.22	93.34
C. Interaction (Hormone × Rate)					
Cn0	100.00a	100.00a	100.00a	100.00a	100.00a
I100	44.40ab	88.90a	100.00a	100.00a	100.00a
I50	77.80a	88.90a	88.90a	88.90a	88.90ab
A100	11.10b	44.40c	55.60b	66.67b	66.67c
A50	11.10b	55.60bc	55.60b	66.67b	77.80bc
M3L	88.90a	100.00a	100.00a	100.00a	100.00a
M5L	66.70ab	77.80ab	100.00a	100.00a	100.00a
Mean	57.14	79.37	85.73	88.89	90.48

Note. Means within a column and factor grouping followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test. D14–D30 = days after sowing; Cn = untreated control; I = indole-3-acetic acid; A = synthetic auxin; M = Polyalthia longifolia (Masquerade tree) leaf extract; 100 mg/L and 50 mg/L = synthetic hormone rates; 1 kg/3 L and 1 kg/5 L = Polyalthia longifolia extract concentrations

3.1 Plant Height

Seedlings from synthetic auxin-treated beans (A) were significantly shorter than all other treatments throughout growth, with the deficit widening over time: by 12 weeks after sowing (WAS), auxin-treated seedlings (17.54 cm) reached less than 55% of control height (32.08 cm), with A100 (8.17 cm) being the shortest overall. This mirrors the emergence trend and aligns with reports that synthetic auxin analogues such as naphthaleneacetic acid and 2,4-D inhibit cereal shoot elongation through disrupted cell expansion and pigment accumulation (Lyubushkina *et al.*, 2025). The supra-optimal auxin load during the 24-hour soaking period appears to have produced lasting stress effects rather than a transient response.

Table 3. Effect of hormone source and application rate on plant height (cm) of cocoa seedlings at 4, 8 and 12 weeks after sowing

Treatment	W4 (cm)	W8 (cm)	W12 (cm)
A. Hormone Source			
Cn	20.25a	25.52a	32.08a
I	17.44a	22.19b	29.35a
A	12.30b	16.04c	17.54b
M	19.88a	24.35a	30.43a
Mean	17.47	22.03	27.35
B. Application Rate			
0 (control)	20.25a	25.51a	32.08a
100 mg/L	13.90c	18.43c	21.17a
50 mg/L	15.84bc	19.81bc	25.72a
1 kg/3 L	19.53ab	23.75ab	24.67a
1 kg/5 L	20.22a	24.95a	36.20a
Mean	17.95	22.49	27.97
C. Interaction (Hormone × Rate)			
Cn0	20.25a	25.52a	32.08ab
I100	16.40bc	21.52b	24.17ab
I50	18.48ab	22.87ab	34.53a
A100	11.40d	15.33c	8.17b
A50	13.20cd	16.75c	16.92b
M3L	19.53ab	23.75ab	24.67ab
M5L	20.21a	24.95a	36.20a
Mean	17.07	21.53	25.25

Note. Means within a column and factor grouping followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test. W4, W8, W12 = weeks after sowing; Cn = untreated control; I = indole-3-acetic acid; A = synthetic auxin; M = Polyalthia longifolia leaf extract

In contrast, *Polyalthia longifolia* extract (M) sustained height gains statistically similar to the control throughout, with M5L producing the tallest seedlings at 12 WAS (36.20 cm), exceeding the control by roughly 13%. Indole-3-acetic acid (I) recovered from an initial lag at 8 weeks (22.19 cm, below control) to parity by 12 weeks (29.35 cm), consistent with the biphasic dose response reported for auxins, where the growth outcome depends on the precise concentration reaching dividing tissue rather than hormone identity (Zhao *et al.*, 2025). These findings reinforce that the botanical extract supplied a gentler, more sustained growth stimulus than synthetic auxin, plausibly because its diffuse phytohormone and cofactor mixture buffers against the sharp dose spikes of single-compound synthetic formulations (Yuniati *et al.*, 2022).

3.2 Number of Leaves

Leaf production followed a pattern broadly consistent with the height data. At 4 weeks after sowing, synthetic auxin-treated seedlings produced significantly fewer leaves (3.0) than the control and other hormone sources, but by 12 weeks after sowing, the hormone-source main effect for leaf number was no longer statistically significant, suggesting a degree of compensatory recovery in leaf initiation once the initial hormonal stress had dissipated. Indole-3-acetic acid and *Polyalthia longifolia* extract, by contrast, promoted leaf production that matched or exceeded the control from the earliest assessment, with I50 (14.7 leaves) and M5L (13.17 leaves) recording the highest leaf numbers at 12 weeks after sowing among the interaction means. Because leaf number is directly linked to the photosynthetic surface area available to a young seedling, this early advantage for the IAA and *Polyalthia longifolia*-treated seedlings has implications for the accumulation of assimilates that support subsequent stem and root development, consistent with the general role of cytokinin-rich biostimulants in promoting cell division at shoot meristems (Mashamaite *et al.*, 2022).

Table 4. Effect of hormone source and application rate on number of leaves of cocoa seedlings at 4, 8 and 12 weeks after sowing

Treatment	W4	W8	W12
A. Hormone Source			
Cn	4.5a	6.5bc	9.2a
I	4.1a	7.8ab	11.3a
A	3.0b	6.3c	7.7a
M	4.1a	8.3a	10.8a
Mean	3.9	7.2	9.8
B. Application Rate			
0 (control)	4.5a	6.5a	9.1a
100 mg/L	3.1b	6.8a	8.3a
50 mg/L	4.0ab	7.3a	10.8a
1 kg/3 L	4.0ab	8.2a	8.5a
1 kg/5 L	4.2ab	8.3a	13.2a
Mean	4.0	7.4	10.0
C. Interaction (Hormone × Rate)			
Cn0	4.5a	6.5bc	9.2ab
I100	3.7ab	7.0abc	8.0b
I50	4.5a	8.5a	14.7a
A100	2.5b	6.5bc	8.7ab
A50	3.5ab	6.2c	6.8b
M3L	4.0a	8.2ab	8.5ab
M5L	4.2a	8.3ab	13.17ab
Mn	3.8	7.3	9.9

Note. Means within a column and factor grouping followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test. W4, W8, W12 = weeks after sowing; Cn = untreated control; I = indole-3-acetic acid; A = synthetic auxin; M = Polyalthia longifolia leaf extract

3.3 Stem Diameter

Stem diameter was the growth parameter least affected by hormone treatment, with no significant differences among hormone sources or application rates at 4 or 8 weeks after sowing. By 12 weeks after sowing, a significant difference emerged only between the control (4.58 mm) and the *Polyalthia longifolia* extract treatment (3.81 mm), with indole-3-acetic acid and synthetic auxin being intermediate and not significantly different from either. This relative insensitivity of radial stem growth, compared with the pronounced treatment effects observed for height, leaf number and, as shown below, leaf area, suggests that cambial activity and secondary thickening in cocoa seedlings are buffered against short-term hormonal perturbation at the seed stage, consistent with the general observation that girth expansion in juvenile cacao is a slower, more conservative growth process than height and canopy expansion (Baligar *et al.*, 2021).

3.4 Leaf Area

Leaf area showed the clearest treatment separation in the study. Synthetic auxin-treated seedlings had the smallest leaf area at every stage, most severely at 4 weeks after sowing (WAS) (7.41 cm², roughly a quarter of the control's 31.69 cm²), with A100 (4.28 cm²) over seven-fold lower than control. As leaf expansion is cell-expansion-driven and sensitive to auxin and gibberellin signalling, this is consistent with disrupted turgor-driven elongation reported under synthetic auxin stress in other species (Lyubushkina *et al.*, 2025). This depressive effect narrowed by 12 WAS (48.16 cm², approaching the control's 52.60 cm²), suggesting partial recovery as the seed-soaking treatment's influence declined.

Table 5. Effect of hormone source and application rate on stem diameter (mm) of cocoa seedlings at 4, 8 and 12 weeks after sowing

Treatment	W4 (mm)	W8 (mm)	W12 (mm)
A. Hormone Source			
Cn	2.98a	3.37a	4.58a
I	2.88a	3.46a	4.28ab
A	2.66a	3.19a	4.18ab
M	2.66a	3.27a	3.81b
Mean	2.80	3.32	4.21
B. Application Rate			
0 (control)	2.98a	3.37a	4.58a
100 mg/L	2.90a	3.35a	4.14a
50 mg/L	2.64a	3.30a	4.33a
1 kg/3 L	2.53a	3.05a	3.48b
1 kg/5 L	2.78a	3.49a	4.13ab
Mean	2.77	3.31	4.13
C. Interaction (Hormone × Rate)			
Cn0	2.98a	3.37a	4.58a
I100	2.98a	3.43a	4.25ab
I50	2.78a	3.48a	4.32a
A100	2.82a	3.27a	4.03ab
A50	2.50a	3.12a	4.33a
M3L	2.53a	3.05a	3.48b
M5L	2.78a	3.49a	4.14ab
Mean	2.77	3.32	4.16

Note. Means within a column and factor grouping followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test. W4, W8, W12 = weeks after sowing; Cn = untreated control; I = indole-3-acetic acid; A = synthetic auxin; M = Polyalthia longifolia leaf extract

Polyalthia longifolia extract (M) produced the largest leaf area throughout, reaching 83.65 cm² at 12 WAS, about 59% larger than the control and 74% larger than the synthetic auxin treatment, with M5L (85.89 cm²) the highest value recorded overall. This is consistent with the flavonoid- and phenolic-rich phytochemical profile of *Polyalthia longifolia* leaves alongside a natural hormone-like fraction (Firdous *et al.*, 2022), broadly analogous to *Moringa oleifera* extract, whose application has repeatedly enhanced leaf expansion and canopy development through combined cytokinin, gibberellin and micronutrient action (Mashamaite *et al.*, 2022; Yuniati *et al.*, 2022). This leaf-area advantage is agronomically significant, as greater leaf area at the nursery stage indicates greater light interception and is a recognized early marker of transplant-ready vigour in cocoa (Odoemelam *et al.*, 2023).

Table 6. Effect of hormone source and application rate on leaf area (cm²) of cocoa seedlings at 4, 8 and 12 weeks after sowing

Treatment	W4 (cm²)	W8 (cm²)	W12 (cm²)
A. Hormone Source			
Cn	31.69a	56.50a	52.60ab
I	31.45a	61.68a	57.90ab
A	7.41b	17.27b	48.16b
M	40.26a	63.97a	83.65a
Mean	27.70	49.86	60.58
B. Application Rate			
0 (control)	31.69a	56.50ab	52.60ab
100 mg/L	11.01b	27.72b	40.98b
50 mg/L	27.86a	51.20ab	65.04ab
1 kg/3 L	40.65a	65.63a	81.40a
1 kg/5 L	39.86a	62.32a	85.89a
Mean	30.21	52.67	65.18
C. Interaction (Hormone × Rate)			
Cn0	31.69b	56.50ab	52.60abc
I100	17.74c	45.06bc	47.30bc
I50	45.17a	78.29a	68.50abc
A100	4.28d	10.37d	34.70c
A50	10.55cd	24.11cd	61.61abc
M3L	40.65ab	65.63ab	81.40ab
M5L	39.86ab	62.32ab	85.89a
Mean	27.13	48.90	61.71

Note. Means within a column and factor grouping followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test. W4, W8, W12 = weeks after sowing; Cn = untreated control; I = indole-3-acetic acid; A = synthetic auxin; M = Polyalthia longifolia leaf extract

3.5 Dry Shoot Weight, Dry Root Weight and Taproot Length

Dry shoot weight mirrored the leaf area and height results, with *Polyalthia longifolia* extract (M) producing the highest shoot biomass (4.07 g), significantly greater than synthetic auxin (2.29 g), while the control and indole-3-acetic acid treatments were intermediate. At the interaction level, M3L (4.35 g) and I50 (4.18 g) were highest, while A100 (1.54 g) was lowest, a more than two-and-a-half-fold difference attributable to hormone source and rate. As shoot dry weight integrates height, leaf number and leaf area over the full growth period, it offers the clearest single index of seedling vigour, confirming that *Polyalthia longifolia* extract produced the most robust seedlings by 12 weeks after sowing.

In contrast, neither dry root weight nor taproot length differed significantly among hormone sources, rates, or their interaction. This insensitivity of root traits is notable, since auxin is classically linked to root initiation and elongation (Kubalóvá *et al.*, 2025). A plausible explanation is that the hormonal signal from a single 24-hour seed soak becomes progressively diluted and metabolised as the root system develops over 12 weeks, so root growth by destructive sampling is driven mainly by endogenous hormone production and substrate resources rather than residual exogenous treatment (Kubalóvá *et al.*, 2025). This shoot-root decoupling is consistent with auxin-gibberellin cross-talk mechanisms, where root elongation is buffered by internal feedback less reactive to a single hormonal pulse than actively expanding shoot tissue (Kubalóvá *et al.*, 2025). Practically, this indicates that pre-sowing treatment chiefly shapes shoot vigour rather than root architecture.

Table 7. Effect of hormone source and application rate on dry shoot weight (DSW), dry root weight (DRW) and taproot length (TRL) of cocoa seedlings at 12 weeks after sowing

Treatment	DSW (g)	DRW (g)	TRL (cm)
A. Hormone Source			
Cn	3.56ab	0.59a	13.47a
I	3.24ab	0.53a	14.85a
A	2.29b	0.42a	11.08a
M	4.07a	0.52a	12.63a
Mean	3.29	0.52	13.01
B. Application Rate			
0 (control)	3.56a	0.59a	13.47a
100 mg/L	1.92b	0.39a	11.08a
50 mg/L	3.61a	0.56a	14.85a
1 kg/3 L	4.35a	0.58a	11.97a
1 kg/5 L	3.79a	0.47a	13.30a
Mean	3.45	0.52	12.93
C. Interaction (Hormone × Rate)			
Cn0	3.56ab	0.59a	12.63a
I100	2.30bc	0.46a	11.40a
I50	4.18a	0.59a	10.80a
A100	1.54c	0.32a	11.70a
A50	3.04abc	0.52a	18.43a
M3L	4.35a	0.58a	11.10a
M5L	3.79ab	0.47a	14.53a
Mean	3.25	0.50	12.94

Note. Means within a column and factor grouping followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test. DSW = dry shoot weight; DRW = dry root weight; TRL = taproot length; Cn = untreated control; I = indole-3-acetic acid; A = synthetic auxin; M = Polyalthia longifolia leaf extract; destructive sampling was conducted at 12 weeks after sowing

4. Conclusion

The findings indicate that pre-sowing treatment influenced cocoa seedling emergence and several measures of early shoot development during the 12-week nursery period. Relative to the untreated control, the commercial synthetic auxin was associated with reduced emergence, shorter seedlings and smaller leaf area, particularly at the higher tested rate. IAA generally produced responses comparable to those of untreated seedlings, although individual responses differed across assessment times and application rates. Polyalthia longifolia leaf extract maintained high emergence and produced favourable shoot-growth responses. At 12 weeks after sowing, the extract treatment had the highest hormone-source mean leaf area (83.65 cm²) and dry shoot weight (4.07 g). Among the individual treatments, the 1 kg/5 L extract produced the greatest recorded plant height and leaf area, whereas the 1 kg/3 L extract produced the greatest dry shoot weight. Treatment differences in dry root weight and taproot length were not statistically significant. These findings support further evaluation of Polyalthia longifolia leaf extract as a nursery treatment for cocoa seedlings, while the observed responses should be interpreted within the specific rates and screen-house conditions studied.

5. Limitations

This study assessed one cocoa seedling type under screen-house conditions over 12 weeks. Accordingly, the results describe early nursery responses rather than post-transplant establishment or field performance. The assessment focused on the tested hormone sources and application rates, so the findings should not be extended to other concentrations or growing conditions without further study. Although differences in shoot growth and emergence were observed, the measurements reported here do not establish the

underlying physiological mechanisms. Further nursery and field evaluations would help determine the consistency of these treatment responses.

6. Recommendation

Nursery operators, especially smallholders, should adopt *Polyalthia longifolia* extract (1 kg/3 L water) over synthetic auxin, given its comparable or superior, lower-cost performance. Further research should characterise its phytohormone composition chromatographically, optimize application rate, assess field performance and test repeated or post-emergence applications to improve root development beyond nursery-stage results.

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Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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