Pelita Perkebunan 42(1) 2026, 13–24
DOI: ....

ISSN: 0215-0212 / e-ISSN: 2406-9574

Improvement of Cocoa Seedlings Growth by Botanical Ethno-Biostimulant Extract (BEBE) and NPK Fertilizer Application

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Received: December 19, 2025 / Accepted: April 14, 2026

Abstract

Cocoa (*Theobroma cacao* L.) is a strategic plantation commodity whose long-term productivity is strongly influenced by seedling quality during the nursery stage. However, cocoa seedling production is frequently constrained by suboptimal nutrient availability, pest and disease pressure, and unstable environmental conditions. This study aimed to evaluate the effects of Botanical Ethno-Biostimulant Extract (BEBE) and NPK fertilizer application on the growth performance of cocoa seedlings. The experiment was arranged in a factorial Completely Randomized Design (CRD) with two factors: BEBE at four levels and compound NPK fertilizer at three levels, resulting in 12 treatment combinations with three replications and three seedlings per replication (108 experimental units). Growth parameters observed included stem diameter, plant height, number of leaves, root traits, fresh biomass weight, and dry biomass weight. The results showed that BEBE and NPK fertilizer significantly affected stem diameter, plant height, fresh weight, and dry weight of cocoa seedlings. The combination of composite BEBE derived from cocoa water shoots, *Conyza sumatrensis*, *Cyperus rotundus*, *Cleome rutidosperma*, and *Ageratum conyzoides* applied at 4 mL L^{-1} with NPK fertilizer at 3 g $polybag^{-1}$ produced the highest vegetative growth and biomass accumulation. In contrast, higher NPK application rates (6 g $polybag^{-1}$) tended to reduce growth performance.

Keyword: Botanical-derived extract, fertilizer, NPK, cocoa, seedlings

INTRODUCTION

Cocoa (*Theobroma cacao L.*) is one of Indonesia's most important plantation crops, supporting rural employment, farmer livelihoods, agro-industrial development, and export revenue. With steady global demand for cocoa-based products, long-term productivity increasingly depends on the quality of planting material produced during the nursery stage. In many cocoa-producing areas, however, nursery performance is still limited by uneven seedling growth, pest and disease pressure, and nutrient supply that does not consistently meet seedling requirements. Seedlings that enter the field with low initial vigor often establish poorly, and this condition may affect plantation performance well beyond the early growth phase.

Although relatively short, the nursery phase represents a decisive period in the cocoa production cycle. Management practices at this stage largely determine seedling vigor, root development, and early adaptability after transplanting. There is therefore a practical need for supplementary inputs that can improve seedling quality without increasing production costs or management complexity. This need is particularly evident in smallholder-based cocoa systems, where the adoption of new practices is strongly influenced by affordability, ease of application, and compatibility with existing nursery routines. In this context, low-cost botanical nutrients inputs offer a realistic option for improving nursery outcomes within an integrated agricultural input management framework.

One approach that has emerged from local practice in Indonesia is the use of Botanical Ethno-Biostimulant Extract (BEBE). BEBE refers to a plant-based biostimulant prepared using ethnobotanical knowledge and simple farmer-led techniques. In this study, the term BEBE is used to position this practice within the scientific concept of plant biostimulants, while acknowledging its origin as a locally developed technology. BEBE is produced from fresh plant biomass using water and manual extraction, without fermentation, heating, or chemical additives. This approach is consistent with current definitions of plant biostimulants as materials that enhance plant physiological processes and nutrient-use efficiency independently of their direct nutrient contribution (du Jardin, 2015; Rouphael & Colla, 2020).

The extraction technique represents a defining feature of BEBE. In contrast to many commercial botanical biostimulants that rely on fermentation, thermal treatment, or solvent-based extraction, BEBE is obtained through low-intensity manual processing. Minimal processing has been reported to preserve labile bioactive compounds, including hormone-like substances and signaling molecules, which may be degraded during prolonged fermentation or exposure to high temperatures (Ertani *et al.*, 2013; Bulgari *et al.*, 2019). Moreover, the effectiveness of biostimulants does not depend on high concentrations of active compounds. Many act through signaling and priming mechanisms, whereby small quantities of bioactive molecules induce measurable physiological responses (Calvo *et al.*, 2014; Yakhin *et al.*, 2017). Biostimulants act primarily through signaling and priming mechanisms rather than direct nutrient supply (Ertani *et al.*, 2013; Yakhin *et al.*, 2017).

From a practical perspective, BEBE is also characterized by operational simplicity and reproducibility under nursery conditions. The absence of fermentation reduces variability associated with microbial activity, temperature fluctuations, and storage duration, factors that often cause inconsistency in fermented botanical extracts. As a result, BEBE can be prepared quickly, applied immediately, and integrated into routine nursery operations without specialized equipment.

The relevance of BEBE is further reinforced by the availability of raw materials within cocoa production systems. Cocoa plantations routinely generate biomass from water shoots (*chupons*), which are removed during pruning to maintain canopy structure and reduce internal competition. These young, actively growing tissues are generally discarded despite their potential richness in endogenous growth regulators. In addition, cocoa plantations in Indonesia host diverse weed communities adapted to disturbed soils and partial shade environments. Species such as *Ageratum conyzoides*, *Cyperus rotundus*, *Conyza sumatrensis*, *Cleome rutidosperma*, and *Acalypha indica* are commonly found in plantation understories and nursery surroundings, making them readily available sources of plant biomass.

Despite the potential benefits of biostimulants, seedling growth remains strongly dependent on adequate macronutrient supply. In Indonesian cocoa nurseries, compound NPK fertilizers—particularly the 16:16:16 formulation—are widely applied due to their balanced nutrient composition and ease of application. However, fertilizer efficiency is highly dependent on dosage, and excessive application may reduce growth performance through nutrient imbalance or phytotoxic effects (van Vliet & Giller, 2017; Wahyudi *et al*., 2023).

Taken together, the integration of BEBE derived from locally available plant biomass with compound NPK fertilization represents a practical approach to improving cocoa nursery management. However, empirical information on the combined effects of BEBE prepared from cocoa water shoots and dominant weed species, particularly when applied alongside compound NPK fertilizer, remains limited. Therefore, this study evaluates the effects of BEBE prepared from (i) cocoa water shoots (*Theobroma cacao* L.) and (ii) mixtures of locally available weed species, applied at 4 mL L^{-1}, in combination with compound NPK fertilizer (16:16:16), on the growth of cocoa seedlings under Indonesian nursery conditions.

MATERIALS AND METHODS

Study Site

The experiment was conducted at the experimental farm of the Indonesian Coffee and Cocoa Research Institute (ICCRI), located in Kaliwining Subdistrict, Jember Regency, East Java Province, Indonesia. The site was selected due to its well-established research facilities and environmental conditions suitable for cocoa nursery experiments. The study was carried out from August to December 2024, encompassing weed collection, preparation of Botanical Ethno-Biostimulant Extract (BEBE), seed germination, seedling establishment, and treatment application.

Experimental Design

Cocoa seedlings (*Theobroma cacao* L.) were grown in polybags under greenhouse conditions. The experiment was arranged in a factorial Completely Randomized Design (CRD) with two factors.

Factor I: Botanical Ethno-Biostimulant Extract (BEBE)

- P0: No BEBE application (control)
- P1: BEBE derived from cocoa water shoots (*Theobroma cacao L.*), applied at 4 mL L^{-1} (water shoot extract)
- P2: BEBE derived from cocoa water shoots (*Theobroma cacao* L.) combined with *Conyza sumatrensis*, *Cyperus rotundus*, *Cleome rutidosperma*, and *Ageratum conyzoides*, applied at 4 mL L^{-1} (water shoot + weed extract)
- P3: BEBE derived from *Conyza sumatrensis*, *Cyperus rotundus*, *Ageratum conyzoides*, *Acalypha indica*, and *Cleome rutidosperma*, applied at 4 mL L^{-1} (weed extract).

Factor II: Compound NPK Fertilizer (16:16:16)

- M0: 0 g $polybag^{-1}$
- M1: 3 g $polybag^{-1}$
- M2: 6 g $polybag^{-1}$

The factorial arrangement resulted in 12 treatment combinations, each replicated three times, with three seedlings per replicate, yielding a total of 108 experimental units.

Prior to the experiment, the greenhouse was cleaned and prepared to minimize environmental variability. Polybags were arranged systematically to ensure uniform spacing among experimental units. The growing media consisted of soil, sand, and cow manure mixed at a 1:1:1 ratio (w/w), with a total weight of 1 kg per polybag. Each polybag was labeled according to its assigned treatment.

Preparation of Botanical Ethno-Biostimulant Extract (BEBE)

Fresh and healthy plant materials corresponding to each treatment were collected and cleaned of adhering soil or debris. For each BEBE formulation, 250 g of fresh plant biomass was immersed in 5 L of non-mineral distilled water (aquadest). The plant material was then manually squeezed for 10–15 minutes to facilitate the release of cellular sap and bioactive compounds. Extraction was terminated when the solution exhibited a brownish coloration accompanied by slight foaming. The extract was subsequently filtered using a fine sieve and applied immediately, without fermentation, heating, or storage.

Seedling Establishment and Treatment Application

Cocoa seeds were sown in polybags and maintained until seedlings reached a uniform growth stage suitable for treatment application. BEBE was applied as a foliar spray at a concentration of 4 mL L^{-1} every two weeks, according to the assigned treatments.

Compound NPK fertilizer (16:16:16) was applied directly to the growing media at the designated dosages. All treatments were applied consistently across replicates following the experimental design.

Observed Variables and Data Analysis

Growth parameters observed included plant height, number of leaves, root length, root volume, fresh biomass weight, and dry biomass weight. Data were analyzed using analysis of variance (ANOVA) to evaluate the effects of BEBE, NPK fertilizer dosage, and their interaction. When significant differences were detected, treatment means were compared using Duncan's Multiple Range Test (DMRT).

RESULT AND DISCUSSION

The preparation of Botanical Ethno-Biostimulant Extract (BEBE) through manual squeezing of fresh green biomass in water resulted in extracts with distinct physical characteristics depending on the botanical materials used (Table 1). Qualitative indicators, including color intensity, aroma, foam formation, gas production, and viscosity, varied consistently among treatments and reflected differences in the composition and complexity of soluble plantderived compounds released during extraction.

The control treatment (P0), which contained no plant biomass, produced a clear and odorless solution without detectable foam, gas formation, or viscosity. This confirms that the physical characteristics observed in other treatments were attributable to compounds released from plant tissues rather than artifacts of the extraction medium.

BEBE prepared from cocoa water shoots alone (P1) showed low to moderate color intensity and aroma, accompanied by slight foam formation during extraction and moderate viscosity. Cocoa water shoots represent young, actively growing tissues characterized by high metabolic activity. Previous studies on cocoa physiology indicate that such tissues contain appreciable levels of endogenous phytohormones, particularly auxins and cytokinins, which regulate cell division, shoot elongation, and source–sink relationships (Wood & Lass, 2001; Taiz *et al.*, 2015). In addition, young cocoa shoots are known to contain soluble carbohydrates, free amino acids, organic acids, and phenolic compounds that are readily released during mechanical disruption (Aikpokpodion & Wilson, 2013; Niemenak *et al.*, 2006). The moderate viscosity observed in P1 therefore likely reflects the presence of dissolved polysaccharides and other low-molecular-weight organic compounds.

More pronounced physical characteristics were observed in BEBE formulations containing mixed plant biomass. Treatment P2, which combined cocoa water shoots with several weed species, exhibited increased color and aroma intensity and slightly higher viscosity compared to P1. This suggests a

Tabel 1. Qualitative physical characteristics of botanical ethno-biostimulant extract (BEBE)

Treatment	Color intensity	Aroma intensity	Foam formation	Gas formation	Viscosity
P0 (control)	–	–	–	–	–
P1 (water shoots)	+	+	+	–	++
P2 (water shoots + weeds)	++	++	+	–	++
P3 (weeds mixture extract)	+++	+++	+	–	+++

Notes: Symbols (+), (++), and (+++) indicate qualitative levels of intensity, representing low, moderate, and high intensity, respectively

higher concentration and broader diversity of dissolved organic compounds. Weed species commonly found in cocoa plantations, such as *Ageratum conyzoides*, *Cyperus rotundus*, and *Conyza sumatrensis*, have been reported to contain phenolic acids, flavonoids, terpenoids, and alkaloid-like substances that are partially water-soluble and biologically active at low concentrations (Xuan *et al*., 2005; Farooq *et al*., 2011; Oyesomi *et al*., 2018). The more complex herbal aroma observed in P2 is consistent with the release of such secondary metabolites.

The strongest physical expression was recorded in BEBE derived exclusively from mixed weed biomass (P3), which showed the highest color intensity, strongest herbal aroma, and greatest viscosity among all treatments. These characteristics reflect the chemical complexity of diverse weed assemblages. Aqueous extracts of mixed weed species have been shown to contain a wide range of secondary metabolites, including phenolics, flavonoids, saponins, and small peptides, which may function as signaling compounds rather than direct nutrient sources (Farooq *et al*., 2011; Bulgari *et al*., 2019).

Across all BEBE treatments, no gas formation was detected, and foam formation was limited to the extraction phase and disappeared after filtration. This confirms that the extraction process remained non-fermentative and stable. According to Sari *et al*. (2024), BEBE can be considered physically homogeneous when it shows no gas formation, limited foam, moderate viscosity, and a non-transparent color corresponding to the plant materials used. All BEBE formulations in this study met these criteria, indicating consistent physical quality across treatments. According to Sari *et al*. (2024), BEBE can be considered physically homogeneous when it shows no gas formation, limited foam formation, moderate viscosity, and a non-transparent color corresponding to the plant materials used.

From a functional perspective, BEBE is better interpreted as a biostimulant rather than a nutrient input. Plant-based biostimulants are widely recognized to act through physiological regulation, including modulation of hormonal balance, enhancement of antioxidant capacity, and priming of plant metabolism, rather than through direct nutrient supply (du Jardin, 2015; Calvo *et al*., 2014; Rouphael & Colla, 2020). Extracts derived from healthy weeds and cocoa water shoots may therefore contain hormone-like substances, phenolic compounds, and organic acids that can be absorbed through leaf surfaces and influence plant responses at low concentrations (Adiwijaya *et al*., 2023).

Although the effects of BEBE were not statistically significant for all growth parameters, the composite formulation represented by treatment P2 consistently tended to produce better seedling performance. This trend may be attributed to the broader spectrum of bioactive compounds present in mixed-species extracts, which may act synergistically to enhance physiological responses. Similar observations have been reported for composite botanical biostimulants derived from multiple plant species, which often show more stable and consistent effects than single-species extracts (Syafitri *et al*., 2024).

Growth Observation

Stem Diameter (mm)

Stem diameter is a fundamental indicator of early seedling vigor in cocoa (*Theobroma cacao* L.), as it reflects the development of vascular tissues, cambial activity, and the plant's capacity to support subsequent canopy growth. Physiologically, stem thickening is closely associated with cell division and expansion processes that depend on balanced water relations and nutrient availability.

At the early growth stage (9 weeks after planting, WAP), stem diameter did not differ markedly among treatments. This uniform response suggests that cocoa seedlings at this stage primarily relied on endogenous reserves and basal substrate fertility, while the physiological effects of BEBE application and NPK fertilization had not yet been fully expressed. Such delayed treatment responses are common in perennial crops, where early growth is often buffered by internal nutrient pools.

As seedlings progressed to 12 WAP, differences among treatments began to emerge. The P2M1 treatment exhibited the largest stem diameter, indicating that the combination of BEBE derived from cocoa water shoots and weeds with a moderate NPK dose provided favorable conditions for radial growth. At this stage, potassium supplied by NPK fertilizer likely contributed to improved osmotic regulation and cell turgor, thereby facilitating cell enlargement and stem thickening. Potassium plays an essential role in regulating cell turgor, water balance, and cambial activity, thereby supporting stem thickening and structural strength during early vegetative growth (Tirta *et al.*, 2025).

Treatment effects became more pronounced at 15 and 18 WAP. The P2M1 combination consistently produced the greatest stem diameter, whereas treatments receiving the highest NPK dose without BEBE support tended to show lower values. This pattern indicates that excessive mineral fertilization in the absence of bioactive stimulation may induce nutrient imbalance or mild physiological stress, ultimately constraining cambial activity and secondary growth. In contrast, the presence of BEBE appeared to enhance nutrient use efficiency, allowing seedlings to respond more effectively to moderate mineral inputs. These findings are consistent with previous reports indicating that the integration of inorganic fertilizers with natural elicitors is more effective in promoting stem growth than single-input approaches.

Table 2. Effect of BEBE and NPK fertilization on stem diameter of cocoa seedlings (mm)

Treatment	Stem diameter (mm)			
	9 WAP	12 WAP	15 WAP	18 WAP
P0M0	5.13 ab	7.37 b	9.43 b	11.13 ab
P1M0	5.17 ab	7.37 b	9.43 b	11.17 ab
P2M0	5.17 ab	7.40 b	9.43 b	11.30 ab
P3M0	5.17 ab	7.37 b	9.47 b	11.23 ab
P0M1	5.20 ab	7.40 b	9.47 b	11.30 ab
P1M1	5.20 ab	7.43 b	9.77 b	11.43 b
P2M1	5.23 b	7.53 b	10.80 c	12.57 c
P3M1	5.20 ab	7.43 b	9.77 b	11.50 b
P0M2	5.07 a	6.80 a	8.87 a	10.87 a
P1M2	5.10 ab	6.83 a	8.87 a	10.90 a
P2M2	5.13 ab	7.37 b	9.43 b	11.00 ab
P3M2	5.15 a	6.83 a	9.43 b	10.90 a

Notes: Means followed by the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$; WAP: Weeks after planting.

Plant Height (*cm*)

Plant height of cocoa seedlings exhibited a consistent increasing trend across all treatment combinations from 9 to 18 weeks after planting (WAP). At 9 WAP, plant height among treatments remained relatively uniform, ranging from approximately 28 to 29 cm, and most treatments did not show significant differences. At 12 WAP, differences among treatments began to emerge. The P2M1 treatment produced the greatest plant height, followed by P3M1, whereas several treatments without NPK application (M0) or with a high NPK dose (M2) remained within the range of approximately 37–40 cm.

Variation among treatments became more pronounced at 15 WAP. At this stage, the highest plant height was observed in treatments involving composite BEBE formulations (P2 and P3), particularly when combined with a moderate NPK dose (M1), with plant height exceeding 59–60 cm. In contrast, other treatments remained within the range of 56–58 cm. By 18 WAP, plant height increased further across all treatments, reaching a range of approximately 69–85 cm. The P2M1 and P3M1 treatments recorded the tallest plants, with heights of approximately 85.17 cm and 82.50 cm, respectively, while treatments such as P1M2 and P2M2 exhibited more moderate growth.

Overall, combinations of BEBE formulations P2 and P3 with a moderate NPK dose (M1) tended to produce superior plant height compared with other treatment combinations, although some treatments did not differ significantly. This response is likely associated with the role of nitrogen supplied at optimal NPK levels. Nitrogen is essential for protein, alkaloid, and chlorophyll synthesis, which promotes cell division and elongation and ultimately contributes to increased plant height (Arsensi *et al.,* 2022). However, excessive NPK application may disrupt the uptake of other nutrients and induce osmotic stress in plants (Prastia, 2022).

BEBE appears to play a protective role by enhancing plant tolerance to osmotic stress induced by high fertilizer concentrations. Secondary metabolites present in BEBE function as elicitors that stimulate plant defense systems and improve nutrient uptake efficiency, thereby supporting plant growth under less favorable conditions (Herman *et al.,* 2024).

Tabel 3. Effect of BEBE and NPK fertilization on plant height of cocoa seedlings (cm)

Treatment	Plant height (cm)			
	9 WAP	12 WAP	15 WAP	18 WAP
P0M0	28.53 a	39.27 ab	56.80 ab	77.52 b
P1M0	28.53 a	39.60 ab	57.60 bc	80.31 cd
P2M0	28.53 a	40.10 ab	57.20 ab	79.24 bc
P3M0	28.53 a	39.37 ab	56.80 ab	79.32 bc
P0M1	28.57 a	40.17 ab	58.30 c	78.48 bc
P1M1	28.60 ab	40.23 ab	58.33 c	80.31 cd
P2M1	29.13 b	44.73 c	60.30 d	85.17 e
P3M1	28.70 ab	41.40 b	59.77 d	82.50 d
P0M2	28.40 a	37.40 a	56.37 a	69.13 a
P1M2	28.50 a	39.13 ab	56.67 ab	69.47 a
P2M2	28.50 a	39.13 ab	56. ab	69.81 a

Notes: Means followed by the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$; WAP : Weeks after planting.

Number of Leaves

The number of leaves is a key determinant of photosynthetic capacity, as leaves represent the primary organs for light interception and assimilate production. Leaf development is highly sensitive to nitrogen availability and the plant's physiological status.

As shown in Table 4, the number of leaves of cocoa seedlings increased across all treatment combinations from 9 to 18 weeks after planting (WAP). At 9 WAP, leaf number ranged around 11 leaves and was relatively uniform among treatments, although the P2M1 treatment exhibited a slightly higher value compared with the others. At 12 WAP, differences among treatments became more apparent. The P2M1 treatment again produced the highest number of leaves (15 leaves), whereas several treatments, such as P0M2 and P2M2, remained within the range of 12–13 leaves. By 15 WAP, leaf number increased consistently across all treatments. The P2M0 treatment recorded one of the highest leaf numbers (18 leaves), followed by P1M1 and P3M1, which also reached approximately 18 leaves.

Treatments under the P2 biosaka formulation consistently produced higher leaf numbers than P0, P1, and P3 across different NPK levels. This trend indicates that BEBE formulated from a combination of cocoa water shoots and several weed species has the potential to significantly enhance vegetative growth of cocoa seedlings. According to Syafitri *et al.* (2024), the use of diverse biosaka materials plays an important role as a natural elicitor capable of stimulating vegetative growth, particularly leaf formation. Therefore, biosaka formulations derived from diverse plant sources can elicit positive responses in leaf development. In addition, nitrogen (N) supplied by NPK fertilizer plays a direct role in photosynthesis through its involvement in amino acid and protein synthesis as well as chlorophyll formation (Wahyudi *et al.*, 2023). Arsensi *et al.* (2022) reported that increased chlorophyll content enhances photosynthetic capacity, resulting in greater assimilate production and subsequent allocation to shoot and leaf development.

At 18 WAP, cocoa seedlings exhibited a further increase in leaf number. The P2M1 treatment achieved the highest leaf number (26 leaves), followed by P2M0 (25 leaves), while P1M0 and P3M1 recorded approximately 22–23 leaves. In contrast, several treatments receiving high NPK doses showed lower leaf numbers, such as P0M2 and P1M2, which produced only 18–19 leaves at 18 WAP. Treatments with high NPK rates (M2) generally resulted in fewer leaves than those receiving the moderate NPK dose (M1). Excessive fertilizer application beyond optimal levels may

Tabel 4. Effect of BEBE and NPK fertilization on leaf number of cocoa seedlings

Treatment	Number of leaves			
	9 WAP	12 WAP	15 WAP	18 WAP
P0M0	11.00 ab	12.68 a	16.33 b	20.33 ab
P1M0	11.33 ab	12.68 a	17.33 bc	22.67 bc
P2M0	11.33 ab	13.33 a	18.00 cd	25.00 cd
P3M0	11.33 ab	12.68 a	17.67 c	22.67 bc
P0M1	11.33 ab	14.33 b	17.33 bc	22.33 bc
P1M1	11.33 ab	14.67 b	18.00 cd	24.67 cd
P2M1	12.33 b	15.00 b	19.00 d	26.00 d
P3M1	11.33 ab	14.68 b	18.33 cd	23.67 cd
P0M2	10.67 a	12.33 a	14.33 a	18.67 a
P1M2	11.00 ab	12.33 a	15.00 a	19.33 a
P2M2	11.00 ab	13.00 a	15.00 a	19.33 a
P3M2	11.00 ab	12.68 a	15.00 a	20.67 ab

Notes: Means followed by the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$; WAP : Weeks after planting.

induce over-fertilization, which disrupts plant metabolism and ultimately suppresses vegetative growth (Farhan *et al*., 2024).

Root and Plant Biomass

The results showed that BEBE and NPK treatments induced significantly different responses in several root-related parameters. Root length and root volume are key indicators of the capacity of the root system to absorb water and nutrients from the growing medium. The P2M1 treatment produced the longest roots (35.17 cm), which was significantly higher than most other treatments. Root volume under the same treatment also reached the highest value (19.33 mL) and differed significantly from the other treatment combinations. In contrast, treatments receiving a high NPK dose (M2) generally exhibited shorter root lengths, ranging from 24 to 25 cm, with no significant differences among them.

The superior root development observed under the P2M1 treatment indicates that the combination of BEBE derived from cocoa water shoots (*Theobroma cacao* L.), *Conyza sumatrensis*, *Cyperus rotundus*, *Cleome rutidosperma*, and *Ageratum conyzoides* with a moderate NPK dose (3 g polybag^{-1}) created optimal conditions for root system development. According to Herman *et al*. (2024), BEBE application can enhance root growth through secondary metabolites that stimulate root cell elongation. In addition, nutrients supplied by NPK fertilizer play complementary roles in root development: nitrogen promotes cell division and elongation, phosphorus accelerates lateral root formation and meristematic tissue development, and potassium regulates cellular osmotic pressure and supports the translocation of photosynthates to the roots. Collectively, these nutrients improve root morphology in terms of both length and volume, thereby enhancing water and nutrient uptake efficiency (Zulkifli *et al*., 2022).

Significant differences were also observed in biomass-related parameters. The P2M1 treatment resulted in the highest fresh weight (92.57 g) and dry weight (29.67 g), both of which were significantly greater than those of most other treatments. In contrast, treatments receiving 6 g NPK generally exhibited lower biomass accumulation, with fresh weights of approximately 59–60 g and dry weights of 14–15 g, and showed no significant differences among treatments. Overall, the combination of BEBE P2 with a moderate NPK dose (M1) consistently produced superior results across all root and biomass parameters evaluated.

The increase in fresh and dry biomass under the P2M1 treatment may be attributed to the presence of bioactive compounds in BEBE, which enhance metabolic activity and improve photosynthetic efficiency. Consequently, BEBE application under P2 supported greater biomass accumulation. This finding is consistent with Andriani *et al.* (2025), who reported that BEBE application increased both fresh and dry biomass through enhanced photosynthetic activity. Fresh weight reflects the uptake and translocation of water and nutrients absorbed by the roots to the shoots, supported by the formation of an active and well-branched root system. High fresh weight indicates a positive plant response to nutrient availability, enabling optimal growth. Meanwhile, dry weight represents the accumulation of photosynthetic products converted into organic compounds such as carbohydrates, proteins, and structural cell components. The accumulation of these organic materials reflects true plant growth, as it is not influenced by tissue water content (Ahmad *et al*., 2016). This interpretation is further supported by Patti *et al*. (2018), who stated that increased nitrogen availability enhances protein synthesis and chlorophyll formation, thereby increasing photosynthetic rate and biomass accumulation.

The results showed that BEBE and NPK treatments induced significantly different responses in several root-related parameters. Root length and root volume are key indicators of the capacity of the root system to absorb water and nutrients from the growing medium. The P2M1 treatment produced the longest roots (35.17 cm), which was significantly higher than most other treatments. Root volume under the same treatment also reached the highest value (19.33 cm^3) and differed significantly from the other treatment combinations. In contrast, treatments receiving a high NPK dose (M2) generally exhibited shorter root lengths, ranging from 24 to 25 cm, with no significant differences among them.

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The increase in fresh and dry biomass under the P2M1 treatment may be attributed to the presence of bioactive compounds in BEBE, which enhance metabolic activity and improve photosynthetic efficiency. Consequently, BEBE application under P2 supported greater biomass accumulation. This finding is consistent with Andriani *et al.* (2025), who reported that BEBE application increased both fresh and dry biomass through enhanced photosynthetic activity. Fresh weight reflects the uptake and translocation of water and nutrients absorbed by the roots to the shoots, supported by the formation of an active and well-branched root system. High fresh weight indicates a positive plant response to nutrient availability, enabling optimal growth. Meanwhile, dry weight represents the accumulation of photosynthetic products converted into organic compounds such as carbohydrates, proteins, and structural cell components. The accumulation of these organic materials reflects true plant growth, as it is not influenced by tissue water content (Ahmad *et al.*, 2016). This interpretation is further supported by Patti *et al.* (2018), who stated that increased nitrogen availability enhances protein synthesis and chlorophyll formation, thereby increasing photosynthetic rate and biomass accumulation.

CONCLUSION

This study demonstrated that the application of BEBE and NPK fertilizer significantly

Tabel 5. Effect of BEBE and NPK fertilization on root length, root volume, and biomass accumulation of cocoa seedlings

Treatment	Root length (cm)	Root volume (cm^3)	Fresh weight biomass (g)	Dry weight biomass (g)
P0M0	28.87 abc	9.33 ab	78.87 b	19.10 b
P1M0	29.03 abc	12.67 bc	78.87 b	19.10 b
P2M0	29.23 abc	12.33 bc	79.10 b	19.43 b
P3M0	28.87 abc	12.33 bc	78.87 b	19.30 b
P0M1	34.50 cd	17.00 cd	87.63 c	20.20 b
P1M1	34.63 c	18.33 d	87.70 c	20.30 b
P2M1	35.17 c	19.33 d	92.57 d	29.67 d
P3M1	33.93 cd	18.67 d	92.10 d	28.07 c
P0M2	24.20 a	7.00 a	59.33 a	14.10 a
P1M2	24.20 a	6.00 a	59.33 a	14.17 a
P2M2	25.80 ab	8.00 ab	59.87 a	14.43 a
P3M2	25.83 ab	6.00 a	59.83 a	14.20 a

Note: Means followed by the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

influenced the growth performance of cocoa seedlings (*Theobroma cacao* L.). The combined application of BEBE and NPK had significant effects on stem diameter, plant height, fresh weight, and dry weight. The treatment combining composite BEBE formulation (derived from cocoa water shoots, *Conyza sumatrensis*, *Cyperus rotundus*, *Cleome rutidosperma*, and *Ageratum conyzoides*) applied at 4 mL L^{-1} with a moderate NPK dose of 3 g $polybag^{-1}$ (P2M1) consistently produced the highest vegetative growth and biomass accumulation compared with other treatments. In contrast, treatments receiving higher NPK rates (M2) tended to exhibit reduced growth responses.

Overall, the integration of BEBE with balanced NPK fertilization proved to be more effective in improving cocoa seedling quality than the use of either input alone. These findings highlight the importance of optimizing nutrient–biostimulant combinations to enhance nursery-stage growth and support sustainable cocoa seedling production.

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