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Physico-Chemical Characterization of Spent Coffee Grounds Compost and Its Effectiveness as a Sustainable Amendment for Improving Maize Seedling Vigor & Leaf Anatomical Development

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ABSTRACT

Spent coffee grounds (SCG) represent a growing waste stream with potential for agricultural valorization, yet their direct soil application is limited by phytotoxic compounds. This study evaluated the physico-chemical properties and agronomic effectiveness of SCG compost enriched with biochar and agricultural residues. Four formulations were composted over 91 days: SCG (Heap 1), SCG with rice husk biochar (Heap 2), SCG with rice husk biochar and empty fruit bunches (Heap 3), and SCG with palm kernel shell biochar (Heap 4). Temperature, pH, weight loss, lead (Pb) concentration, germination index (GI), root elongation, and leaf cell wall thickness of maize (Zea mays L.) were assessed. Heap 3 exhibited the highest thermophilic activity ($p = 0.0029$ vs. Heap 1) and GI (95.3%). Biochar-amended treatments stabilized pH near neutrality, whereas SCG alone remained acidic. Linear regression confirmed significant weight reduction across all treatments ($p < 0.001$), with biochar enhancing mass retention. Lead (Pb) concentrations declined to undetectable levels by day 91 in all heaps. Root elongation was significantly greater in biochar-amended composts (6.46–7.50 cm) compared to SCG alone (4.48 cm; $p < 0.001$). Transmission electron microscopy revealed that Heap 4 significantly increased maize leaf cell wall thickness relative to the control (mean difference = 0.201 µm, $p = 0.001$). These findings demonstrate that biochar-enriched SCG composting produces mature, non-phytotoxic amendments that enhance maize seedling vigor and leaf structural development.

Keywords: **Biochar, spent coffee grounds, palm kernel shell biochar, empty fruit bunches**

INTRODUCTION

The escalation of global coffee consumption in recent years has contributed to the production of vast quantities of organic waste, particularly spent coffee grounds (SCG) (Becze *et al.*, 2024; Kumar *et al.*, 2025). In 2023, worldwide coffee waste was estimated at 6 to 8 million tons annually, with Asia responsible for approximately 1.43 million tons due to its consumption of 22% of the world's coffee (Yusufoğlu *et al.*, 2024; Tsigkou *et al.*, 2025). The industry generates roughly 650 kg of discarded SCG for every ton of processed coffee beans, signifying a substantial burden on existing waste management systems (Bevilacqua *et al.*, 2023; Chavhan *et al.*, 2024).

This continuous increase in coffee consumption, propelled by shifting consumer lifestyles, the trend toward specialty coffee, and expanding markets in both developed and emerging economies, has exacerbated SCG generation since 2020 (Czekała *et al.,* 2023; Lachenmeier *et al.,* 2024). In Malaysia, the coffee sector has experienced significant growth, driven by rising cafe culture and consumer demand for distinctive coffee experiences (Zakaria and Mohd, 2024; Ellisha and Ami, 2024).

The projected number of branded coffee outlets is expected to exceed 3,330 by 2025, marking a 28% increase from 2024, alongside a forecasted 1.4% market expansion through 2029, which will likely amplify food and coffee waste production (Kaur *et al.,* 2025). Local observations, such as a seven-day study in Sungai Petani, Kedah, reported the generation of 93.04 kg of food waste from cafés, potentially including SCG, highlighting the tangible environmental consequences related to inadequate waste management practices (Mohd-Iszairi *et al*., 2023; Kohli *et al*., 2024).

Although SCG is known to be a massive contributor of organic waste, it cannot be directly used in agriculture because it contains compounds like caffeine that are phytotoxic and may adversely impact the health of plants and soil (Marín-Tello *et al.,* 2020; Rahim *et al.,* 2025). Improper disposal of SCG has been shown to cause greenhouse gas emissions, soil and water contamination, and offensive odors, while the persistence of phytotoxic compounds such as caffeine, tannins, and polyphenols further inhibits seed germination, disrupts soil microbial dynamics, and promotes soil acidification (Kueh, 2021; Franca *et al.,* 2022; Pérez-Burillo *et al.,* 2022).

A variety of management strategies have been suggested for SCG, including its utilization as a raw material for manufacturing, incorporation into food and cosmetic products, conversion to biofuel, and its use in composting and biochar production (Gebreeyessus, 2022; Ahmed *et al.,* 2024; Elezović *et al.,* 2025). Among these, composting and biochar synthesis offer the most promising solutions for sustainable waste valorization, as their end products can be reapplied in agricultural contexts, thereby mitigating environmental harm (Marín-Tello *et al.,* 2020; Gamaralalage *et al.,* 2025).

Composting SCG has demonstrated improved seed germination and root development compared to direct field application, although drawbacks such as stunted plant growth, disrupted microbial communities, and reduced root-to-shoot ratios in some crops have also been reported (Picca *et al*., 2023; Ozer *et al*., 2024; Sinclair *et al.,* 2024). The integration of biochar into the composting process enhances the sustainability and efficacy of SCG management by promoting soil health, accelerating compost maturation, and improving compost quality through pathogen suppression and support of beneficial microbial taxa (Medina *et al*., 2021; Karam *et al*., 2022; Murtaza *et al.,* 2023). Adoption of these practices within broader technological and policy frameworks can advance the circular economy and food waste management in Malaysia (Abdul-Rahman *et al.,* 2020).

Specifically, biochar-enriched SCG composts have been noted to facilitate humification, alleviate phytotoxicity, increase phosphate and potassium concentrations, optimize nitrogen retention, and elevate chlorophyll content, resulting in more favorable crop performance (Ozer *et al*., 2024; Abban-Baidoo *et al.,* 2024). SCG composting also enhances microbial activity and produces bioactive peptides, thus supporting sustainable soil amendment practices and plant nutrition (Zhang *et al*., 2023; Xu *et al.,* 2023). Nonetheless, the combined effects of SCG and biochar in composting applications, particularly regarding maize growth performance, remain underexplored in current research. This study seeks to address this gap by investigating the agronomic potential of SCG-biochar compost, focusing on improvements in soil fertility, plant physiological characteristics, nutrient uptake, and anatomical structure within maize cultivation systems.

MATERIALS AND METHODS

The spent coffee grounds were sourced from a local establishment in Bandar Baru Bangi, Malaysia, to serve as a representative model for urban lignocellulosic waste. Following collection, the SCG were homogenized to minimize batch variability. The selection of this specific waste stream aligns with sustainable waste valorization strategies, aiming to transform high-volume urban residues into value-added products (Yusof *et al.,* 2019; Amato-Lourenco *et al.,* 2024).

Three primary organic amendments were utilized in this study including rice husk biochar (RHB), palm kernel shell biochar (PKSB), and oil palm empty fruit bunches (EFB). The RHB was sourced from Sendi Enterprise (Tanjung Karang, Selangor), while the PKSB was supplied by Vata VM Synergy (M) Sdn Bhd. The EFB was supplied by the Fermanagh Oil Palm Mill. To ensure a uniform substrate matrix, all materials underwent an initial air-drying process to reach a constant moisture. Following dehydration, the materials were mechanically homogenized. This preparation protocol was critical to maintaining consistent physicochemical properties across all experimental composting treatments (Zainudin *et al.,* 2020; Alarefee *et al.,* 2021).

All composting materials were weighed to 100 kg per treatment heap, with initial moisture levels adjusted to approximately 60% using rainwater (Sokaˇc *et al.,* 2022; Mageshwaran *et al.,* 2024). Standard laboratory equipment, such as pH meters, temperature probes, and analytical balances, were used for physico-chemical characterization throughout the experiment, whilst chemical reagents were used for nutrient analysis and heavy metal quantification. This methodological approach ensured the reproducibility and reliability of all substrate preparation and analytical procedures (Kazeem *et al.,* 2024).

Composting process

Composting was performed via a fast-composting method under all-weather sheltered conditions. The exclusion of rainfall through this sheltered approach ensured the integrity of the moisture-to-solids ratio and prevented uncontrolled water saturation, thereby maintaining these sheltered parameters, the study ensured consistent microbial activity and temperature profiles across all experimental treatments throughout the decomposition period (Zainudin *et al.,* 2022). Four distinct treatment heaps, each with a total mass of 100 kg, were prepared using specific substrate combinations as follows: (i) spent coffee grounds (SCG) as a control; (ii) SCG amended with rice husk biochar; (iii) SCG integrated with rice husk biochar and empty fruit bunches; and (iv) SCG combined with palm kernel shell biochar. The precise mixing ratios and compositions for each formulation are summarized in **Table 1**.

Table 1: Composition and ratios of materials in experimental spent coffee ground compost treatments

Treatment heap	Material	Ratio	Heap initial weight (kg)
Heap 1 (H1)	SCG only	1	
Heap 2 (H2)	SCG + RHB	9:1	
Heap 3 (H3)	SCG + RHB +dried shredded EFB	8:1:1	100
Heap 4 (H4)	SCG + PKSB	9:1	

Note: SCG, spent coffee grounds; RHB, rice husk biochar; EFB, empty fruit bunches; PKSB, palm kernel shell biochar

The composting materials were arranged in a series of successive layers to a total vertical height of 100 cm. This systematic configuration was designed to optimize the physical architecture of the compost pile, ensuring enhanced decomposition through improved aeration and heat retention within the 100 kg substrate matrix (Morad *et al.,* 2008). Each layer thoroughly mixed every two to three layers using a pitchfork to ensure uniform distribution, and additionally, a 2.5 cm layer of soil was incorporated to introduce soil microorganisms, thereby fostering efficient microbial activity and accelerating organic matter breakdown (Trinklein, 2022; Aguilar-Paredes *et al*., 2023). All compost heaps were turned at seven-day intervals to maintain adequate aeration, ensure uniform distribution of materials, and stimulate microbial activity (Zainudin *et al.,* 2020; Manga *et al*., 2023).

Assessment of compost weight reduction was performed by recording the initial mass of each compost heap at the commencement of the experiment and subsequently measuring the heaps at regular seven-day intervals throughout the 91-day composting period. All mass determinations were conducted using an analytical balance. The percentage reduction in compost mass was calculated by comparing the final weight to the initial weight, thus enabling quantitative evaluation of organic matter decomposition and moisture loss for each compost formulation (Díaz *et al.,* 2021). This systematic protocol facilitated consistent monitoring of mass loss and provided dependable data for subsequent analysis of compost stability and maturity.

Physico-chemical analyses

Temperature measurements were conducted by inserting a temperature meter probe into the core of each composting pile. During every turning of the compost, 10 g samples were collected, with one representative composite sample obtained from randomized locations, primarily from the middle and bottom sections of the pile (Medina *et al*., 2021; Zainudin *et al.,* 2022). All analyses were performed on a dry basis, with each sample measured in triplicate to ensure reliability. Lead (Pb) tracing in compost was conducted using Atomic Absorption Spectroscopy (AAS), comprising sample preparation, digestion, and analytical detection steps to quantify Pb concentrations within the compost matrix (Indhumathy and Muthulakshmi, 2024). All experimental procedures adhered to the relevant guidelines and regulations.

Compost maturity and phytotoxicity testing

Compost maturity and phytotoxicity evaluations were performed using a maize (*Zea mays* L.) seed germination bioassay (Zahrim *et al.,* 2021; Manaf *et al.,* 2024). For the germination assay, 100 seeds per treatment were distributed across three replicates (10 seeds per container) on autoclaved cotton pads. Following the methodology of Oktiawan *et al.* (2018), compost extracts were prepared at a 1:10 (w/v) ratio with distilled water, shaken, and filtered. Seeds were moistened with these extracts, while distilled water served as the negative control. Each petri dish was sealed with Parafilm to minimize moisture loss while permitting air exchange, and incubated in a dark location to facilitate seed germination (Simol et al., 2022; Taha *et al*., 2024).

After 72 hours, seed germination was recorded for all treatments and controls, root and shoot lengths were measured using a ruler and calliper (Oktiawan *et al.,* 2018; Angyu and Lanki, 2025). Root lengths for all germinated seeds varied within each replicate, were documented and mean root elongation values were calculated for each treatment (Zahrim *et al.,* 2021; Nozari *et al.,* 2021). The maturity and safety of compost are determined by its Germination Index (GI), which serves as a standardized measure for seedling establishment (Saypariya *et al.,* 2024). A GI above 80% indicates that the compost is mature and non-phytotoxic, making it safe for agricultural use. In contrast, values below 50% suggest that the compost is still immature and contains phytotoxic substances that could harm young plants (Yang *et al.,* 2021; Saypariya *et al.,* 2024). To evaluate these levels, the GI is calculated by comparing the germination percentage and root length of a treated sample against a control group using the following formula:

$$GI\ (\%) = \left[\left(\frac{\text{number of seeds germinated in compost extract}}{\text{number germinated in control}}\right) \times \left(\frac{\text{average root length in compost extract}}{\text{average root length in control}}\right)\right] \times 100.$$

To ensure statistical reliability and minimize experimental bias, each treatment replicate utilized a cohort of approximately 100 seeds, with root length serving as a critical indicator of compost maturity and the degradation of low-molecular-weight organic acids (Kong *et al*., 2022; Kong *et al*., 2023).

Plant growth experiment

Following Alarefee *et al*. (2021), a greenhouse pot experiment was conducted to evaluate the impact of organic amendments on the growth performance of sweet maize (*Zea mays* L.). The study utilized Munchong Series topsoil collected from Field 10, Universiti Putra Malaysia and placed in a 20 kg polybag, and Leckat Sugar Rich 592 (F1 Hybrid), a selected maize variety for the growth performance. The experiment consisted of five groups: a control (unamended topsoil) and four treatments amended with compost (H1, H2, H3, and H4) (**Table 1**). Each treatment was replicated five times using a randomized complete block design (RCBD). After germination, plants were thinned to one seedling per pot.

Maize growth was monitored for 50 days until the early tasselling stage to evaluate performance under controlled conditions. Following the growth stages outlined in **Table 2**, irrigation and fertilization were applied (Alarefee *et al*., 2021; Reeza *et al.,* 2023). Compost amendments were incorporated at a 20% (w/w) ratio by thoroughly mixing with the soil prior to potting (Abdullah *et al.,* 2022; Xu *et al.,* 2023). Visual health assessments of the maize were conducted weekly to monitor physiological responses

Table 2: Irrigation and basal fertilization (NPK 15-15-15) schedules based on maize developmental stages

Stage/Week	Irrigation (per day)	Fertilization (NPK-15-15-15)
Weeks 1-2	200 mL	5 g / plant / week
Weeks 3-4	400 mL	10 g / plant / week
Weeks 5	400 mL	10 g / plant / week
Week 6+ (Flowering)	600-700 mL	15 g / plant / week

Histological analyses

Fresh, fully expanded maize leaves without signs of damage or disease were sampled from each treatment group. A small section was excised from the midpoint of each leaf blade, avoiding edges and tips for consistency. Samples were dipped in 3% glutaraldehyde and 1% osmium tetroxide mixture to preserve tissue structure, followed by dehydration and resin embedding. Ultramicrotomy was used to prepare ultra-thin sections (~100 nm), which were subsequently stained with heavy metals and mounted on grids for Transmission Electron Microscopy (TEM) observation. The epidermal cell wall thickness was measured from TEM images, with mean values calculated for each treatment to assess the impact of compost amendments (Xu *et al*., 2025).

Statistical analyses

All statistical analyses were conducted using R software (version 4.5.1). Data normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. One-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$) was used to compare compost temperature, pH, germination index, root elongation, and leaf cell wall thickness among treatments. The Kruskal–Wallis test was applied where normality assumptions were not met. Simple linear regression was used to model compost weight loss over the 91-day period, with residual normality verified via the Shapiro–Wilk test. Results are expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Compost temperature

Figure 1 illustrates the temporal temperature fluctuations of the compost heaps over the 91-day monitoring period. Heap 3 which a mixture of spent coffee grounds, rice husk biochar, and EFB, exhibited superior thermal performance, particularly during the thermophilic phase (between 41–70°C). This pronounced thermal response indicates intensified microbial metabolic activity and optimized aeration, which collectively accelerated organic matter degradation and shortened the duration to reach compost maturity relative to other treatments (Mohammed *et al.,* 2025). Statistical analysis via one-way ANOVA confirmed that these temperature variations between heaps were significant ($p = 0.0033$).

Tukey's HSD post hoc test indicated that this significant difference was specifically between Heap 1 and Heap 3 ($p = 0.0029$). Heap 3 which comprising spent coffee grounds, rice husk biochar, and dried shredded empty fruit bunches (EFB), displayed the highest average temperatures, indicating a more pronounced thermophilic phase. This enhanced thermal performance is likely due to improved compost structure, with optimal texture and reduced compaction supporting greater microbial activity and heat production (Zahra *et al.,* 2023; Abban-Baidoo *et al.,* 2024; Mussa *et al.,* 2025).

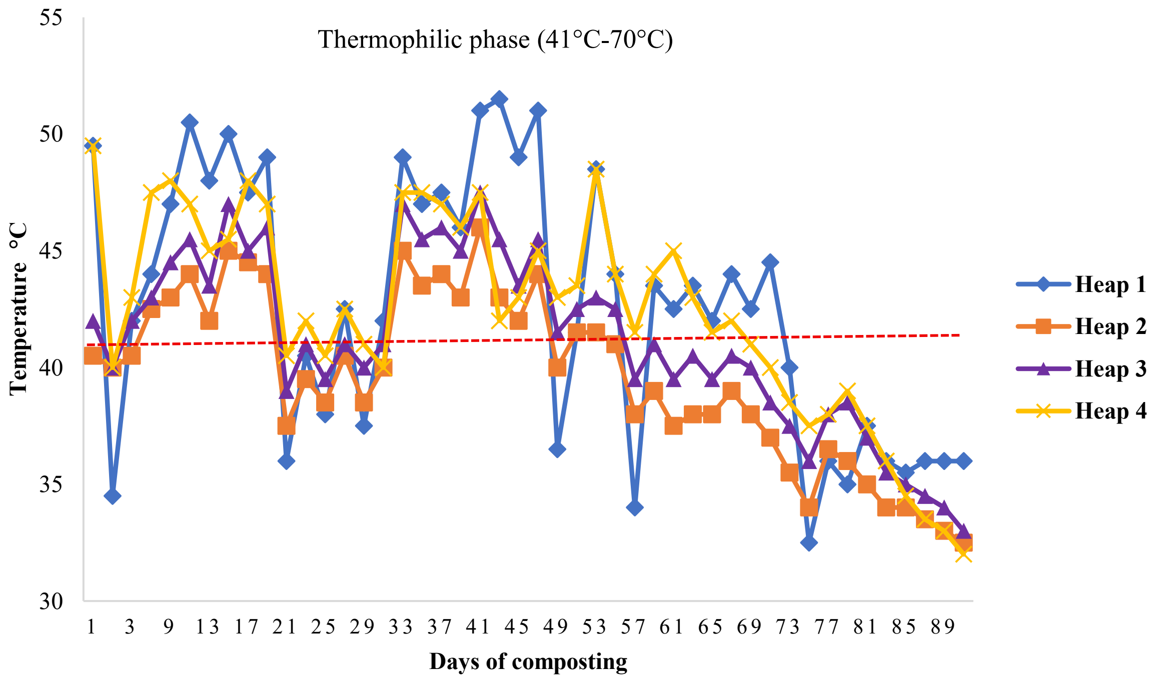

Figure 1: Temperature changes over time for each compost heap during the 91-day composting period
Note: The horizontal dashed line indicates the thermophilic onset threshold for compost treatments monitored over 91 days: Heap 1 (spent coffee grounds), Heap 2 (spent coffee grounds + rice husk biochar), Heap 3 (spent coffee grounds + rice husk biochar + dried shredded empty fruit bunches), and Heap 4 (spent coffee grounds + palm kernel shell biochar).

Physico-chemical changes characteristic of composting, such as a reduced carbon-to-nitrogen (C/N) ratio, sustained elevated temperatures (typically reaching 50–60°C for several weeks), and increased concentrations of nitrogen and micronutrients, have been reported in empty fruit bunch (EFB) compost piles and are crucial for efficient organic matter decomposition (Krishnan *et al.,* 2017; Zainudin *et al.,* 2022). The process is further accelerated by the regulation of moisture, strategic turning frequency, and adequate aeration, all of which stimulate microbial metabolism and promote greater heat production, thereby facilitating the transformation of EFB into mature compost (Alkarimiah and Suja, 2020; Peng *et al.,* 2023). In this study, the higher temperature levels and enhanced decomposition observed in Heap 3 were also likely attributable to the active presence of thermophilic and cellulolytic bacteria, which play a key role in degrading the lignocellulosic structure of EFB (Zainudin *et al.,* 2022; Saypariya *et al.,* 2024).

Substrate formulations incorporating EFB and biochar significantly modulate temperature dynamics and catalyze microbial kinetics within composting systems (Wu *et al*., 2020; Zahari *et al.,* 2023). The integration of EFB promotes the proliferation of thermophilic microbiota capable of degrading recalcitrant lignocellulosic structures, which accelerates carbon and nitrogen mineralization and facilitates prolonged thermophilic phases (Alkarimiah and Suja, 2020; López *et al*., 2021). This synergistic effect optimizes organic matter decomposition and reduces the composting time, thereby fulfilling agricultural requirements for the timely application of stabilized soil amendments (Tahir *et al*., 2022; Luo *et al.,* 2025).

Compost pH

The pH values across all compost treatments exhibited a significant initial increase, a trend primarily attributed to the accelerated mineralization of organic matter and the subsequent release of ammonia through microbial ammonification (**Figure 2**). Heap 4 attained the maximum alkalinity, peaking at pH 8.40 by day 56, followed by a gradual decline as nitrification potentially commenced. Conversely, Heap 2 maintained sustained alkaline conditions (pH > 7.0) for the majority of the study period. While Heap 3 displayed moderate alkalinity, Heap 1 remained consistently below the neutral threshold (pH < 7.0), representing the lowest pH values throughout the composting process.

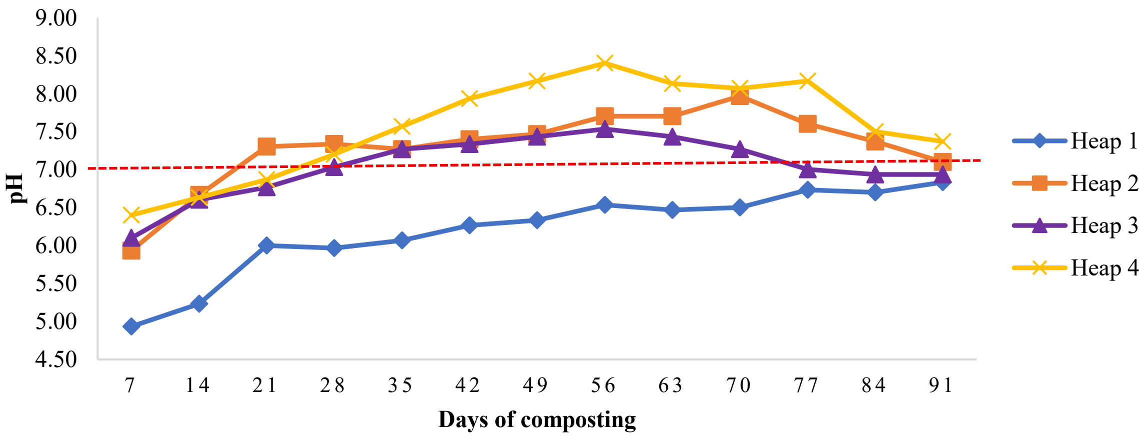

Figure 2: Trend of pH over time for each compost heap during the 91-day composting period

Note: The horizontal dashed line indicates threshold for the onset of acidic phase (pH 7) for compost treatments monitored over 91 days: Heap 1 (spent coffee grounds), Heap 2 (spent coffee grounds + rice husk biochar), Heap 3 (spent coffee grounds + rice husk biochar + dried shredded empty fruit bunches), and Heap 4 (spent coffee grounds + palm kernel shell biochar).

A one-way analysis of variance (ANOVA) revealed a statistically significant difference in mean pH across the four compost heaps, F (3, 48) = 15.62, $p < 0.001$. Tukey's HSD post hoc analysis demonstrated Heap 1 (spent coffee grounds only) had significantly lower mean pH compared to Heap 2 ($p < 0.001$), Heap 3 ($p = 0.0012$), and Heap 4 ($p < 0.001$), and no significant differences were observed among Heaps 2, 3, and 4 ($p > 0.05$). These temporal pH changes reflect essential processes such as ammonia production, organic acid degradation, and microbial succession, which are pivotal for compost maturation (Ghinea and Leahu, 2020; Aji *et al*., 2021).

The distinct pH profiles observed among the compost heaps underscore the considerable impact of substrate composition and amendments, particularly the inclusion of biochar and EFB, on the chemical setting within the compost. These factors support effective decomposition and stabilization (Cooper *et al.,* 2020; Bello *et al.,* 2026). The consistently more acidic environment of Heap 1, attributable to the natural acidity of coffee residues likely resulted in slower microbial activity and delayed compost maturation (Vela-Cano *et al.,* 2019; Devi *et al*., 2020). Meanwhile, the remaining treatments attained near-neutral pH by day 91, aligning with optimal parameters for maize productivity and soil microbial vitality while underscoring the critical role of substrate optimization in modulating compost chemistry to meet specific agronomic and pedological requirements (Tandzi *et al*., 2018; Sharma *et al.,* 2025).

Compost weight

All four composting treatments commenced at 100% of their initial weight and exhibited a progressive decline in mass over the 91-day composting period. The magnitude and rate of weight loss differed substantially among treatments (**Figure 3**), reflecting the influence of biochar type and co-amendment on organic matter decomposition dynamics (Moghimi *et al*., 2023).

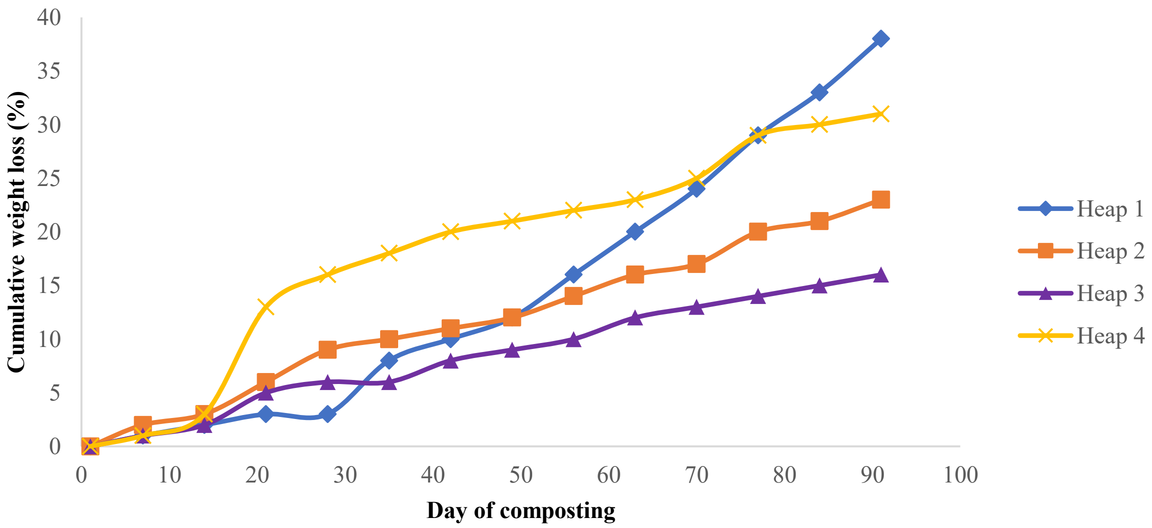

Figure 3: Cumulative weight loss (%) of four compost heaps over a 91-day composting period

Note: Heap 1 (spent coffee grounds), Heap 2 (spent coffee grounds + rice husk biochar), Heap 3 (spent coffee grounds + rice husk biochar + dried shredded empty fruit bunches), and Heap 4 (spent coffee grounds + palm kernel shell biochar) are different compost treatment groups monitored over the 91-day period

The control treatment (Heap 1: SCG only) exhibited the greatest total weight loss at 38% (62% residual mass), with a mean weight retention of 85.79 ± 12.74% and a coefficient of variation (CV) of 14.85%, indicating substantial mass fluctuations during composting. In contrast, biochar-amended treatments demonstrated enhanced mass retention, following a total weight loss hierarchy of Heap 4 (PKSB; 31%, mean = 82.00 ± 10.39%, CV = 12.67%) > Heap 2 (RHB; 23%, mean = 88.29 ± 7.26%, CV = 8.22%) > Heap 3 (RHB + EFB; 16%, mean = 91.64 ± 5.26%, CV = 5.74%). These results indicate that while the SCG control decomposed most rapidly, the integration of biochar and lignocellulosic amendments significantly stabilized compost mass over the 91-day period (Moghimi *et al.,* 2023).

This observation aligns with existing literature reporting that biochar incorporation stabilizes organic matter through its recalcitrant carbon structure, thereby reducing microbial decomposition rates (Alkarimiah and Suja, 2020; Gross *et al.,* 2024). Specifically, the combination of rice husk biochar (RHB) with lignocellulosic empty fruit bunch (EFB) in Heap 3 was the most effective for mass retention, suggesting a synergistic effect between biochar and fibrous co-amendments in moderating decomposition (Wu *et al*., 2020). Biochar enhances carbon stabilization through both its intrinsic recalcitrance and its capacity to adsorb labile carbon, which minimizes CO_2 emissions and organic carbon loss during composting (Wu *et al*., 2020; Wang *et al*., 2025).

Table 3: Linear regression for weight loss during 91-day composting

Treatment	R^2	Adj. R^2	$F(1,12)$	*p*-value
Heap 1 (SCG)	0.948	0.943	217.25	<0.001
Heap 2 (SCG+RHB)	0.991	0.990	1303.62	<0.001
Heap 3 (SCG+RHB+EFB)	0.990	0.989	1206.07	<0.001
Heap 4 (SCG+PKSB)	0.925	0.919	148.53	<0.001

All four linear models were highly significant ($p < 0.001$), confirming that composting duration was a robust predictor of mass loss across all treatments (**Table 3**). Heap 2 (R^2 = 0.991) and Heap 3 (R^2 = 0.990) demonstrating near-perfect linearity, suggesting that decomposition proceeded at a highly uniform rate throughout the 91-day period. Meanwhile, Heap 1 (R^2 = 0.948) and Heap 4 (R^2 = 0.925) showed lower coefficients of determination, likely due to rapid initial degradation phases that decelerated over time. Residual diagnostics validated the regression assumptions, as the Shapiro-Wilk test confirmed normally distributed residuals for all treatments ($p > 0.05$). Recent studies validate that the incorporation of biochar enhances compost carbon retention, as its stable aromatic structures are resistant to microbial degradation, similar to other recalcitrant carbon forms that contribute to a long-term carbon sink (Pongsiriyakul *et al*., 2024; Ramli *et al*., 2025; Zou *et al.,* 2025).

Compost Pb toxicity

Heavy metal concentrations across all treatments remained significantly below Malaysian regulatory thresholds for safe agricultural application. Although the guidelines focus primarily on paddy, their 85 mg/kg lead (Pb) threshold is equally applicable to maize due to comparable translocation rates from soil to grain, which pose long-term dietary health risks (Zulkaflee *et al*., 2022).

Table 4. Detection of the measured parameter in compost heaps over 12 weeks of composting

Week of composting	Pb Concentration in Compost (mg/g)			
	Heap 1	Heap 2	Heap 3	Heap 4
3	nd	1.46	nd	nd
6	nd	0.37	nd	nd
9	nd	nd	nd	nd
12	nd	nd	nd	nd

Lead (Pb) were detected in Heap 2, which exhibited values of 1.46 mg/g at week 3 and 0.37 mg/g at week 6 (**Table 4**). The substantial reduction in these concentrations suggests a dilution effect or the immobilization of metallic ions within the stabilizing organic matrix, reinforcing environmental safety as no detectable values (nd) were recorded for Heaps 1, 3, or 4 throughout the study, nor for Heap 2 beyond week 6, indicating the compound was only transiently present or degraded (Zhou *et al*., 2018; Palansooriya *et al.,* 2022).

Lead (Pb) can accumulate in soils from compost application, posing health risks by entering the food chain through crops grown on contaminated soil, and its exposure is associated with neurological and other health concerns, making the monitoring of heavy metal levels in compost before use essential (Rai *et al*., 2019; Moshchenko *et al.,* 2022; Sahen *et al*., 2025). Compost maturation significantly decreases Pb bioavailability, driven by increased humic content and a stabilized pH approaching neutral or slightly alkaline values ideal for crop growth (Ko *et al*., 2008; Smith *et al*., 2009; Udovic *et al*., 2012). Mature compost reliably elevates soil pH by 0.5–0.75 units, which improves nutrient uptake and mitigates acidification risks, whereas immature compost may exhibit pH fluctuations due to ongoing organic acid production and nitrogen transformations (Bożym and Siemiątkowski, 2018). Thus, elevated compost pH significantly reduces Pb bioavailability, ensuring safer amendments.

Compost maturity

To validate the transition from active decomposition to biological stability, several maturity indices were assessed, focusing on the reduction of phytotoxic compounds and the promotion of plant growth potential.

Seed germination index (%)

A germination index above 80% is recognized as the benchmark for non-phytotoxic, mature compost, in agreement with current research (Thomas *et al.,* 2024; Angyu and Lanki, 2025). Post hoc Tukey HSD analysis revealed two distinct groups among the compost treatments. Heap 2, Heap 3, and Heap 4 were grouped together, as no significant differences in GI were observed among them ($p > 0.05$) (**Figure 4**). In contrast, Heap 1 was significantly lower than the other three treatments ($p < 0.05$), forming a separate group.

The reduction in the germination index (GI) for Heap 1 indicates significant phytotoxicity directly attributed to bioactive compounds such as caffeine, tannins, and polyphenols characteristic of spent coffee grounds and documented to inhibit seed germination and root development which resulted in GI values consistently below the established 80% threshold required to denote compost maturity and non-phytotoxicity (Cervera-Mata *et al*., 2020; Lara-Ramos *et al*., 2023; Thomas *et al*., 2024). In contrast, composts containing biochar yielded significantly higher germination indices. This improvement is attributed to biochar's high surface area and porosity, which facilitate the adsorption and immobilization of phytotoxic organic compounds including caffeine and polyphenols thus mitigating their negative impacts on seeds (Rizwan *et al.,* 2023; Ebrahimi *et al*., 2024). Furthermore, biochar enhances cation exchange capacity, fostering a more supportive and less phytotoxic environment for seed germination and early seedling growth (Zafar-ul-Hye *et al.,* 2021; Riniarti *et al.,* 2025).

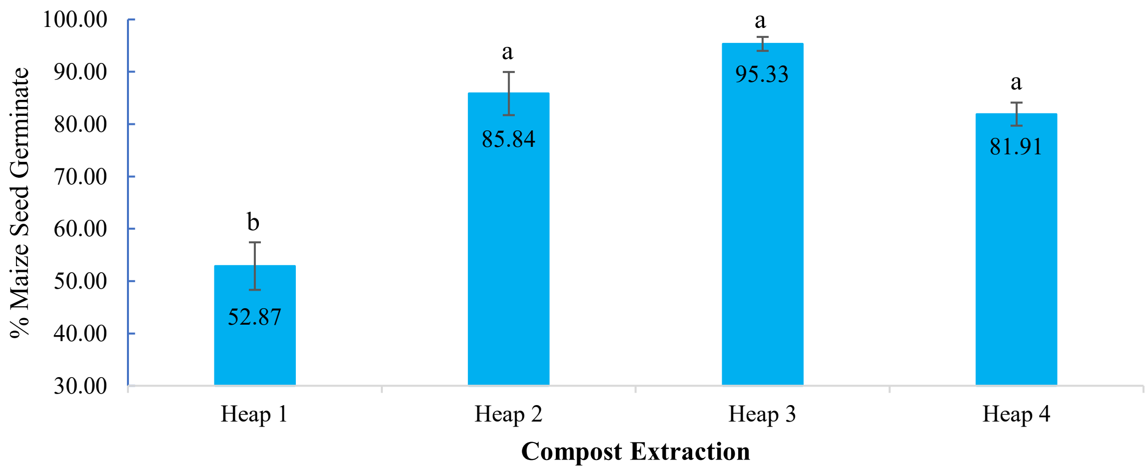

Figure 4: Maize seed germination index (%) for four compost extracts

Note: Compost extracts from Heap 1 (spent coffee grounds), Heap 2 (spent coffee grounds + rice husk biochar), Heap 3 (spent coffee grounds + rice husk biochar + dried shredded empty fruit bunches), and Heap 4 (spent coffee grounds + palm kernel shell biochar) were used to evaluate maize seed germination.

Seed root lengths

The phytotoxic impact of the compost treatments on early plant development is shown in **Table 5**, which presents the mean radicle elongation of maize (*Zea mays* L.) seedlings following a five-day exposure period. To ensure statistical reliability and minimize experimental bias, each treatment was evaluated using a substantial cohort of approximately 100 seeds per replicate. The observed variations in root development across the aqueous compost extracts serve as a critical indicator of maturity and the successful degradation of low-molecular-weight organic acids.

Table 5. Effect of compost extract treatments on average root length (cm) of maize seedlings

Treatment	n	Mean (cm)	SD	SE	Tukey Group
Heap 1	3	4.48	0.51	0.29	a
Heap 2	3	6.68	0.29	0.17	bc
Heap 3	3	7.50	0.24	0.14	c
Heap 4	3	6.46	0.26	0.15	b

The effect of compost extract treatments on root length of maize seedlings was assessed using a one-way analysis of variance (ANOVA). Root length was measured from 100 seeds per replicate, with three replicates per treatment (N = 1,200). Replicate means (n = 3) served as the experimental unit. The assumptions of normality (Shapiro-Wilk test, all $p > 0.05$) and homogeneity of variance (Levene's test, $F = 0.23$, $p = 0.871$) were satisfied. The ANOVA revealed a highly significant difference among treatments ($F_{3,8} = 42.19$, $p < 0.001$). As shown in **Table 5**, Heap 3 recorded the highest mean root length (7.50 ± 0.14 cm), followed by Heap 2 (6.68 ± 0.17 cm), Heap 4 (6.46 ± 0.15 cm), and Heap 1 (4.48 ± 0.29 cm). Tukey's HSD post-hoc test indicated that Heap 1 was significantly inferior to all other treatments ($p < 0.001$). Heap 3 differed significantly from Heap 4 ($p = 0.024$) but not from Heap 2 ($p = 0.073$), while Heap 2 and Heap 4 were statistically similar ($p = 0.857$). These results suggest that compost extracts from Heap 3 and Heap 2 were more effective in promoting root elongation compared to Heap 1 and Heap 4. These results from root length analysis strongly indicate Heap 3 provides the most effective, plant-friendly compost, whereas Heap 1 may require further maturation or modification (Afriliana *et al.,* 2021; Manaf *et al.,* 2024).

Direct application of SCG to soil, without adequate composting or maturation, consistently leads to reduced seed viability and stunted root growth, as demonstrated in experimental trials with urban agricultural plants and standardized germination assays (Hardgrove and Livesley, 2016; Jeníˇcek *et al.,* 2022; Angeloni *et al.,* 2024). The addition of biochar and EFB to spent coffee compost has been shown to significantly enhance maize root development by increasing root length and surface area, thereby promoting more resilient root systems and optimal plant growth (Mardhiana *et al.,* 2021; Alghamdi *et al.,* 2024; Rahim *et al.,* 2025).

Maize leaves cell wall thickness

The pot study consisted of five treatment groups to assess differences in plant cellular anatomy, namely unamended topsoil (control) and topsoil amended with compost from Heaps 1 through 4 (H1–H4). Each treatment was replicated five times and arranged in a Randomized Complete Block Design (RCBD). As shown by the **Figure 5** boxplot, compost amendment led to a progressive increase in median cell wall thickness and upper-quartile values. Heap 4 produced the most significant structural enhancement, suggesting its specific nutrient profile better supports secondary cell wall biosynthesis, while Heaps 2 and 4 showed broader spreads in thickness values that indicated more variability compared to the highly consistent results of Heap 3 (Hu *et al*., 2024; Khan *et al*., 2023).

A one-way ANOVA showed a significant effect of treatment on cell wall thickness ($F = 5.998$, $p = 0.0011$). Tukey HSD test indicated that Heap 4 had significantly thicker cell walls than the Control (mean difference = 0.201 µm, $p = 0.0011$) and Heap 1 (mean difference = 0.164 µm, $p = 0.010$).

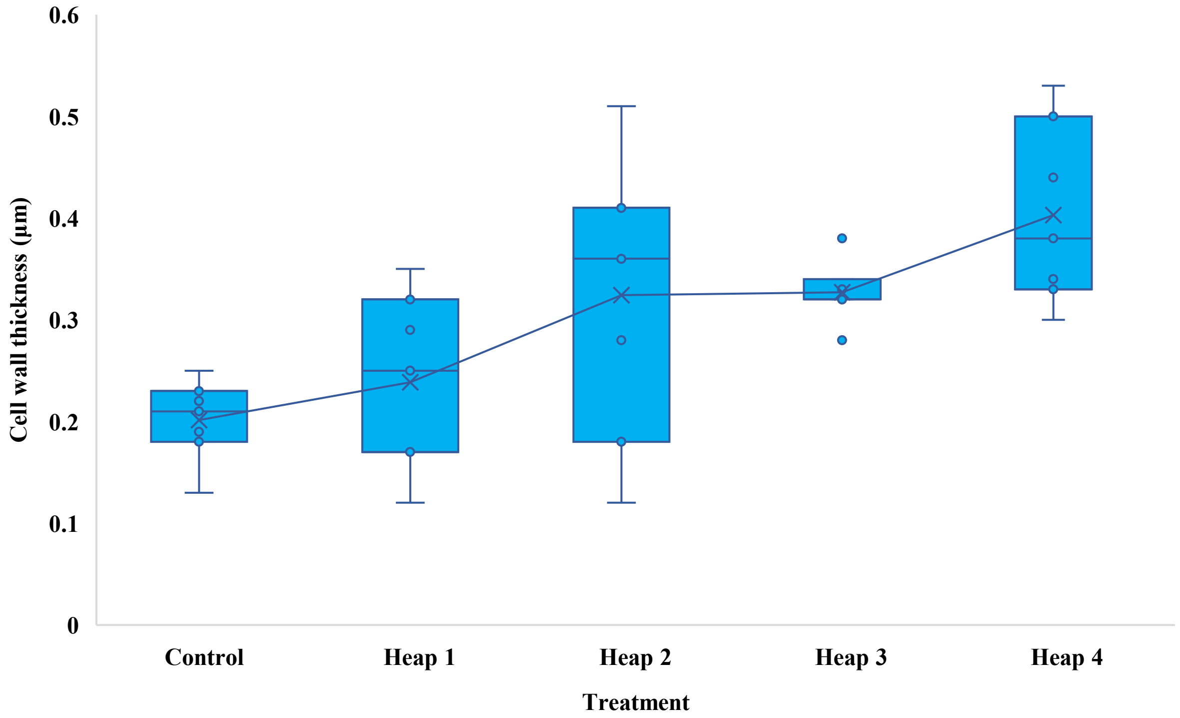

Figure 5: The graph presents maize leaf cell wall thickness (µm) across different compost treatment groups, including a control.

Note: Data are presented as box-and-whisker plots with overlaid individual measurements (n = 5 per group). The horizontal line indicates the median and the 'X' represents the mean. A connecting line illustrates the mean cell wall thickness trend across treatments.

Figure 6 (a–e) shows the TEM micrographs of maize leaf cell wall thickness across different treatment, where the biochar-enriched Heap 4 treatment produced the largest increase in maximum maize cell wall thickness despite higher experimental heterogeneity, a finding supported by reported improvements in wall thickness, breaking force, and the co-deposition of silica, hemicellulose, and lignin driven by gene upregulation (Miao et al., 2022; Fallah et al., 2023). In contrast, Heap 3 demonstrated the most consistent promotion of secondary cell wall deposition across all replicates, indicating a more uniform physiological response. This observed variability, particularly within the heavily amended cohorts, may be attributed to fluctuations in compost maturity, localized microbial metabolic activity, or the intrinsic heterogeneity of nutrient distribution within the compost matrix (Li and Delvaux, 2019; Listyarini *et al*., 2023).

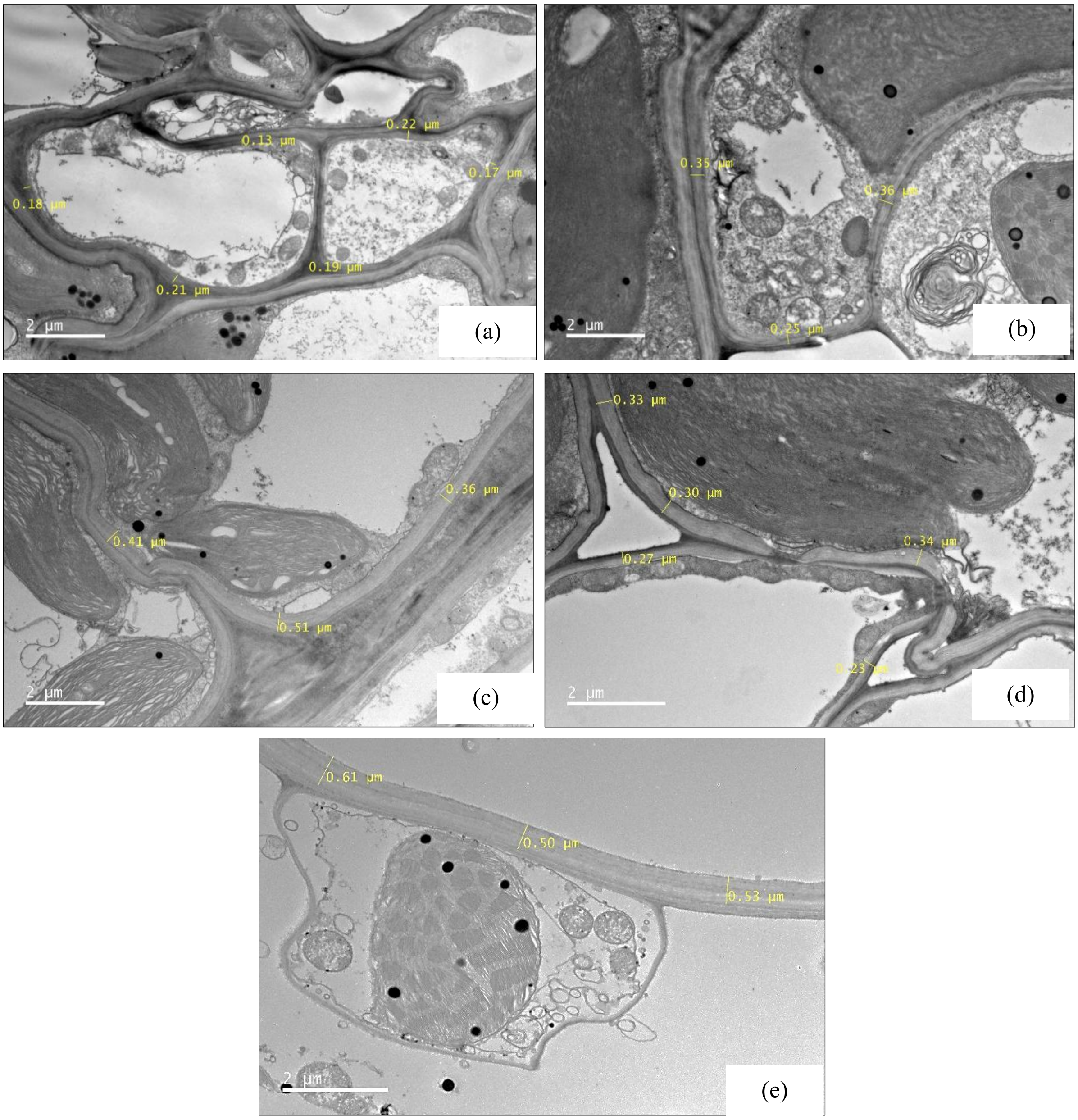

Figure 6: TEM micrographs showing maize leaf cell wall thickness across treatments: (a) unamended control, (b) SCG only, (c) SCG with RHB, (d) SCG with RHB and EFB, and (e) SCG with PKSB.

Note: Yellow annotations indicate individual cell wall thickness measurements (µm). Cell wall thickness increased progressively from Control (0.13–0.22 µm) to Heap 4 (0.50–0.61 µm), suggesting that biochar-enriched compost amendments promoted greater cell wall deposition in maize leaf tissue. Scale bars = 2 µm.

CONCLUSION

This study demonstrated that co-composting spent coffee grounds with biochar and lignocellulosic residues significantly improved compost maturity, pH stability, and overall amendment quality compared to non-composted spent coffee grounds alone. Among the formulations tested, the combination of spent coffee grounds with rice husk biochar and empty fruit bunches exhibited the most favourable composting performance, characterized by enhanced thermophilic decomposition, superior germination index, and greater root elongation in maize seedlings. Biochar incorporation effectively buffered compost acidity toward neutrality, a desirable property for maintaining soil pH balance and nutrient availability upon field application. The progressive mass reduction observed across all treatments confirmed active organic matter stabilization, while Pb immobilization to undetectable levels ensured the safety of the final compost products as soil amendments.

Palm kernel shell biochar compost further enhanced maize leaf cell wall thickness, indicating that compost nutrient composition directly influenced plant cellular development through improved soil–plant nutrient transfer. In contrast, unamended spent coffee ground compost remained acidic and phytotoxic, underscoring the importance of substrate optimization in compost formulation for soil application.

These findings affirm that biochar-enriched spent coffee ground composting produces stable, non-phytotoxic organic amendments capable of improving soil fertility and supporting crop establishment. The utilization of biochar derived from agricultural by-products such as rice husk, empty fruit bunches and palm kernel shell further contributes to waste valorization, positioning this approach as a dual-purpose strategy for organic waste management and soil improvement. Future research should investigate the long-term influence of these composts on soil organic matter turnover, nutrient retention capacity, and soil biological activity under field conditions, to further optimize compost formulations for sustainable soil management and crop production.

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