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ORIGINAL PAPER

Microbial-Enhanced Peel and Seed Wastes of *Dimocarpus longan* Bokashi Fertilizers: Unlocking Soil Health and Bioactive Constituents in *Trigonella foenum-graecum*

Esraa E. Ammar[1,2] · Mai A. El-Esawy[2] · Xiaobo Zou[1] · Soumya Ghosh[3,4] · Helen Onyeaka[5] · Doaa E. Elsherif[2]

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Abstract

This study evaluates a modified Bokashi fermentation system utilizing a specific microbial consortium (*Saccharomyces cerevisiae*, *Lactococcus lactis*, and *Rhodopseudomonas palustris*) to bio-transform *Dimocarpus longan* (longan) fruit peels and seeds into bio-fertilizer. The fermentation stability was assessed via total organic matter (OM) and carbon-to-nitrogen (C/N) ratios. The resulting bio-compost teas, Longan Peel (LP), Longan Seed (LS), and a 1:1 mixture (LP: LS), were applied at 1% (w/v) to *Trigonella foenum-graecum* (fenugreek) in a pot experiment. The 15-day-old seedlings were collected for analysis. All fermented treatments significantly enriched soil nutrients and optimized the C/N ratio compared to the control. The LS treatment yielded the highest nutrient concentrations, while the LP: LS treatment resulted in the highest microbial activity, specifically increasing fungal counts (e.g., *Aspergillus*, *Cladosporium*, and *Penicillium* spp.) and bacterial/actinomycete populations. Physiologically, LP: LS-treated fenugreek exhibited the highest increases in chlorophyll pigments, carbohydrates, protein content, and secondary metabolites (phenolics and flavonoids contents). Furthermore, RT-PCR analysis confirmed the up-regulation of key biosynthetic genes, including *MVK*, *PAL*, and *FLS* (polyphenolic/terpenoid pathways) and *CHLH* (chlorophyll biosynthesis). Oxidative stress, measured via malondialdehyde (MDA) levels, remained within control ranges, indicating no phytotoxicity. These data demonstrate that the *S. cerevisiae*, L. *lactis*, and *R. palustris* Bokashi system effectively stabilizes longan waste into a nutrient-rich bio-fertilizer. The up-regulation of metabolic genes and improved soil microbial profiles provide a technical basis for using this waste-to-resource strategy as a measurable alternative to synthetic chemical inputs in sustainable agriculture.

✉ Esraa E. Ammar
esraa_ammar@science.tanta.edu.eg

✉ Helen Onyeaka
h.onyeaka@bham.ac.uk

1 School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China

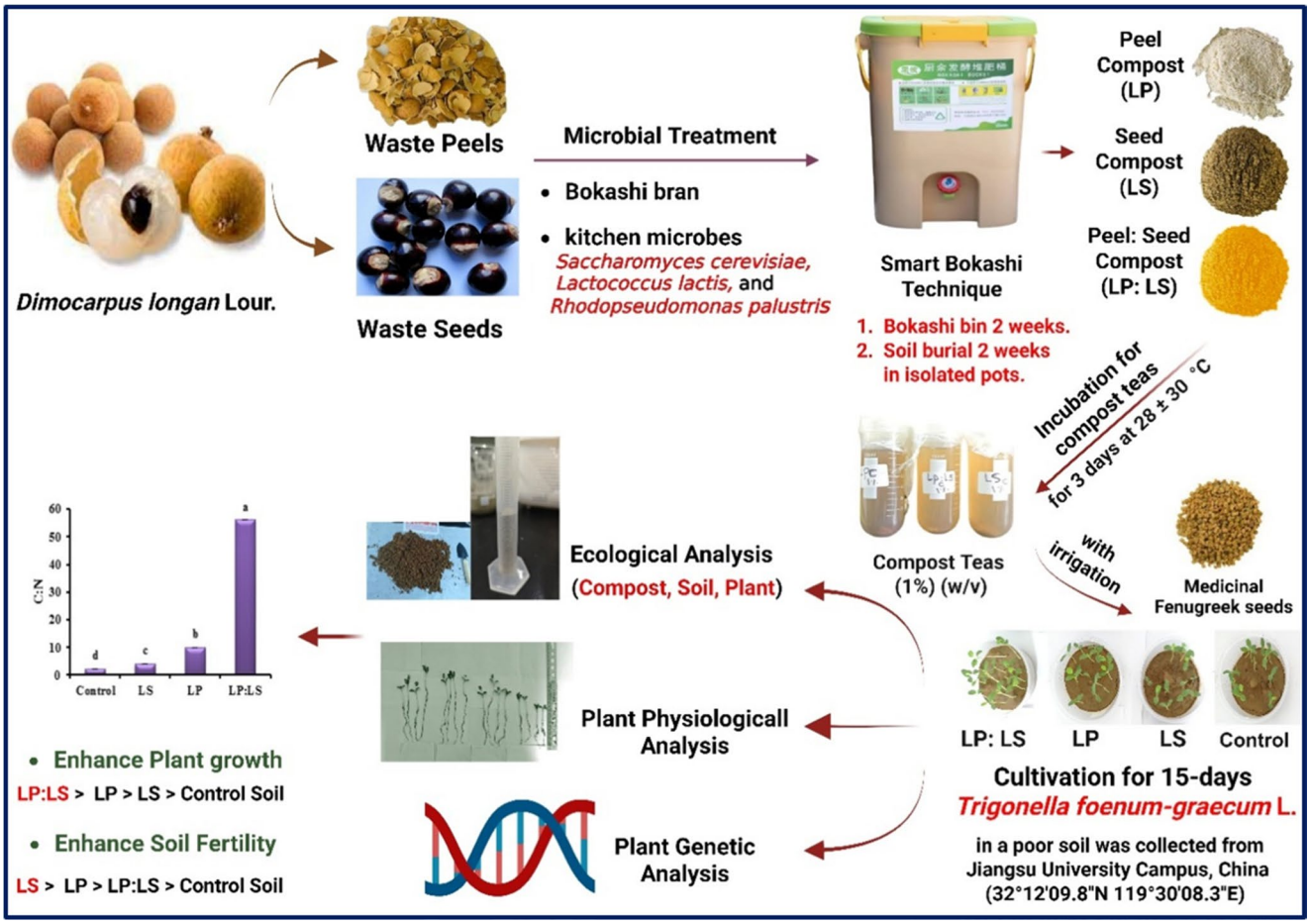
2 Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

3 Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Oman

4 University of the Free State, Bloemfontein 9301, South Africa

5 School of Chemical Engineering, University of Birmingham, Birmingham B152 TT, UK

Graphical Abstract



Highlights

- Modified production of environmentally sustainable fertilizers utilizing peel and seed residues of *Dimocarpus longan* Lour. through the Bokashi composting method.
- All prepared (w/v) (1%) Bokashi longan waste composts [LP (peel), LS (seed), and LP: LS (peel: seed)] exhibited the highest levels of essential nutrients and total organic matter compared to the control soil, along with favorable C: N ratios.
- Essential nutrients, total organic matter, plus C: N ratio are elevated in the treated soil with LS, then LP, plus LP: LS.
- Treated soils with LP and LP: LS showed the highest average fungal colony counts, featuring six fungal species, while LS and LP: LS treated soils had the highest bacterial colony counts, representing two bacterial groups.
- In *Trigonella foenum-graecum* L., LP: LS compost tea achieved the highest nutrient content, total organic matter, C: N ratio, germination rate, mineral absorption, growth criteria, and secondary metabolites.
- RT-PCR revealed that LP: LS treatment activated key secondary metabolite genes in the plants.

Keywords *Dimocarpus longan* Lour. Wastes · Bokashi composting technique · Eco-friendly fertilizers · *Trigonella foenum-graecum* L. · Soil fertility · Secondary metabolites

1 Introduction

Fenugreek is a Fabaceae plant commonly referred to as *Trigonella foenum-graecum* L. and is an annual plant (Ammar 2022). Fenugreek has been in widespread culinary and folk medicinal use since ancient times, using the green leaves and seeds (Chi et al. 2025). The fenugreek extract demonstrates the existence of a large number of phyto-compounds. It indicates the presence of steroids, terpenoids, alkaloids, flavonoids, phenolics, glycosides, saponins, and tannins

(Wangui et al. 2025). These polyphenolic compounds are termed phenylpropanoids because they are formed out of phenylalanine. The use of flavonoids and phenolic compounds has many beneficial biological effects (Bonta 2020; Dias et al. 2021; Mutha et al. 2021). Terpenoids are heterogeneous groups of primary and secondary plant metabolites such as pigments used in photosynthesis, phytohormones, and essential oils (Pichersky and Raguso 2018).

Global interest in the circular economy, sustainability, and zero waste, driven by the UN's Sustainable Development Goals, focuses on recycling waste to promote economic growth and minimize environmental harm. (Qi et al. 2024). Modern science integrates these concepts to develop affordable, eco-friendly solutions for major climate change problems like food shortages, soil infertility, and desertification, by turning waste into valuable opportunities. (Ammar 2021; Younis et al. 2022). Bio-fertilization stands as a key method, leveraging biodegradable materials to enhance soil fertility (Ammar et al. 2023). Bio-fertilizers use biodegradable materials like microalgae, fungi, and various plant residues (e.g., fruit peels, coffee grounds, tea powder), which often contain beneficial compounds like antioxidants and alkaloids (Ammar 2022, 2023; Chakravarty and Mandavgane 2021; Shahrajabian et al. 2019; Tarashkar et al. 2023). This approach is particularly effective when applied to Chinese longan fruit (*Dimocarpus longan* Lour.) waste, whose thick shells possess a rich composition of natural biochemical biodegradable constituents, such as polysaccharides, flavonoids, alkaloids, carotenoids, vitamins that act as antioxidants, anti-bacterial, or nutrients. (Ammar et al. 2025; Shahrajabian et al. 2019). All while these compounds have traditional medicinal or cosmetic applications, they are ideally suited as natural soil amendments for sustainable ecology. (Ammar et al. 2025).

To efficiently transform this waste into a usable resource, the Bokashi technique is employed. This method is an organic fertilizer extensively utilized in many agricultural locations, noted for its rich nutrient content, helpful microbes, and organic materials. This fertilizer promotes and activates beneficial microorganisms in the soil, boosts its physical characteristics, and provides nutrients for plants. Unlike traditional organic fertilizers, such as chicken manure or compost, which require several weeks or months for decomposition, the bokashi fermentation process occurs within 14 to 21 days (Quiroz and Céspedes 2019). In certain instances, the end outcome is a semi-stabilized organic compound, which refers to a partially degraded and biochemically unstable substance (Quiroz and Céspedes 2019). The stability of bokashi persists after its application to the soil (Quiroz and Céspedes 2019). The use of a non-stabilized substance may adversely affect plant growth by reducing oxygen availability, immobilizing nitrogen in the soil, or causing phytotoxicity due to harmful chemicals (Christel 2017; López-atanacio et al. 2025). Consequently, this research focuses on employing the cost-effective Bokashi technique to manage longan fruit waste, including peels and seeds, through fermentation using Bokashi bran inoculated with *Saccharomyces cerevisiae* EC-1118 and *Lactococcus lactis*. This approach aims to produce novel, eco-friendly bio-fertilizers and to evaluate their effects on the growth of *Trigonella foenum-graecum* L., while investigating the associated molecular and physiological mechanisms, bioactive compound accumulation, and improvement of soil fertility.

2 Materials and Methods

2.1 Longan Waste Management and Preparation of Kitchen Bokashi Composts and Teas

Kitchen waste, consisting of peels and seeds from longan fruit (*Dimocarpus longan Lour.)*, was collected from local Chinese marketplaces during January and February 2024. The waste was cleaned with distilled water, sorted, and dehydrated in a forced-air oven at 40 °C for 72 h. To ensure surface area consistency, the dried materials were milled and sieved to a particle size of 0.2–2.0 mm. The experimental setup utilized three 9 L polypropylene (PP) Bokashi bins (dimensions: 20 cm × 20 cm × 23 cm). Each bin featured an automated monitoring and dispensing system controlled by an ATmega328 microcontroller. This system integrated an ultrasonic sensor for waste levelling, a DHT11 sensor for real-time temperature (maintained at ± 28 °C) and humidity monitoring, and a moisture sensor. An automated DC motor-driven spindle was used to ensure the precise dispensing of Bokashi bran. Each treatment consisted of 10 g of waste powder mixed with 50 mL of distilled water to form a paste. The treatments were: (1) longan peel (LP), (2) longan seed (LS), and (3) a 1:1 ratio of peel and seed (LP: LS). To initiate fermentation, each bin received 15 g of kitchen microbes: 5 g *Saccharomyces cerevisiae* EC-1118, 5 g of *Lactococcus lactis*, and 5 g *Rhodopseudomonas palustris* to enhance the starter culture. The bins were hermetically sealed to maintain anaerobic conditions. The primary fermentation lasted 14 days at ambient temperature, with leachate (Bokashi tea) collected via the drainage tap every 3 days to prevent moisture saturation and maintain the anaerobic environment. Following the initial 2-week fermentation, fermented pastes were then buried ($n = 3$ replicates per treatment) in autoclaved, clean soil basins (pH 5–6.5) to complete maturation. The final Bokashi tea exhibited a pH

of 4.8, confirming successful acidic fermentation (Lew et al. 2021; López-atanacio et al. 2025). Three Bokashi compost teas (1% w/v in distilled water: LP, LS, LP: LS) were prepared from matured composts, incubated for 3 days at ± 28 °C, then filtered (Yin et al. 2025).

2.2 Experimental Skeleton

Twenty seeds of *Trigonella foenum-graecum* L. (Egyptian Commercial cultivar) were subjected to surface sterilization using 10% commercial bleach [5% (w/v) sodium hypochlorite] for 10 min, followed by three washes with distilled water before sowing. The seeds were cultivated in poor soil collected from Jiangsu University Campus, Zhenjiang, Jiangsu Province, China (32°12'09.8"N 119°30'08.3" E) for 15 days during winter 2024 at ambient temperature and in darkness within transparent pots (14.9 × 8.5 × 10 cm, with a capacity Of 1000 mL) till germination then transferring to light under normal day conditions (maximum average temperature (12 °C), and daylight average (10–11 h) . The experiment consisted of four treatments: (1) Control (distilled water), (2) LP, (3) LS, and (4) LP: LS. Each treatment was performed in triplicate, utilizing three independent pots per group. Twenty seeds were sown in each pot to ensure statistical robustness. Three replicates were arranged vertically under the same conditions that involved administering 5 cc of irrigation water for each treatment separately in an alternating fashion until seedling harvesting. The seedlings were harvested 15 days after germination for physiological and molecular analysis.

2.3 Physicochemical and Nutrient Analysis of Compost and Soil

Samples were made at a 1:5 (w/v) ratio to evaluate electrical conductivity (EC) in mS cm^{-1} and pH values using a conductivity meter (60 Sensor Operating Instruction Corning) and a glass electrode pH meter (Model HI 2211), respectively. Atomic Absorption Spectrometry (AAS) was utilized to examine soil extracts obtained from 5 g of air-dried soil samples, employing 2.5% (v/v) glacial acetic acid. Total nitrogen was quantified with the Micro-Kjeldahl technique. Following digestion, samples were allowed to cool to room temperature, typically resulting in a colorless or occasionally pink coloration. The molybdenum blue method was employed for phosphorus analysis via a spectrophotometer (CECIL CE 1021). Potassium was measured using a flame photometer (CORNING M410) (Allen et al. 1974). The ratio of total organic matter (OM) and the carbon-to-nitrogen (C/N) ratio was assessed (Allen et al. 1974). The apparatus was set up and utilized according to the manufacturer's guidelines.

2.4 Microbial Soil Analysis and Classification

Around microbial soil analysis and classification, concerning soil microbiology, the microbial community composition in soil was assessed as an indication of soil fertility through Colony-forming units (CFU g^{-1}) for both bacteria and fungi; furthermore, morphological classifications of fungi and bacteria were examined (Acea et al. 1988; Paul 2007; Söderberg et al. 2004; Vieira and Nahas 2005).

2.5 Plant Analysis

2.5.1 Main Nutrient Plant Analysis

Regarding mineral analysis; Minerals were extracted from 0.5 to 1 g dry weight plant samples using 20 mL of a nitric acid and hydrogen peroxide solution (3:2 v/v) until a transparent solution was achieved. Total nitrogen was measured using the Micro-Kjeldahl method. The Molybdenum blue method was utilized to measure total phosphorus using a spectrophotometer (CECIL CE 1021). Potassium was measured using a flame photometer (CORNING M410), whereas iron was evaluated at 239.562 nm by inductively coupled plasma (ICP) (Perkin Elmer, Optima 7000 DV). All of these approaches, together with the total organic matter (OM%) and C/N ratios, were delineated by Allen (1974). The equipment was configured and operated according to the manufacturer's specifications. The concentrations of phosphorus, nitrogen, and potassium in the plant data are presented as percentages.

2.5.2 Assessment of Growth Patterns

For assessment of growth patterns, after a thorough washing, the seedlings (15 days old) were gathered, and their fresh weights were estimated. Fresh seedlings were desiccated for three days at 50 °C to ascertain their dry weight.

2.5.3 Pigment Measurements

About pigments measurements, fresh tissue (50–100 mg fresh weight) from the two latest-grown leaves was sampled for chlorophyll and carotenoids quantification via a spectrophotometer after extraction in 80% acetone, following (Lichtenthaler and Wellburn 1983). Authors measured the absorbance at 645, 663, and 470 nm.

2.5.4 Total Soluble Sugar and Total Soluble Protein Contents

The total soluble sugar concentration was assessed in the produced borate buffer extract. The total soluble sugar

concentration was assessed at 490 nm and subsequently quantified as mg g^{-1} dry weight using a predefined calibration curve.

The total soluble protein concentration was quantitatively evaluated. The absorbance was quantified spectrophotometrically at 595 nm. The protein concentration was quantified in mg g^{-1} dry weight using a developed calibration curve of bovine serum albumin (BSA).

2.5.5 Measurement of Phenolic and Flavonoid Contents

the ethanolic extract of the shoot was utilized to quantify total phenolics and flavonoids. Desiccated leaves were ground into a fine powder. At ambient temperature, tissues (0.1 and 0.5 g) were homogenized and incubated for 30 min in 70% acetone, followed by centrifugation at 4 xg. The phenolic content was measured utilizing the Folin-Ciocalteu reagent. The extract was combined with Folin-Ciocalteu reagent in a 1:1 volume ratio. After 5 min, a 7.5% sodium carbonate solution was added at a volume ratio of 2:1. After 2 h, the absorbance was measured at 725 nm. 100 µl of the same extract was amalgamated with 4 cc of distilled water for the flavonoid assay, followed by the addition of 0.3 mL of 5% sodium nitrite. After five minutes, 0.3 cc of 10% aluminum chloride was added. After 5 min, 2 mL of 1 M sodium hydroxide was added to the mixture. The absorbance was measured against a blank at 510 nm. Catechin was employed for the calibration curve.

2.5.6 Evaluation of Stress Biomarkers

Malondialdehyde (MDA) concentration was quantified according to the methodologies established. Leaf was extracted using a 5% (w/v) TCA solution to evaluate MDA. Following 20 min of heating, the sample extract and 0.67% (w/v) TBA were then chilled. The sample's absorbance was quantified at two wavelengths: 532 nm and 600 nm.

2.5.7 Genetically Dimensions

Total RNA was extracted from 100 mg of the fully expanded leaves, and then leaf samples were immediately snap-frozen in liquid nitrogen, stored at −80 °C, and then pulverized into a fine powder using a pre-chilled mortar and pestle under liquid nitrogen to prevent RNA degradation. Extraction was performed utilizing the Qiagen RNase Mini Kit. Total RNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Germany) following the manufacturer's protocol. cDNA was synthesized in a 20 µl reaction volume using M-MLV Reverse Transcriptase (Promega, USA), 5x RT buffer, 10 mM dNTPs, 50 ng μL^{-1} oligo(dT)18 primer, 40 U μl^{-1} RiboLock RNase inhibitor, and 200 U μl^{-1} M-MLV RT enzyme. The thermal cycling conditions were: 5 min at 65 °C (primer annealing/DNA denaturation), 60 min at 42 °C (reverse transcription), and 10 min at 95 °C (enzyme inactivation). Quantitative real-time PCR was conducted in triplicate using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA) in a 25 µl reaction volume containing 1 µl cDNA (1:10 dilution), 12.5 µl 2x Master Mix, 0.5 µM each primer (Table S1), and nuclease-free water. Thermal cycling on the Rotor-Gene Q (Qiagen, Germany) consisted of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s (combined annealing/extension). Relative gene expression was quantified using the 2^(-ΔΔCt) method with ACTIN as the reference gene (Elsherif et al. 2024a, b a; Livak and Schmittgen 2001).

2.6 Statistical Investigation

The results are presented as the mean of three replicates ± standard error (SE). Differences between treatments for the different measured variables were tested by one-way analysis of variance (ANOVA), followed by Tukey's test with significant differences found ($p < 0.05$) using XLSTAT software (version 2014.5.03).

3 Results

3.1 Analysis of Bokashi Composts and Teas

All compost teas exhibited acidic pH values, remaining below the control soil (7.5) yet above 5. The measured pH values were 5.8 for LP, 5.2 for LS, and 5.4 for LP: LS (Table 1A). Similarly, all composts showed higher EC means compared to the control (0.77). LP had the maximum EC (1.46), followed by LP: LS (1.17) and LS (1.12) (Table 1A).

Analysis of the Bokashi composts (LP, LS, and LP: LS) confirmed that all formulations contained significantly greater proportions of essential minerals compared to the control soil. The most dramatic increase was observed in N^+ %, which recorded an exceptional spike in the LP (1.2%), then LS (1.1%) formulations, representing an increase of approximately 40-fold over the minimal control level (0.029%) (Table 1A). For K^+ %, the LP treatment yielded the highest value (5.32%), with all other treatments (LP: LS at 4.38% and LS at 3.64%) substantially surpassing the control soil (2.89%) (Table 1A). The Total C^{+4}% content was also markedly enriched, peaking in the LS formulation (36.59%), followed by LP: LS (33.47%) and LP (30.20%), all significantly exceeding the control (20.20%) (Table 1A). Finally, while all composts contributed to P^{+5}% availability,

Table 1 Summarization of ecological and Atomic Absorption Spectrometry (AAS) analyses, where (A) Prepared Bokashi composts and teas control soil and prepared Bokashi composts, (B) Treated soil samples, (C) Treated plant samples

A. Prepared Bokashi composts and teas				
Ecological Analyses	C	LS	LP	LP: LS
pH means	7.5 ± 0.0088^{a}	5.2 ± 0.0058^{d}	5.8 ± 0.0088^{b}	5.4 ± 0.0115^{c}
EC means (mS cm $^{>-1}$)	0.8 ± 0.0088^{d}	1.1 ± 0.012^{c}	1.5 ± 0.0058^{a}	1.2 ± 0.0058^{b}
K %	2.9 ± 0.115^{d}	3.7 ± 0.011^{c}	5.3 ± 0.0010^{a}	4.4 ± 0.0400^{b}
N %	0.1 ± 0.0015^{d}	1.1 ± 0.058^{c}	1.2 ± 0.058^{a}	0.8 ± 0.0153^{b}
P %	28.0 ± 0.592^{d}	30.4 ± 0.1453^{a}	28.1 ± 0.067^{c}	28.4 ± 0.058^{b}
C %	20.2 ± 0.029^{d}	36.6 ± 0.0318^{a}	30.2 ± 0.4460^{c}	33.5 ± 0.253^{b}
OM%	4.9 ± 0.0088^{d}	36.6 ± 0.7265^{c}	56.4 ± 0.4460^{b}	59.7 ± 0.39^{a}
C: N	20.2 ± 0.0291^{d}	36.6 ± 0.0088^{a}	30.2 ± 0.4680^{c}	33.5 ± 0.2805^{b}
B. Treated soil samples				
pH means	7.3 ± 0.0088^{b}	7.4 ± 0.0088^{a}	7.3 ± 0.0088^{c}	7.2 ± 0.0088^{d}
EC means (mS cm $^{-1}$)	0.08 ± 0.0009^{d}	0.17 ± 0.0006^{a}	0.11 ± 0.0006^{b}	0.09 ± 0.0006^{c}
N %	0.04 ± 0.0014^{c}	0.14 ± 0.00385^{a}	0.14 ± 0.0038^{a}	0.07 ± 0.0009^{b}
P %	30.5 ± 0.029^{d}	35.7 ± 0.1155^{a}	33.1 ± 0.0581^{b}	32.0 ± 0.0418^{c}
K %	2.7 ± 0.064^{d}	3.4 ± 0.015^{a}	3.3 ± 0.114^{b}	3.2 ± 0.0318^{c}
OM%	2.9 ± 0.029^{c}	7.2 ± 0.012^{a}	6.7 ± 0.2800^{a}	4.5 ± 0.3003^{b}
C: N	18.8 ± 0.063^{d}	52.6 ± 0.3358^{a}	39.4 ± 0.2887^{b}	29.9 ± 0.0203^{c}
C. Treated plant samples				
N %	1.5 ± 0.0441^{c}	1.7 ± 0.0289^{b}	1.7 ± 0.0416^{b}	2.3 ± 0.1935^{a}
P %	1.3 ± 0.3467^{c}	3.6 ± 0.0569^{b}	4.8 ± 0.0536^{b}	6.0 ± 0.0736^{a}
K %	0.8 ± 0.0233^{c}	0.9 ± 0.0436^{b}	0.9 ± 0.0033^{b}	1.5 ± 0.0145^{a}
OM%	10.2 ± 0.1013^{c}	10.3 ± 0.0441^{c}	10.4 ± 0.0473^{b}	13.5 ± 0.0458^{a}
C: N	2.2 ± 0.0088^{d}	4.1 ± 0.067^{c}	9.9 ± 0.060^{b}	56.0 ± 0.012^{a}

Informatively, *C* control soil, *LS* Longan seed compost, *LP* Longan peel compost, and *LP* LS: Longan (peel: seed) compost. The data represents means ± standard error ($n = 3$). Different lowercase letters are used to indicate statistically significant differences at Tukey's test ($P < 0.05$). Informatively, *pH* Potential of hydrogen, *EC* Electrical conductivity, *K* potassium, *N* nitrogen, *P* phosphorus, *C* carbon, OM%: Organic matter percentage, and C: N: Carbon: Nitrogen ratio

the LS formulation registered the largest percentage (30.37%) compared to the control soil (28.01%) (Table 1A).

All prepared composts demonstrated an extreme elevation in the total organic matter (OM %) compared to the control soil (4.9%). The LP: LS formulation recorded the maximum OM percentage (59.7%), followed by LP (56.4%), then LS (36.6%) (Table 1A). Similarly, the Carbon-to-Nitrogen (C: N) ratio was markedly higher in the records across all composts than in the control soil (20.20%). The most substantial ratio was observed in LS (36.55%), followed by LP: LS (33.45%), and then LP (30.16%) (Table 1A). These high OM % and C: N values confirm the composts' capacity to significantly enrich the soil's organic content and nutrient cycling potential.

3.2 Soil Analysis

3.2.1 Main Nutrient Soil Analysis

Round soil pH means; a control soil was 7.32, while a slightly with treatment with the compost teas as follows: it was slightly augmented in the LS sample till 7.41 and lessened slightly in LP till 7.28, plus LP: LS 7.18 (Table 1B**)**. Around soil EC mean, a treated control soil was 0.08, but it increased in LS till 0.17, while in LP was 0.11, and LP: LS was 0.09 (Table 1B**)**.

All compost treatments successfully increased the levels of essential growth minerals and organic components in the soil compared to the untreated control (Table 1B). The LS treatment consistently showed the most significant enhancements across all measured parameters. Specifically, K^{+} % surged highest in LS (3.40%), followed by LP (3.21%) and LP: LS (3.20%), all exceeding the control (2.71%). N^{+} % also peaked with LS (0.140%), closely followed by LP (0.139%), and then LP: LS (0.073%), marking a substantial rise over the control (0.042%). Similarly, P^{+5}% was highest in LS (35.7%), followed by LP (33.1%), and LP: LS (32%), relative to the control (30.5%). Furthermore, all composts elevate the total organic matter (OM %) of the soil that recorded in the control (2.85%) only, while with LS elevated to (7.23%), LP (6.73%), and LP: LS (4.53%) (Table 1B). All composts elevate the C: N ratio of the soil, as recorded in the control (18.81) only, while with LS elevated to (52.64), LP (39.40), and LP: LS (29.93) (Table 1B).

3.2.2 Microbial Soil Analysis and Classification

About fungal colony-forming unit CFU g^{-1}, treated soil with LP: LS compost tea recorded the highest fungal CFU (1112 CFU g^{-1}), then LP (800 CFU g^{-1}) and LS (400 CFU g^{-1}) compared to the control soil (100 CFU g^{-1}) (Fig. 1a). Six fungal species were recorded in soil samples after treatment with compost teas as follows: *Aspergillus spp.*, *Cladosporium spp.*, *Penicillium spp.*, *Rhizopus spp.*, *Scopulariopsis spp.*, and *Saccharomyces spp.*) (Table 2).

Both LP: LS and LP treated soils recorded the whole 6 fungal species, and 3 in LS. The control soil contains only *Aspergillus spp.* (Table 2). About bacterial colony-forming

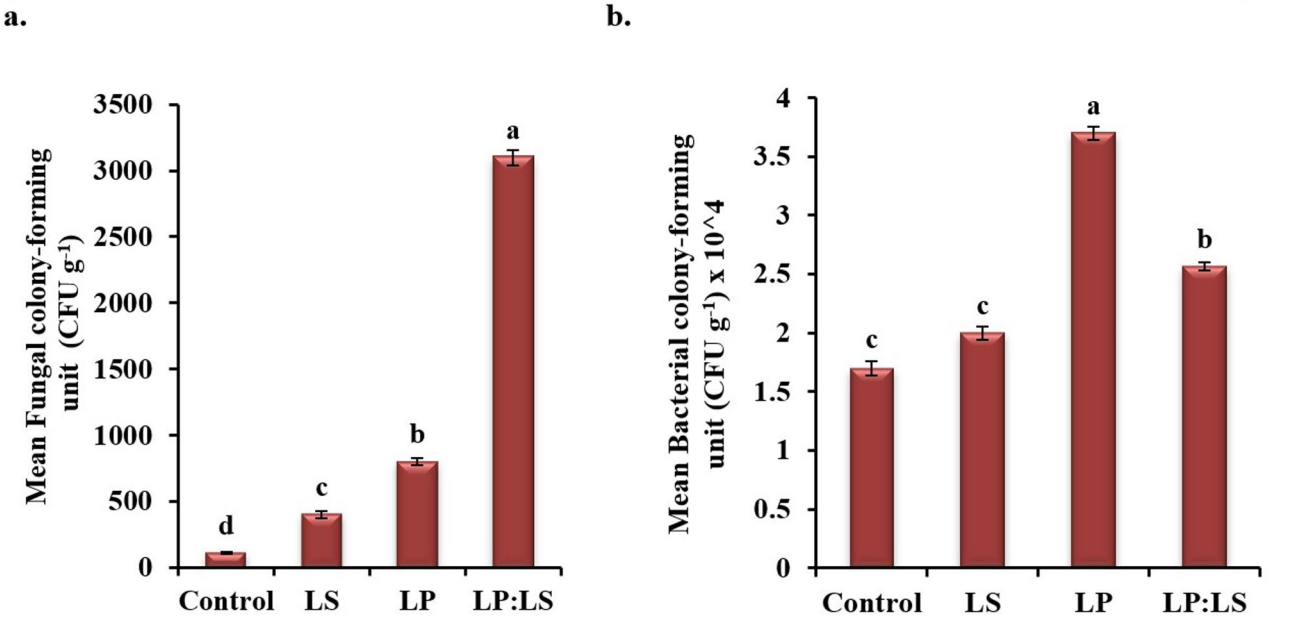


Fig. 1 Assessment of the total Microbial colony-forming Unit (CFU g^{-1}) in the control soil and treated samples by composts., (**a**) Mean fungal; colony-forming unit (CFU g^{-1}), plus (**b**) Mean bacterial colony-forming unit (CFU g^{-1}) x $^{10\wedge4}$. The data represents means ± standard error ($n=3$). Different lowercase letters are used to indicate statistically significant differences at Tukey's test ($P<0.05$). Iinformatively, C. control soil, LS: Longan seed compost, LP: Longan peel compost, LP: LS: Longan (peel: seed) compost, and CFU: colony-forming unit

Table 2 Mean of fungal colony-forming unit CFU g^{-1} of treated soil samples with Bokashi compost teas

Fungal Species	Mean of CFU g^{-1} in soil samples			
	Control	LS	LP	LP: LS
Aspergillus spp.	100	300	100	412
Cladosporium spp.	0	0	200	200
Penicillium spp.	0	0	200	200
Rhizopus spp.	0	100	100	100
Scopulariopsis spp.	0	0	100	100
Saccharomyces spp.	0	0	100	100
Total count (CFU g^{-1})	100	400	800	**1112**
Number of fungal species	1	3	**6**	**6**

Informatively, *C* control soil, *LS* Longan seed compost, *LP* Longan peel compost, *LP LS* Longan (peel: seed) compost, and *CFU* colony-forming unit

Table 3 Mean of bacterial colony-forming units CFU g^{-1} of treated soil samples with Bokashi compost teas

Bacterial Species	Mean of CFU g^{-1} in soil samples			
	Control	LS	LP	LP: LS
Bacillus spp.	1.7 × 10^4	1.8 × 10^4	3.7 × 10^4	1.7 × 10^4
Actinomycetes	0	2 × 10^4	0	0.9 × 10^4
Total count (CFU g^{-1})	1.7 × 10^4	2 × 10^4	3.7 × 10^4	2.6 × 10^4
Number of Bacterial species	1	**2**	1	**2**

Informatively, *C* control soil, *LS* Longan seed compost, *LP* Longan peel compost, *LP: LS* Longan (peel: seed) compost, and *CFU* colony-forming unit

unit CFU g^{-1}, treated soil with LP compost tea recorded the highest bacterial CFU (3.7 × 10^4 CFU g^{-1}), then LP: LS (2.6 × 10^4 CFU g^{-1}) and LS (2 × 10^4 CFU g^{-1}) comparing to the control soil (1.7 × 10^4 CFU g^{-1}) (Fig. 1b). Both *Bacillus spp.* and Actinomycetes were recorded in LS and LP: LS soil treated samples, while the control soil and LP treated soil had Bacillus spp. only (Table 3).

3.3 Plant Analysis

3.3.1 Main Nutrient Plant Analysis

The essential growth minerals showed higher concentrations in the compost-amended soils compared with the control. Potassium (K^+) displayed the greatest increase in the LP: LS treatment (1.45%), followed by LP (0.89%) and LS (0.88%), whereas the control recorded 0.83% (Table 1C). Likewise, nitrogen (N^+) reached its highest level in LP: LS (2.34%), then LP (1.68%) and LS (1.65%), compared with 1.47% in the control (Table 1C). Phosphorus (P^{5+}) also peaked in LP: LS (6.04%), followed by LP (4.83%) and LS (3.62%), while the control showed only 1.31% (Table 1C). All compost treatments increased the organic matter (OM %) of plant samples above the control value (10.18%), reaching 13.49% in LP: LS, 10.37% in LP, and 10.28% in LS (Table 1C). Similarly, the C: N ratio rose markedly with compost application compared with the control (2.21), increasing to 56.02 in LP: LS, 9.92 in LP, and 4.13 in LS (Table 1C).

3.3.2 Seed Germination and Seedling Growth Criteria

About seed germination and seedlings growth criteria; the primary test of seed germination of 20 seeds of *Trigonella foenum-graecum* with different treatments after 5 days shows that the control soil (10/20 seeds) (50%), LS (11/20 seeds) (55%), LP (12/20 seeds) (60%), and LP: LS (14/20 seeds) (70%). The data in Fig. 2a, b, c, d show the effect of peel and seed wastes of *Dimocarpus longan* on seed germination, lengths, fresh and dry weights of *Trigonella foenum-graecum* seedlings after 15 days of growth. Application of peel and seed wastes of *Dimocarpus longan* significantly increased the seedling germination after 15 days to become 13 seeds at LS, 14 seeds at LP, and 15 seeds at LP: LS compared to 12 seeds at the control soil (Fig. 2a). In addition, application of LS, LP, and LP: LS significantly increased the seedling total plant length corresponding to the control, showing the highest increase with LP: LS application (Fig. 2b). In the same way, the results showed that application of LS, LP, and LP: LS significantly augmented the fresh plus dry weights for 15-d-old *Trigonella foenum-graecum* seedlings in comparison to the control (Fig. 2c). The ratios of increase were 29%, 44%, and 81%, respectively, for fresh weight, besides 18%, 33%, and 48%, for the dry weights (Fig. 2d).

3.3.3 Photosynthesis Pigment Content

The data represented in Table 4 show the effect of peel and seed wastes of *Dimocarpus longan* on chlorophyll a, besides chlorophyll b, and carotenoid contents of 15-d old *Trigonella foenum-graecum* seedlings. Application of LS, LP, and LP: LS significantly amplified Chl *a*, Chl *b*, and carotenoid contents correspondingly about the control. The ratios of increase were 29%, 32%, and 38%, respectively, for Chl *a* content, 20%, 153%, and 180%, for Chl *b* content, and 14%, 40%, and 60%, respectively, for carotenoid content.

Table 4 Effect of peel and seed wastes of *Dimocarpus longan* (LS, LP and LP: LS) on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids contents (mg g^{-1} d.wt) of 15-d old *Trigonella foenum-graecum* seedling

Treatments	Chl a	Chl b	Carotenoids
Control	1.21 ± 0.02^{c}	0.64 ± 0.01^{d}	0.42 ± 0.01^{d}
LS	1.52 ± 0.02^{b}	0.77 ± 0.01^{c}	0.48 ± 0.01^{c}
LP	1.6 ± 0.03^{ab}	1.62 ± 0.05^{b}	0.59 ± 0.01^{b}
LP: LS	1.67 ± 0.02^{a}	1.79 ± 0.03^{a}	0.67 ± 0.01^{a}

The data represents means ± standard error ($n = 3$). Different lowercase letters are used to indicate statistically significant differences at Tukey's test ($P < 0.05$). Informatively, *C* control soil, *LS* Longan seed compost, *LP* Longan peel compost, *LP*: *LS* Longan (peel: seed) compost, and *Chl a* chlorophyll *a* and *Chl b* chlorophyll *b*

3.3.4 Total Soluble Protein and Total Soluble Sugar

Figure 3a, b elucidates the impact of peel and seed waste from *Dimocarpus longan* on the total soluble protein (TSP) and total soluble sugar (TSS) content in 15-day-old *Trigonella foenum-graecum* and seedlings. Application of LS, LP, and LP: LS significantly augmented TSP and TSS contents correspondingly with the control. The ratios of increase were 30%, 76%, and 117%, respectively, for TSP content and 12%, 58% (Fig. 3a), and 143%, for TSS content (Fig. 3b).

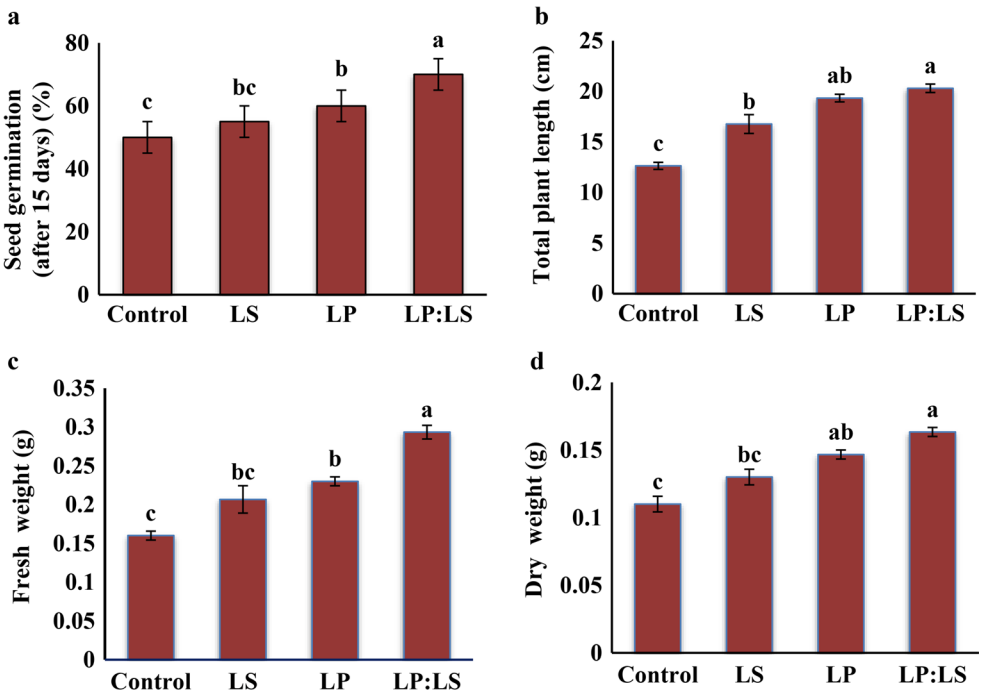


Fig. 2 Effect of peel and seed wastes of *Dimocarpus longan* (LS, LP, and LP: LS) on seed germination (%), seedlings length (cm), and fresh and dry weights (mg) of 15-d old *Trigonella foenum-graecum* seedlings. The data represents means ± standard error ($n = 3$). Different lowercase letters are used to indicate statistically significant differences at Tukey's test ($P < 0.05$). Informatively, C. control soil, LS: Longan seed compost, LP: Longan peel compost, and LP: LS: Longan (peel: seed) compost

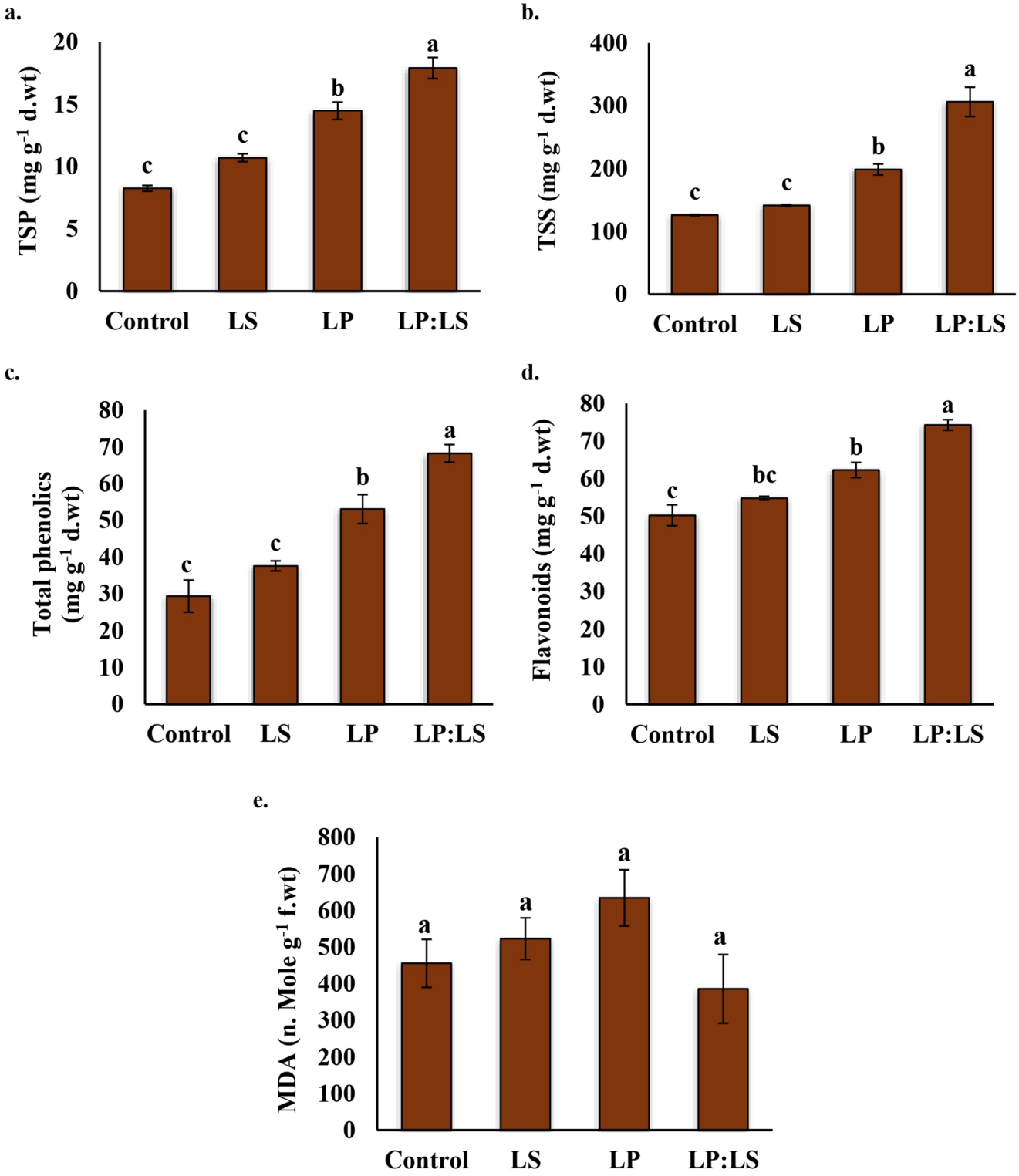


Fig. 3 Effect of peel and seed wastes of *Dimocarpus longan* (LS, LP and LP: LS) on **(a)** The total soluble soluble sugars contents (TSS), and **(b)** The total soluble protein (TSP), additionally, **(c)** the total phenolics content, **(d)** the flavonoids content, and **(e)** malondialdehyde contents (MDA) towards the old *Trigonella foenum-graecum* seedling. The data represents means ± standard error ($n = 3$). Different lower-case letters are used to indicate statistically significant differences at Tukey's test ($P < 0.05$). Informatively, C. control soil, LS: Longan seed compost, LP: Longan peel compost, LP: LS: Longan (peel: seed) compost, TSS: the total soluble sugars contents, TSP: The total soluble protein contents, and MDA: Malondialdehyde

3.3.5 Phenolics, Flavonoids, and Malondialdehyde (MDA) Content

The data represented in Fig. 3c, d show the effect of peel and seed wastes of *Dimocarpus longan* on total phenolics and flavonoids contents of 15-d old *Trigonella foenum-graecum* seedlings. Application of LS, LP, and LP: LS significantly increased total phenolics (Fig. 3c) and flavonoid contents (Fig. 3d), corresponding to the control. The ratios of increase were 28%, 81%, and 132%, respectively, for the total phenolics content and 9%, 24%, and 48%, respectively, for the total flavonoids content. Moreover, the application of peel and seed wastes did not result in a statistically significant change in MDA content across treatments (Fig. 3e), indicating that the compost applications-maintained lipid peroxidation at levels comparable to the control.

3.3.6 Genetic Measurement

For gene expression, this study looked into gene expression of several important enzymes involved in secondary metabolites, such as *CHLH* (Fig. 4a), *PAL* (Fig. 4b), *FLS* (Fig. 4c), and *MVK* (Fig. 4d). In comparison to the control, the outcomes demonstrated that plants treated with LP: LS had much greater transcript levels and increased expression of several genes. In particular, the highest *CHLH*, *MVK*, *FLS*, and *PAL* expression increases were 16.12, 3.73, 4.5, and 4.55-fold, respectively, with the LP: LS treatment dosage.

4 Discussion

Soil organic fertility is universally vital for global production, as organic matter regulates the crucial physical, chemical, and biological balances within the soil-plant system. Therefore, converting organic waste into eco-friendly fertilizers, such as Bokashi, is an essential management strategy that improves ecosystem health and increases crop yield (Gashua et al. 2023). While conventional organic decomposition typically requires an extended period for nutrient release, Bokashi offers a rapid solution. It is derived from fermented organic matter infused with beneficial microbes (Effective Microorganisms or EM-4) that accelerate the decomposition and mineralization process, making nutrients swiftly available for plant absorption, often within just one to two weeks (Gashua et al. 2023; Luyima et al. 2022). Our results demonstrate a concomitant increase in both nutrient availability and microbial density. While the experimental design does not decouple the individual impacts of the inoculum from the indigenous nutrient pool, the correlation between elevated CFU counts and improved growth suggests that the microbial consortia may play a supportive role in the mineralization process of the longan residues. Soil

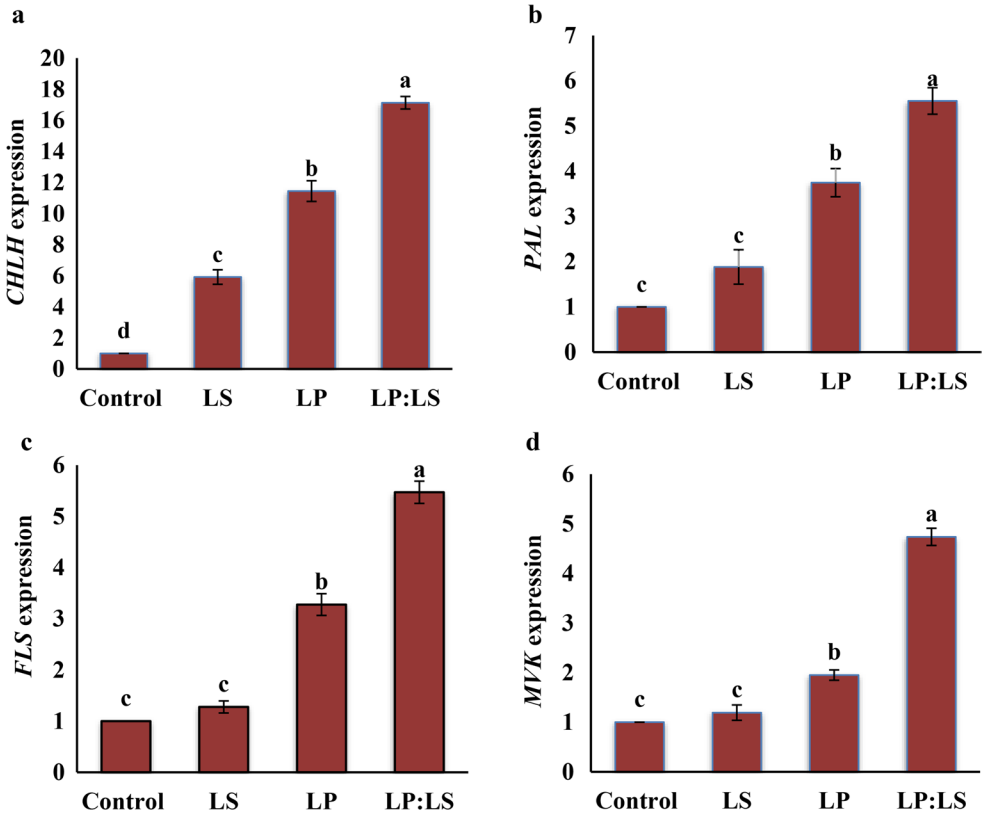


Fig. 4 The effect of peel and seed wastes of longan compost (LS, LP and LP: LS) on **(a)***CHLH*, **(b)***PAL*, **(c)***FLS* and **(d)***MVK* genes expression of *Trigonella foenum-graecum* seedlings. The data represents means ± standard error ($n = 3$). Different lowercase letters are used to indicate statistically significant differences at Tukey's test ($P < 0.05$). Informatively, C. control soil, LS: Longan seed compost, LP: Longan peel compost, and LP: LS: Longan (peel: seed) compost, PAL: Phenylalanine ammonia lyase, MVK: Mevalonate kinase, CHLH: H subunit of Mg-chelatase, and FLS: Flavonol synthase

treated with all composts exhibited an increase in fungal colony counts, particularly in LP and LP: LS treated soils, which contained six fungal species: *Aspergillus spp.*, *Cladosporium spp.*, *Penicillium spp.*, *Rhizopus spp.*, *Scopulariopsis spp.*, and *Saccharomyces spp.* In addition to the treated soils with all composts increased bacterial colony counts, particularly in LS and LP: LS treated soils, which exhibited the highest bacterial colony counts, representing two bacterial groups: *Bacillus spp.* and Actinomycetes. Microorganisms are vital participants in various ecosystem processes, notably enhancing soil fertility. Soil bacteria enable the biogeochemical cycling of important mineral nutrients, such as nitrogen, phosphorus, and sulfur, vital for plant growth. The bacteria employ organic carbon as their energy source to enable the recycling process (Basu et al. 2021; Gashua et al. 2023). The incorporation of actinomycetes to improve soil fertility and plant yield is an effective strategy for developing sustainable agricultural systems, due to their efficiency, environmental safety, and low production costs. Actinomycetes demonstrate antagonism through the production of 1,3-β-glucanase and enzymes that hydrolyze cell walls. Conversely, actinomycetes directly affect plant growth by excreting plant growth-promoting compounds, mineral nutrients, or facilitating the multiplication of beneficial microorganisms. Actinomycetes improve soil nutrient concentrations, increase organic matter content, and strengthen soil microbial communities.

The findings of this study suggested that the growth criteria (seed germination, seedlings length, root length, seedlings fresh and dry weights) of Fenugreek plants were increased in all treatments relative to the control. The most favourable enhancing impact was recorded with LP: LS relative to the non-treated control. The enhanced growth criteria observed in the LP: LS treatment is likely attributable to the combined influence of a lower pH and a higher mineral content, as reflected by the elevated EC. The acidity of the compost teas potentially enhanced the solubility of micronutrients, while the microbial presence likely accelerated the release of N, P, and K from the organic seed and peel matrix. This finding means that all prepared Bokashi composts possessed high levels of minerals and nutrients that elevate the soil fertility and promote plant growth. According to Atomic Absorption Spectrometry (AAS), essential minerals K^+ %, N^+ %, and P^{+5}% for all manufactured composts recorded higher levels than the control soil; thus, after treatment of the soil, the soil nutritional content and fertility were elevated. Additionally, C^{+4}%, C: N ratio, and total organic matter (OM%) of all prepared composts were determined more than the control soil, thus elevating the soil fertility of all treated soils and enhancing their nutritional contents. (Senesi 1989; Singbah et al. 2025). Moreover, all manufactured compost teas were acidic, with their pH less than the control soil (7.5), but more than 5, while all of them maintained the pH level of the soil after treatments more than 7. This outcome is suitable for soil fertility for the growth of a wide range of suitable pH for most crops, e.g., wheat, corn, soybeans, oats, barley, and alfalfa (from 4.7 to 7.5) (Singbah et al. 2025; Tong et al. 2021). Furthermore, all composts elevation of the fungal colony counts, especially LS and LP: LS treated soils had the highest bacterial colony counts. Soil microbes in LP: LS treated, such as *Aspergillus spp.*, *Cladosporium spp.*, *Penicillium spp.*, *Rhizopus spp.*, *Scopulariopsis spp.* facilitate the biogeochemical cycling of mineral nutrients, including phosphorus, sulfur, plus nitrogen, are essential for plant growth. The bacteria employ organic carbon as their energy source to enable the recycling process (Basu et al. 2021; Gashua et al. 2023). *Bacillus spp.* can significantly alter the soil microbiome, perhaps enhance plant development, and increase soil microbial diversity. Furthermore, they serve a vital role in conferring resistance to plants against biotic and abiotic stressors (Zhang et al. 2025; Tsotetsi et al. 2022; Gurikar et al. 2023).

The application of prepared seed compositions has led to substantial increases in the levels of photosynthetic pigments (chl *a*, chl *b*, and carotenoids) and the *CHLH* gene in Fenugreek seedlings. The most advantageous enhancement was observed in LP: LS compared to the untreated control. Chlorophyll production depends on the initial committed step, which entails the incorporation of Mg^{2+} into protoporphyrin (Reid and Hunter 2002; Sirijovski et al. 2008). This process is mediated by the H subunit of magnesium chelatase (CHLH) and exhibits a conserved mechanism across plants. To enhance adaptability to variable terrestrial environments, CHLH develops several biological activities, encompassing chlorophyll production, retrograde signaling, and abscisic acid (ABA) responses. The primary benefit of seed is the provision of essential nutrients that function during seed establishment (Elsherif et al. 2024a, b b). The elevation of chlorophyll levels and their biosynthesis would be associated with improved photosynthetic activity and growth rates. The peels and seeds of longan compost minerals may be directly integrated into or indirectly enhance the biosynthetic pathway of chlorophylls (El-Shenody et al. 2023; Elsherif et al. 2025). Furthermore, elevated carotenoid levels are typically associated with mitigating oxidative stress as antioxidants, which may indicate appropriate cellular function (Nessem et al. 2023). Enrichment of LP: LS soil with actinomycetes boosts photosynthesis and subsequently increases production. Actinomycetes increased nitrogen availability in soil, thereby enhancing the activity of crucial nitrogen-metabolizing enzymes, including glutamine synthetase, glutamate synthase, and nitrate reductase.

The impact of longan peel and seed compost application on Fenugreek seedlings was evaluated by assessing major

metabolic indicators. The results indicated that seeds germinated with soil treatments using produced compost teas considerably enhanced TSS and TSP, with LP: LS exhibiting the highest values compared to the control. The elevated TSS and TSP mostly correspond to enhanced metabolic activities and elevated rates of photosynthesis, evidenced by increased biomass and pigment levels. Likewise, seeds germinating with natural compost were found to provide plants with vital nutrients and essential metabolites such as carbohydrates and proteins, thereby enhancing growth rate (El-Shenody et al. 2023). Furthermore, the enhancement of LP: LS soil by actinomycetes improves photosynthetic activities, resulting in increased levels of nitrogen-containing amino acids and enhanced concentrations of sugars (Jog et al. 2016). The LP: LS soil treatment can improve seed germination and seedling growth due to the presence of growth-promoting substances such as polyphenols, which facilitate enhanced root and shoot development, thereby strengthening and promoting the health of plants from an early stage. Although Total Soluble Protein (TSP) can be considered a general measure of plant metabolic activity, its antioxidant activity is directly associated with its ability to alleviate oxidative stress by enzymatic and non-enzymatic mechanisms. Physiologically, a high upsurge of TSP is likely to be indicative of an enhanced antioxidant defense system of the plant, especially when the plant is confronted with environmental pressures or when the plant has been subjected to bio-stimulants (Quan et al. 2025).

The stress marker malondialdehyde (MDA) was used to determine the metabolic status after the use of longan peel and seed compost teas and induction of secondary metabolites. Use of compost teas of longan peels and seeds did not show any significant change in MDA concentration, which is consistent with the control levels and shows a non-stress situation of the plant tissues. The results of this discovery indicate that longan peel and seed compost teas usage is two-fold in curbing the oxidative stress and enhancing beneficial plant responses. This is attributed to the fact that CHLH is incorporated into various signaling pathways of the chloroplast, including retrograde and hormonal signaling, which help in suppressing past reactive oxygen species (ROS) (Sun and Shen 2024; Li et al. 2025).

While the rapid mineralization of Bokashi-treated longan residues facilitates swift nutrient availability, we acknowledge that the maturity and stability of such organic amendments are complex processes involving humification and carbon sequestration. The transition from raw residue to stabilized compost involves significant shifts in organic matter quality and humic substance formation. In our study, although direct humification indices (e.g., E4/E6) were not measured, the high germination rate and lack of oxidative stress (MDA) in Fenugreek seedlings serve as a practical indicator of compost stability and the absence of phytotoxic organic acids. This suggests that the fermentation process effectively transformed the longan peels and seeds into a bio-stimulant-rich substrate that would provide study-able insights into the soil-carbon dynamics by (Xing et al. 2025).

The cumulative number of phenolic compounds and flavonoids present in the seedlings improved considerably when they were subjected to longan peel and seed compost. Plant phenolics and flavonoids have high potentials of application in various-medical and food industries, thus making modern research focus on their biosynthetic pathways. In comparison to the control, the gene levels of the PAL, the main enzyme in the phenylpropanoid pathway, increased significantly in the LP: LS therapy. PAL activation is an indication of the augmentation of secondary metabolism as a direct response to primary metabolism (Barros and Dixon 2020). In this way, overexpression of PAL was an indication of activation of the phenylpropanoid pathways, which was supported by the above-mentioned surge in the levels of phenolic and flavonoid caused by the use of LP: LS. Several genes of enzymes that are predominantly linked to flavonoid production were evaluated in terms of relative expression to aid in the progression of flavonoid production after the LP: LS soil application. The current data demonstrates elicitation ability of plants under LP: LS soil, which powers the number of flavonoids and phenolics in the leaves of the Fenugreek. In addition, compared to the control, the LP: LS therapy was associated with a considerable increase in the FLS gene expression that was subsequently projected to their functions and enzyme activity. FLS is a rate-limiting enzyme in the production of flavonols and it encodes the major enzyme in the process of flavonoid metabolite synthesis (Liu et al. 2022; Shi et al. 2022). The hydroxylated flavonoid derivatives at C-3 position such as myricetin, quercetin, and kaempferol are produced as FLS catalyzes the desaturation of dihydroflavonols (Lei et al. 2023). The greater expression of FLS leads to production of too much quercetin and kaempferol in tobacco (Park et al. 2020). As a result, the findings showed that the LP: LS treatment had an outstanding capability to trigger the secondary metabolic pathways which led to the proliferation of bioactive substances.

Terpenoids, or isoprenoids, are structurally built up of active isoprene precursors generated by the methylerythritol phosphate (MEP) and mevalonate (MVA) pathways, which take place in the cytoplasm and plastids, respectively (Tabatabaee et al. 2021). The MVK gene encodes mevalonate kinase, which plays a crucial role in phosphorylating MVA to 5-phosphate mevalonate, which is a precursor in the synthesis of isoprenoid precursors to several other terpenoids, including sterols and sesquiterpenes (Rodriguez-Concepcao and Boronat 2015; Silva et al. 2020). Therefore, we found that the MVK gene improved significantly in the treated plants using LP: LS compost tea, which participates

in the terepenoid biosynthetic pathway. This is counterbalanced by the fact that a recorded increase in gene expression of MVK in *Arabidopsis thaliana* plant results in a significant increase in terpenoids (Lluch et al. 2000). The innovative functional amendment LP: LS compost tea was found to have a strong impact on the expression of MVK genes, which showed that the plant production pathway of isoprenoid biosynthesis was triggered by the treatment.

5 Conclusion

This research paper presents a successful move to a circular economy whereby the *Dimocarpus longan* peel and seed wastes are converted to high-efficiency and eco- friendly bio-fertilizers through the Bokashi composting process and kitchen microbes. The Longan (peel: seed) compost mixture (LP: LS) turned out to be the most effective treatment that boosted soil nutrients and microbial diversity in a mound and, at the same time, enhanced biomass, nitrogen assimilation, and secondary metabolite production in *Trigonella foenum-graecum* L. These physiological gains were further confirmed by molecular analysis, which affirmed the up-regulation of major genes that regulate secondary metabolic pathways. By transforming the organic waste of food into a value-added fertilizer that has resulted in an improved fertility of the soil and quality of the crops, this study provides a model of limited-scale research where zero-waste agricultural methods will result in zero-waste solutions and will directly align with the United Nations Sustainable Development Goals (SDGs) 2030. In the future, we plan to conduct further research to investigate the long-term toxicological effects of this bio-fertilizer on both the plants and the soil.

Abbreviations

LP	Longan peel compost
LS	Longan seed compost
LP: LS	Longan (peel: seed) compost
pH	Potential of hydrogen
EC	Electrical conductivity
C: N	Carbon: Nitrogen ratio
AAS	Atomic Absorption Spectroscopy
PAL	Phenylalanine ammonia lyase
MVK	Mevalonate kinase
CHLH	H subunit of Mg-chelatase
FLS	Flavonol synthase
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
ANOVA	Analysis of Variance
EM	Micro-organism
Chl *a*	Chlorophyll*a*
Chl *b*	Chlorophyll*b*

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Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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