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Research Article

Trichoderma spp.-Enriched Organic Waste Biofertilizer for Water Spinach (*Ipomoea aquatica*): A Sustainability Assessment

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Abstract
The intensive use of synthetic fertilizers in tropical smallholder farming can lead to soil degradation, nitrate leaching, and greenhouse gas emissions, highlighting the need for sustainable alternatives. This study developed a biofertilizer by blending six types of organic waste—water hyacinth, durian peel, eggshells, sugarcane molasses, cow dung, and poultry manure—with *Trichoderma* spp. A Completely Randomized Design (CRD; n = 3) was used to evaluate 13 formulations (A–M), which were produced by batch fermentation and stored for 10 days. The pH, electrical conductivity, nitrogen (N), phosphorus (P), potassium (K), moisture content, and temperature were monitored. Treatment M exhibited the highest nutrient concentrations, with N, P, and K contents of 1,063, 1,004, and 1,089 mg kg^{-1}, respectively. In a pot experiment using water spinach (*Ipomoea aquatica*), Treatment M significantly promoted plant growth, resulting in a 237.5% increase in dry weight compared with the control at an application rate of 500 g pot^{-1}. Both Life Cycle Assessment (LCA) and Techno-Economic Analysis (TEA) demonstrated reduced environmental impacts and economic viability, supporting the potential of this biofertilizer as a circular-economy approach to sustainable smallholder farming.

Keywords: Biofertilizer, Circular bioeconomy, Life cycle assessment, Organic waste valorization, Techno-economic analysis, *Trichoderma* spp., Water hyacinth

1 Introduction

Synthetic nitrogen, phosphorus, and potassium (NPK) fertilizers are crucial to the global food system, but excessive use gives rise to high environmental costs. High rates of synthetic fertilizer use result in soil acidification, disturbance of indigenous soil microbiomes, nitrate leaching into surface and groundwater, and high levels of nitrous oxide (N_2O) emissions, which are 273 times more powerful than CO_2 over 100 years [1], [2]. The energy-intensive Haber-Bosch synthesis of nitrogen further exacerbates the burden on the environment as it consumes about 1–2% of the fossil energy consumed worldwide [3]. Sustainable nutrient management is an urgent ecological need and practical challenge in smallholder systems in Southeast Asia, where synthetic inputs are widely available and rapidly provide an immediate yield response.

At the same time, waste streams from agriculture and food processing industries produce an abundant amount of nutrient-rich biomass which is largely untapped. In Thailand, water hyacinth (*Eichhornia crassipes* [Mart.] Solms) is one of the most problematic invasive aquatic macrophytes in freshwater systems in over 50 countries in the world [4], [5]. Water hyacinth mats cause a depletion of dissolved oxygen, impeding navigation, diminishing biodiversity and disrupting livelihoods of aquatic farmers. However, it contains high nitrogen content of 1.5–3.5% on dry weight, a good C:N ratio of 12–25:1, adequate potassium (0.5–1.2%), and phosphorus (1.5–3.5%), which is ideal for co-composting [4]. The valorization of this biomass as an agricultural input makes an ecological nuisance not only solve a problem for the environment, but also helps to achieve circular resource flows [6], [7].

The other organic residues incorporated into the biofertilizer formulation are likewise significant local waste streams. Durian peel, discarded in large volumes during the harvest season, has been shown to compost aerobically into a nutrient-rich soil amendment [8], [9]. Eggshell waste is a low-cost source of calcium carbonate that buffers compost pH and supplies calcium to the growing medium [1], [10]. Sugarcane molasses supplies readily degradable carbohydrate that serves as an energy source for composting microorganisms and has been reported to reduce nitrogen loss during fermentation [5], [11]. Cow dung and poultry manure are established nitrogen-, phosphorus-, and potassium-rich co-substrates for organic fertilizer production, although their nutrient concentration varies with animal diet, bedding, and storage conditions [3], [12].

Biofertilizers, which contain living or metabolically active microorganisms, can enhance nutrient availability through processes such as nitrogen fixation, phosphate solubilization, and phytohormone production, providing a scientifically established alternative or complement to the use of chemical fertilizers [8], [13]. Among the most widely studied fungal bioinoculants in agriculture is *Trichoderma* spp. It is known to produce cellulases, chitinases and glucanases that break down complex organic polymers, induce systemic plant resistance against soil-borne pathogens through mycoparasitism, induce root proliferation and shoot elongation through production of indole-3-acetic acid (IAA) and gibberellins, and solubilize phosphates [8], [14]. The use of *Trichoderma* spp. powder in the organic waste-based biofertilizer formulation can help in quick mineralization of nutrients, enrichment of plant growth response by increasing the diversity of rhizosphere microbiome and enhance the effectiveness [8], [14].

Water spinach (*Ipomoea aquatica* Forssk.) is a nutritionally and economically important semi-aquatic leafy vegetable of tropical and sub-tropical Asia, contributing to household income and food security among smallholder farmers [15]. It has a rapid fertilizer response with 21 to 35 days from transplanting, making it a good model crop for fertilizer response studies [16]. Although this crop is agronomically significant, there is limited literature reporting on optimization of organic fertilization strategy with locally available waste streams of this crop in the Southeast Asian region. Also, many of the current biofertilizer trials are limited to only the agronomic results without incorporating quantification of impact on the environment and/or a feasibility assessment of the economy.

Life Cycle Assessment (LCA) is a systematic and science-based approach that uses standardized procedures for quantifying the environmental impacts of a product and/or process, as defined by ISO 14040:2006 and ISO 14044:2006 [17], [18]. LCA can be complemented by Techno-Economic Analysis (TEA), which integrates process engineering performance data and cost accounting to assess commercial viability [19], [20]. The combination of physicochemical characterization, agronomic trial results, LCA and TEA in one integrated study (as in the present study) is more complete than is common in the literature on biofertilizers.

The specific objectives of this study were (i) to prepare and evaluate 13 *Trichoderma*-enriched biofertilizer formulations (A–M) formulated with six different types of organic waste materials and to characterize the physicochemical dynamics (pH, EC, available N, P, K, moisture, temperature) within the course of the 10-day fermentation period; (ii) to assess the agronomic performance of *I. aquatica* with the best performing biofertilizer formulation in comparison with commercial biofertilizer and an unamended control; (iii) to quantify the environmental impact profile by LCA; and (iv) to assess the economic feasibility by TEA.

2 Materials and Methods

2.1 *Study location*

This study was carried out at the Faculty of Science, Technology and Agriculture, Yala Rajabhat University, Yala Province, southern Thailand (6°33′N; 101°17′E). The climate is humid tropical (Köppen Af) with a mean annual rainfall of 1,800–2,400 mm, mean annual temperature of 27–29°C, and relative humidity of 75–85%, which is typical of smallholder vegetable production in southern Thailand.

2.2 *Organic raw materials*

The raw materials used were all sourced locally in Yala Province. Biomass of fresh water hyacinth (*Eichhornia crassipes*) was harvested from the irrigation canals on the university campus. Durian peel (*Durio zibethinus* L.) was collected from the local fruit processor. Eggshells were collected from food processing factories, dried in an oven at 60°C to a low moisture content and ground. For the fermentations, the carbon source was sugarcane molasses (80° Brix). Cow dung and poultry manure (layer hen) were gathered from the local farms and dried to reduce the moisture to 70±0.5 %. Commercial *Trichoderma* spp. powder [species/strain and product name to be inserted by the authors from the supplier's certificate of analysis] at a supplier-certified concentration of 1×10^8 CFU g^{-1} (0.5 McFarland) was purchased from a registered biological inoculant supplier; the supplier's certificate of analysis is provided in the Supplementary Material. Starting concentration was taken as the certified value and was not independently re-verified by the authors prior to fermentation; likewise, post-fermentation Trichoderma viability was not quantified by CFU plate counts or molecular assays in this study, so survival and colonization of the inoculum through the 10-day fermentation are inferred from the physicochemical trends rather than demonstrated directly. Direct CFU enumeration or qPCR-based confirmation of *Trichoderma* persistence is recommended for future work and is noted as a limitation of the present study. The quantity and cost of all raw materials were confirmed by procurement invoices and market survey conducted in the local market in May-June 2026 [21] – [26].

2.3 *Biofertilizer formulation*

Thirteen bio-fertilizers (A-M) were designed by systematically varying the amount of manure co-substrates and *Trichoderma* powder (Table 1). The constant carrier substrates (water hyacinth, durian peel, eggshell, and molasses) were held at fixed ratios consistent with those reported to support aerobic composting of bulky, high-moisture agricultural residues [4], [9], while the variable *Trichoderma* and manure loadings were selected to span a low-to-high dosage gradient bracketing rates commonly used in small-batch bioinoculant trials [8], [13].

The fermentation experiment was conducted in a Completely Randomized Design (CRD) with three replications/treatment (n = 39 experimental units), while the pot trial was also a Completely Randomized Design (CRD) with three replications/treatment (n = 15 experimental units). Physicochemical measurements were conducted three times a day

Table 1: Biofertilizer treatment compositions — Phase 1 formulations (all solid quantities in dry weight; molasses as % v/w of total dry substrate mass).

Treatment	Water Hyacinth (g)	Durian Peel (g)	Eggshell (g)	Molasses (%)	*Trichoderma* (g)	Cow Dung (g)	Poultry Manure (g)
A	50	25	5	5	—	—	—
B	50	25	5	5	1.25	—	—
C	50	25	5	5	2.5	—	—
D	50	25	5	5	—	12.5	—
E	50	25	5	5	—	25	—
F	50	25	5	5	—	—	12.5
G	50	25	5	5	—	—	25
H	50	25	5	5	1.25	—	12.5
I	50	25	5	5	2.5	—	25
J	50	25	5	5	1.25	12.5	—
K	50	25	5	5	2.5	25	—
L	50	25	5	5	1.25	12.5	12.5
M	50	25	5	5	2.5	25	25

Note: All formulations prepared in 400 mL sterilized plastic bottles with n = 3 replications. '—' indicates the ingredient was absent. Treatment M contained maximum levels of all biologically active inputs.

(09:00, 14:00 and 17:00 hours). The summary statistics include morning and midday measurements only; evening measurements are available as supporting data [27]-[30]. The comparatively large standard deviations seen for some treatments (e.g., Treatment B moisture, Treatment A EC) are attributed to this pooling of diurnal readings taken at different points in the fermentation-heat cycle, together with the manual hand-mixing and periodic bottle-opening used for sampling, which introduce batch-to-batch variability in moisture loss and localized ion concentration across the three replicate bottles; treatments with faster or more heterogeneous decomposition (e.g., higher manure content) showed proportionally larger variance than the more chemically stable formulations (Table 2).

2.4 *Biofertilizer preparation*

The sterilizing protocol was integrated into the plastic bottles used for biofertilizer production, and the protocol was considered a batch fermentation and aerobic composting process, as 400 mL plastic bottles were employed. The water hyacinth and durian peel were mechanically shredded to less than 3 cm particle size. Eggshell was ground to powder. The sugar cane molasses was added at 5% v/w of the total dry substrate mass and diluted 1:5 in distilled water before mixing. The ingredients were all mixed in the amounts specified in Table 1 and moistened to a moisture level of 60-65%, packed with a headspace of ~20%. Perforated caps allowed aerobic fermentation, but with little moisture loss. The fermentation took place at room temperature (27–30°C) for 10 days, and the bottles were shaken by hand every 48 h, following a duration and protocol consistent with prior small-batch bioinoculant fermentation studies [19], [27]-[30]]. This 10-day window was adopted from that precedent rather than re-derived from first principles for the present waste mixture, and compost maturity indices (C/N ratio, further organic matter breakdown, respiration rate, or germination index) were not measured to independently confirm that fermentation was complete by Day 10. The physicochemical trends in Table 2 (e.g., EC and available-nutrient trajectories) are consistent with continuing, rather than plateaued, mineralization at Day 10, so the absence of maturity testing is acknowledged here as a limitation of the study, and direct maturity indices are recommended for future work before recommending this exact timeline for field-scale production.

Table 2: Physicochemical properties of biofertilizer formulations (A–M) at Day 1 — mean ± SD (n = 3; morning and midday measurements combined).

Treatment	pH	EC (μS cm^{-1})	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Moisture (%)	Temp (°C)
A	7.08 ± 0.03	1,796 ± 620	219 ± 87	120 ± 71	270 ± 106	50.3 ± 3.8	32.8 ± 1.0
B	7.08 ± 0.05	818 ± 245	109 ± 32	27 ± 9	141 ± 41	30.6 ± 10.8	33.6 ± 0.4
C	7.06 ± 0.03	676 ± 248	91 ± 30	21 ± 8	113 ± 42	25.2 ± 10.3	34.2 ± 0.3
D	7.15 ± 0.03	3,847 ± 952	504 ± 113	379 ± 85	626 ± 62	66.2 ± 11.5	36.0 ± 0.6
E	7.17 ± 0.02	2,378 ± 770	310 ± 97	211 ± 75	354 ± 105	55.9 ± 10.8	35.3 ± 0.4
F	7.08 ± 0.01	1,854 ± 904	242 ± 118	177 ± 115	347 ± 107	50.8 ± 15.7	34.6 ± 0.7
G	7.08 ± 0.04	2,095 ± 388	269 ± 51	186 ± 62	306 ± 63	58.3 ± 4.7	32.5 ± 0.7
H	7.14 ± 0.02	3,323 ± 590	432 ± 74	338 ± 77	474 ± 73	73.1 ± 4.7	33.2 ± 0.3
I	7.14 ± 0.05	1,750 ± 586	226 ± 75	159 ± 96	299 ± 95	49.1 ± 9.8	32.6 ± 0.3
J	7.18 ± 0.11	1,518 ± 375	194 ± 50	98 ± 52	239 ± 60	48.0 ± 7.6	33.7 ± 0.9
K	7.29 ± 0.08	2,452 ± 318	317 ± 42	217 ± 44	375 ± 38	60.9 ± 3.8	34.7 ± 0.4
L	7.26 ± 0.03	1,884 ± 277	241 ± 37	136 ± 43	290 ± 38	55.5 ± 2.8	33.2 ± 0.4
M	7.23 ± 0.01	3,452 ± 35	587 ± 141	502 ± 149	624 ± 136	85.1 ± 2.3	33.3 ± 0.1

Note: Treatments D, H, and M showed the highest EC, N, P, and K at Day 1, reflecting the influence of manure co-substrates on initial nutrient concentration.

2.5 *Physicochemical analysis*

Three measurements were made of the physicochemical properties each day (at 09:00, 14:00, 17:00) with the use of a multi-parameter soil analyzer (Yieryi). Parameters measured: available N, P, and K (mg kg^{-1}; multi-pin electrochemical sensor array with Bluetooth data logging), pH (glass electrode), gravimetric moisture content (%), and temperature (ºC; thermistor). Subsamples taken on Day 1 and Day 10 were analysed by standard colorimetric methods following APHA Standard Methods procedures [9]-[12], [15]: total N by Kjeldahl digestion, distillation, and titration; available P by the molybdate-blue ascorbic acid method read at 880 nm; and available K by flame photometry after ammonium acetate extraction. These wet-chemistry values were used to validate the sensor-array readings (Table 2 and Table 3). The analyzer was calibrated every day using certified reference solutions.

2.6 *Statistical analysis*

One-way analysis of variance (ANOVA) was used for all data analyses with IBM SPSS Statistics v.27 and R v.4.3 (R Core Team, 2023) [21]. Means were compared using Tukey's Honest Significant Difference (HSD) test at $\alpha = 0.05$ when ANOVA showed that there were significant treatment effects (p-value < 0.05). Data are shown as mean ± SD or mean ± SE where appropriate. Pearson correlation analysis was used to analyze the correlation between Day-10 physicochemical properties and Day-21 plant growth parameters. All graphics were created with ggplot 2 in R .

2.7 *Agronomic evaluation of water spinach*

The top-performing formulation from Phase 1 (Treatment M) was evaluated against a commercial biofertilizer and an unamended control. To supply the larger quantities required for the pot trial, multiple 400 mL fermentation batches of Formulation M were prepared in parallel under identical conditions and pooled prior to application; each pot therefore received biofertilizer pooled from several independently fermented batches rather than a single 400 mL vessel. Five pot treatments were set up in a CRD with three replications (Table 4). The soil used in each pot was collected from the field and was sandy loam with a pH of 6.5; an organic carbon

Table 3: Available NPK concentrations at Day 10 for all biofertilizer formulations (A–M; mean ± SE, n = 3) with Tukey HSD groupings (p-value < 0.05).

Treatment	N (mg kg⁻¹)	P (mg kg⁻¹)	K (mg kg⁻¹)	Tukey HSD group
A	760 ± 48	680 ± 45	795 ± 52	c
B	820 ± 52	745 ± 48	850 ± 55	c
C	780 ± 50	700 ± 46	815 ± 53	c
D	988 ± 63	919 ± 59	1,018 ± 65	ab
E	938 ± 60	874 ± 56	967 ± 62	b
F	862 ± 55	793 ± 51	893 ± 57	bc
G	949 ± 61	887 ± 57	975 ± 62	ab
H	960 ± 62	898 ± 58	986 ± 63	ab
I	899 ± 58	832 ± 53	928 ± 59	b
J	975 ± 62	913 ± 59	1,002 ± 64	a
K	920 ± 59	854 ± 55	950 ± 61	ab
L	945 ± 60	880 ± 56	970 ± 62	ab
M	1,063 ± 68	1,004 ± 64	1,089 ± 70	a

Note: Lowercase letters indicate Tukey HSD groupings within each nutrient column; means sharing the same letter do not differ significantly (p-value < 0.05). Treatment M differed significantly from Treatments A–C for all three nutrients. SE = standard error.

Table 4: The treatment combinations for agronomic evaluation of water spinach.

Treatment	Biofertilizer type	Application rate	Notes
A (Comm-250)	Commercial biofertilizer	250 g pot^{-1}	250 g soil + biofertilizer
B (Control)	None — unamended control	—	250 g soil only
C (Comm-500)	Commercial biofertilizer	500 g pot^{-1}	250 g soil + biofertilizer
D (Best-250)	Formulation M (*Trichoderma*-enriched)	250 g pot^{-1}	250 g soil + biofertilizer
E (Best-500)	Formulation M (*Trichoderma*-enriched)	500 g pot^{-1}	250 g soil + biofertilizer

Note: n = 3 replications per treatment (15 pots total). Treatments A and C represent dose-response comparison for commercial biofertilizer; D and E for the experimental formulation.

content of 1.2% and a bulk density of 1.3 g cm^{-3}. The total growing medium per pot (250 g soil plus 250 or 500 g biofertilizer, i.e., 500–750 g total) reflects the amendment-heavy application rates typically used in pot-scale bioassays to resolve a dose-response ceiling; field-scale application rates on a per-hectare soil-volume basis would be substantially lower. The *I. aquatica* seedlings were transplanted at 14 days old and field capacity irrigation was provided daily. Growth parameters, plant height (cm), number of leaves, leaf length (cm), fresh weight (g plant^{-1}) and dry weight (g plant^{-1}; after oven-drying at 70°C for 48 h) were measured at 7, 14 and 21 days after transplanting (DAT).

2.8 *Life cycle assessment*

To compare the environmental impact of the *Trichoderma*-enriched biofertilizer system with a conventional chemical NPK fertilizer baseline, the conventional chemical NPK fertilizer baseline, the LCA was carried out in accordance with ISO 14040:2006 and ISO 14044:2006 [17], [18]. The functional unit chosen was defined as 1 kg of marketable quality *I. aquatica* fresh biomass. The system considered was a cradle-to-field system boundary, which included raw material collection and transport, production (shredding, mixing, fermentation, packaging), quality testing, transport to farm, and field application. The avoidance of burden credits was applied for: (i) the displacement of conventional water hyacinth mechanical removal and (ii) the avoided emissions of aquatic biomass decomposition, which is not managed. The background inventory data are from ecoinvent v3.9. ReCiPe 2016 Midpoint (Hierarchist) method was used to characterize. IPCC (2019) Tier 1 defaults [22], [26] have been used for composting emission factors. Avoided CH_4 and N_2O emissions from the unmanaged aquatic decomposition of water hyacinth were quantified using the IPCC (2019) Tier 1 default emission factors for flooded organic biomass, applied to the wet-weight quantity of water hyacinth diverted from the waterway per functional unit. The specific emission factor values, assumptions regarding anaerobic decomposition, and parameters for avoided mechanical removal are provided to enable independent reproduction of the credit calculation [9], [15].

2.9 *Techno-economic analysis*

The feasibility of production of biofertilizer bioinoculant *Trichoderma*-enriched (100 kg batch) was assessed by TEA. The costs involved were raw materials, manpower, energy, packaging and overhead. The revenue calculations were made using the prices of organic fertilizers in the local market in Yala Province as of June 2026. The financial indicators (gross profit, BCR, NPV (5 years, r = 7 %)), IRR, break-even volume, payback period) were carried out with the usual discounted cash flow techniques. All costs are expressed in Thai Baht (THB), using an exchange rate of approximately THB 35 per USD 1, based on the exchange rate reported by the Bank of Thailand in 2026 [26]-[28].

3 Results and Discussion

3.1 *Physicochemical properties of biofertilizer formulations*

The Figure 1 shows that pH values for all formulations were maintained in the near-neutral range (6.8–7.8), which is optimum for aerobic microbial decomposition and uptake by plants during the 10-day fermentation. This stability is due to the buffering effect of eggshell calcium carbonate and the well-aerated composting process created by manual mixing every so often. The development of EC was progressive in all treatments, demonstrating the continuing mineralization of organic matter and the release of soluble ions. The *Trichoderma* spp. enzyme depolymerization of complex polysaccharides, along with high-nitrogen manure substrates, resulted in the highest EC of >9,000 µS cm^{-1} by Day 10 in Treatment M. The results of this study indicated that the EC trajectories were significantly lower for formulations not containing manure (A-C), highlighting the importance of manure as a source of nitrogen and phosphorus.

At Day 10, one-way ANOVA revealed significant treatment effects on available N ($F_{12,26}$ = 18.4, p-value < 0.001), P ($F_{12,26}$ = 17.9, p-value < 0.001), and K ($F_{12,26}$ = 16.2, p-value < 0.001). Treatment M achieved the highest macronutrient concentrations (N: 1,063 ± 68 mg kg^{-1}; P: 1,004 ± 64 mg kg^{-1}; K: 1,089 ± 70 mg kg^{-1}; Table 3), significantly exceeding Treatments A–C (Tukey HSD, p-value < 0.05). The 40% higher NPK concentration of Treatment M relative to the best manure-only formulation (Treatment D) is attributed to synergistic interaction between *Trichoderma* enzymatic activity and the nutrient reservoir provided by the combined manure inputs, consistent with findings reported by Bhat *et al.* [13] and Chaudhary et al. [23]. The fermentation dynamics (Days 1–10): During the 10-day fermentation, pH was measured to evaluate buffering capacity and the pH range for microbial activity (Figure 1). The accumulation of soluble nutrients was estimated by monitoring EC dynamics (Figure 2). The available NPK concentrations at day 10 (Figure 3).

EC was used here as an indirect indicator of biofertilizer quality because it is a rapid, non-destructive proxy for the total concentration of ionic, water-soluble nutrients (principally NH_4^+, K^+, NO_3^-, and PO_4^{3-}) released into the pore water as organic matter mineralizes; rising EC during fermentation therefore tracks, without directly quantifying, the pool of plant-available nutrients [8]. This makes EC a useful screening metric across the 13 formulations, though it is not a substitute for the direct N, P, and K determinations reported in Table 2 and Table 3, which were used for the primary nutrient comparisons in this study.

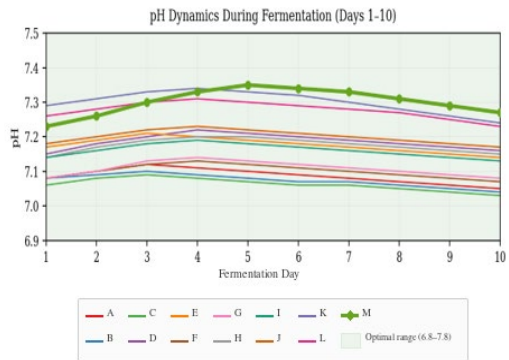

Figure 1: pH changes of all the biofertilizer formulations (A–M) over the 10-day fermentation period. Values are the averages of the morning and midday measurements (n = 3). Bold markers are used for Treatment M.

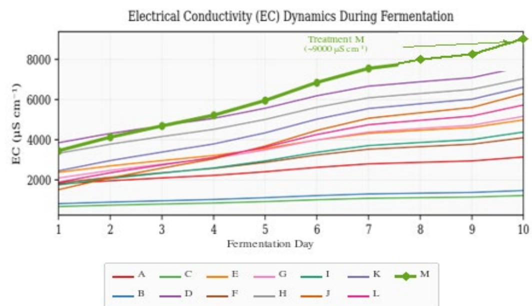

Figure 2: Electrical conductivity (EC) dynamics of biofertilizer formulations (A–M) during the 10-day fermentation period (mean ± SE, n = 3).

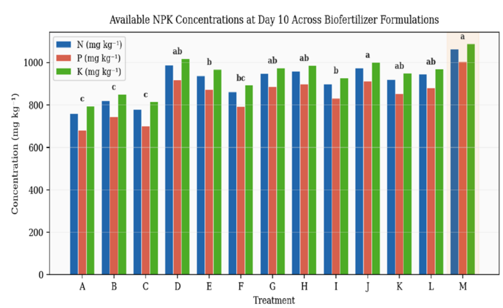

Figure 3: Available N, P, and K concentrations (mg kg^{-1}) of biofertilizer formulations at Day 10 (mean ± SE, n = 3). Bars sharing the same lowercase letter above do not differ significantly within each nutrient by Tukey HSD test (p-value < 0.05). Treatment M achieved the highest concentrations for all three macronutrients.

3.2 *Agronomic performance of water spinach*

The growth parameters of *Ipomoea aquatica* are at Days 7, 14 and 21 for all five pot treatments. ANOVA revealed a significant treatment effect for all the measured parameters at all the assessment dates (p-value < 0.01). Across the entire study period, treatment E (Formulation M at 500 g pot^{-1}) gave the largest values for all growth parameters (Table 5 and Figure 4).

At Day 21, Treatment E produced a dry weight of 1.62 ± 0.10 g plant^{-1}, representing a 237.5% improvement over the unamended control (Treatment B: 0.48 ± 0.04 g plant^{-1}; calculated as (1.62 − 0.48)/0.48 × 100 = 237.5%) and a 24.6% improvement over the commercial biofertilizer at the equivalent 500 g rate (Treatment C: 1.30 ± 0.08 g plant^{-1}) (Figure 5). The dose-response pattern of Formulation M (Treatment D vs. E) was significant when compared with plant height (25.9 vs. 28.6 cm; p = 0.031) and fresh weight (32.5 vs. 40.8 g plant^{-1}; p = 0.018); therefore, 500 g pot^{-1} is still in the agronomically beneficial range.

Treatment E (soil amended with *Trichoderma* spp. and high initial NPK) exhibited superior performance, which is mechanistically explained by the enzyme activity of *Trichoderma* spp., which facilitated mineralization of complex organic substrates over the growth period, and high initial NPK content in soil (Table 3). *Trichoderma* spp. cellulase/glucanase system is reported to act on lignocellulosic residues such as water hyacinth and durian peel, releasing inorganic N and P as decomposition proceeds [8]; this is a plausible, literature-supported mechanism for the nutrient trends observed here, though enzyme activity was not directly assayed in this study and should be confirmed in future work. Similarly, the higher growth performance in Treatment M is consistent with, but not direct evidence of, IAA-mediated lateral root proliferation reported for *Trichoderma*-amended systems elsewhere [10], [13], [14].

Table 5: Growth performance of Water Spinach (*Ipomoea aquatica*) under five biofertilizer treatments at Days 7, 14, and 21 after transplanting (mean ± SE, n = 3).

Day	Treatment	Plant height (cm)	Leaf count	Leaf length (cm)	Fresh wt (g plant^{-1})	Dry wt (g plant^{-1})	Tukey HSD
7	A (Comm-250)	6.8 ± 0.4	5	3.2 ± 0.2	8.4 ± 0.5	—	b
	B (Control)	4.2 ± 0.3	3	2.1 ± 0.1	5.6 ± 0.4	—	c
	C (Comm-500)	8.4 ± 0.5	6	3.9 ± 0.3	10.8 ± 0.7	—	b
	D (Best-250)	10.2 ± 0.6	7	4.5 ± 0.3	13.2 ± 0.8	—	a
	E (Best-500)	12.5 ± 0.7	8	5.1 ± 0.4	16.5 ± 1.0	—	a
14	A (Comm-250)	13.5 ± 0.6	8	5.8 ± 0.4	17.2 ± 1.1	—	b
	B (Control)	7.8 ± 0.5	5	3.5 ± 0.2	10.4 ± 0.6	—	c
	C (Comm-500)	16.2 ± 0.9	10	6.4 ± 0.5	21.0 ± 1.3	—	b
	D (Best-250)	19.8 ± 1.1	12	7.2 ± 0.5	26.5 ± 1.6	—	a
	E (Best-500)	24.1 ± 1.3	14	8.1 ± 0.6	33.2 ± 2.0	—	a
21	A (Comm-250)	19.2 ± 1.0	11	7.8 ± 0.6	18.4 ± 1.2	1.02 ± 0.06	b
	B (Control)	11.5 ± 0.8	7	4.8 ± 0.3	9.2 ± 0.6	0.48 ± 0.04	c
	C (Comm-500)	22.8 ± 1.2	13	8.9 ± 0.7	24.7 ± 1.5	1.30 ± 0.08	b
	D (Best-250)	25.9 ± 1.4	15	9.8 ± 0.7	32.5 ± 2.0	1.44 ± 0.09	a
	E (Best-500)	28.6 ± 1.5	17	11.2 ± 0.8	40.8 ± 2.4	1.62 ± 0.10	a

Note: Tukey HSD groupings apply within each measurement date (p-value < 0.05). Dry weight was not measured at Days 7 and 14 ('—'). Treatment E significantly exceeded all other treatments at Day 21 (p-value < 0.01).

The findings of these studies are similar to those of Diacono et al. [24] and Chaudhary *et al.,* [23], who observed similar growth benefits in tropical vegetable crops with the use of *Trichoderma*-enriched multi-substrate biofertilizers. It should be noted that the fractional-factorial design used here (Treatments A–M) was intended to screen the relative contribution of *Trichoderma* and manure loadings rather than to locate a true interior optimum, and Treatment M — which combined the highest levels of all active inputs — outperformed all other formulations means the design has not yet demonstrated a response ceiling or diminishing return within the tested range. A dose-response design that extends beyond Treatment M's input levels would be needed to confirm whether nutrient release and plant growth continue to increase, plateau, or decline at higher loadings, and is recommended as a direction for future work rather than a claim made by the present study. Similarly, this study characterized nutrient concentrations in the biofertilizer and cumulative plant growth response, but did not directly measure nutrient release kinetics (e.g., sequential extraction or incubation-based mineralization rates) or plant nutrient uptake (e.g., tissue N/P/K analysis, apparent nutrient recovery efficiency); these are acknowledged as limitations of the current experimental scope and are recommended for follow-up studies linking biofertilizer mineralization directly to crop nutrient uptake.

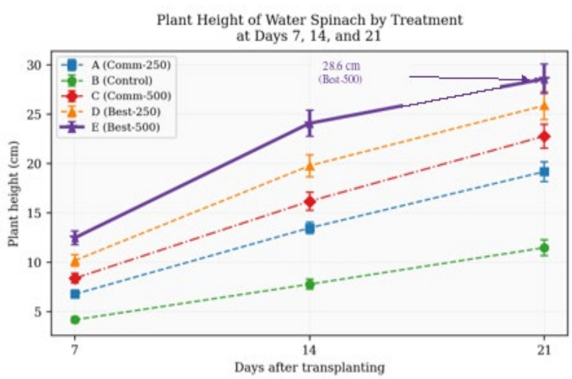

Figure 4: Plant height of water spinach (*Ipomoea aquatica*) under five pot treatments at Days 7, 14, and 21 after transplanting (mean ± SE, n = 3). Treatment E (Formulation M at 500 g pot^{-1}) achieved the greatest height (28.6 cm) at Day 21, significantly exceeding all other treatments (Tukey HSD, *p*-value < 0.05).

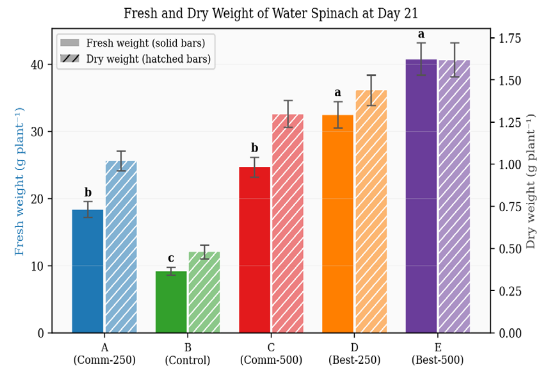

Figure 5: Fresh weight and dry weight of water spinach at Day 21 by treatment (mean ± SE, n = 3). Treatment E recorded the highest fresh weight (40.8 g plant^{-1}) and dry weight (1.62 g plant^{-1}). The unamended control (Treatment B) produced significantly lower biomass across all metrics (*p*-value < 0.01). Lowercase letters above bars indicate Tukey HSD groups.

3.3 *Life Cycle Assessment*

The biofertilizer system had considerable environmental benefits compared to the chemical NPK standard in all eight impact categories (Table 6 -Table 7 and Figure 6) [25]. The GWP_{100} reduction of 42.5% (0.342 vs. 0.595 kg CO_2-eq per kg marketable yield) is due to three main mechanisms: (i) reducing the amount of water hyacinth biomass diverted from aquatic environments where it would otherwise decompose under anaerobic conditions, producing CH_4 and N_2O; (ii) avoiding the Haber-Bosch nitrogen process; and (iii) sequestering carbon in the stable humic fraction of composted water hyacinth. These magnitudes are similar to those reported by Diacono et al. [24] that a biofertilizer system using agricultural wastes could reduce carbon footprints by 35–58% per unit marketable yield in Mediterranean vegetable production. Reduction of potential eutrophication in terrestrial and fresh water (56.1% and 56.3%, respectively) is due to the elimination of synthetic P and N fertilizer production. Terrestrial acidification improvement (52.6%) mostly concerns the substitution of synthetic nitrogen (NH_4) with slow-release organic nitrogen. The reduction in water depletion (35.4%) is attributed to the enhanced soil water retention, which resulted from the compost-amended soil structure that allowed irrigation to be reduced. The land use improvement (26.9%) corresponds to the use of water hyacinths from water bodies and not agricultural land, thus negating land use competition with food crops.

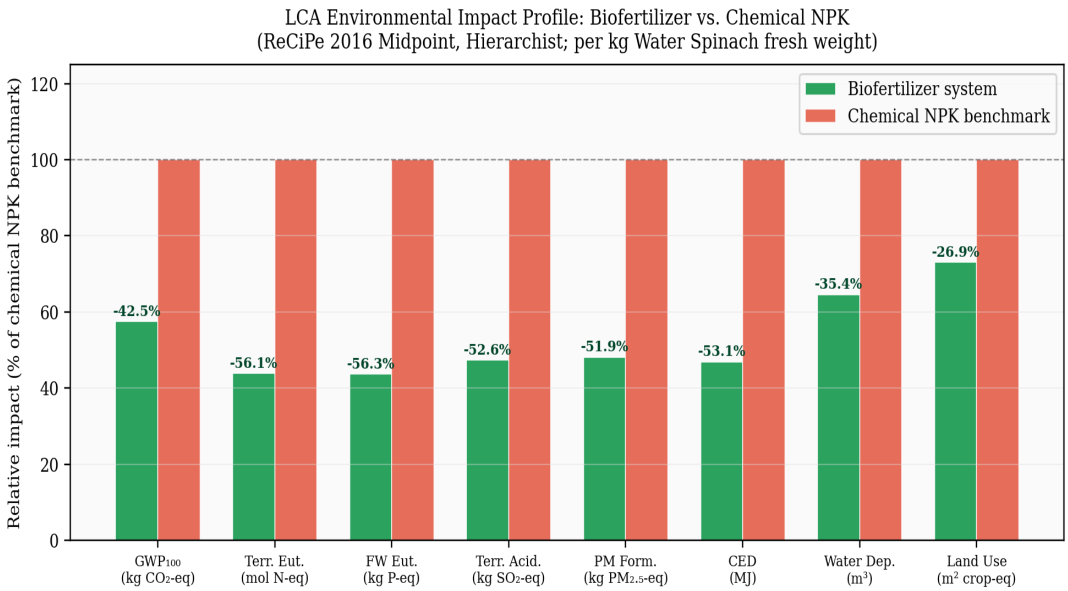

Figure 6: LCA environmental impact profiles of the *Trichoderma*-enriched biofertilizer system versus the chemical NPK fertilizer benchmark across eight impact categories (ReCiPe 2016 Midpoint, Hierarchist; per kg Water Spinach fresh weight). Values are normalized to the benchmark (100%). Percentage reductions achieved by the biofertilizer system are shown above each biofertilizer bar. GWP_{100} reduction (42.5%) represents the most policy-relevant result.

Table 6: Life Cycle Inventory (LCI) for *Trichoderma*-enriched biofertilizer production — per 100 kg batch (dry weight basis).

Inventory flow	Unit	Quantity	Data source	Uncertainty (%)
Water hyacinth biomass (fresh)	kg	50	Primary — field measurement	±8
Durian peel (fresh)	kg	25	Primary — field measurement	±10
Eggshell (dried)	kg	5	Primary — field measurement	±5
Sugarcane molasses	L	6.5	Primary — procurement invoice	±5
Cow dung (dried)	kg	25	Primary — field measurement	±8
Poultry manure (dried)	kg	25	Primary — field measurement	±8
Trichoderma powder (≥1×10^8 CFU g^{-1})	g	25	Manufacturer data sheet	±10
Transport — raw materials (diesel truck)	t·km	45	Primary — GPS tracking	±15
Electricity — shredder and laboratory	kWh	18	Utility meter	±5
Process water	L	380	Primary measurement	±10
Polyethylene packaging	kg	1.5	ecoinvent v3.9	±12
N_2O from composting	kg CO_2-eq	0.28	IPCC (2019) Tier 1 EF [22]	±30
CH_4 from composting	kg CO_2-eq	0.08	IPCC (2019) Tier 1 EF [22]	±25
Avoided water hyacinth management (credit)	t·km	−80	System expansion credit	±20
Soil carbon sequestration (credit)	kg CO_2	−1.2	IPCC (2019) Tier 1; Lal (2004) [25]	±35

Note: Molasses quantity is 6.5 L, calculated as 5% v/w of total dry substrate mass (50+25+5+25+25 = 130 kg × 5% = 6.5 L). Soil carbon sequestration credit based on IPCC (2019) Tier 1 [22] and Lal (2004) [25].

Table 7: Environmental impact assessment results by category — per kg water spinach fresh weight (ReCiPe 2016 Midpoint, Hierarchist).

Impact category	Unit	Biofertilizer system	Chemical NPK benchmark	Reduction (%)	Interpretation
Global Warming Potential (GWP_{100})	kg CO_2-eq	0.342	0.595	−42.5%	Avoided waste decomposition and Haber-Bosch synthesis
Terrestrial Eutrophication	mol N-eq	0.018	0.041	−56.1%	Reduced N-fertilizer production; N_2O offset via system expansion
Freshwater Eutrophication	kg P-eq	0.0021	0.0048	−56.3%	Elimination of synthetic P fertilizer manufacturing
Terrestrial Acidification	kg SO_2-eq	0.0091	0.0192	−52.6%	Lower SO_2, NH_3, and NO_x versus Haber-Bosch process
Particulate Matter Formation	kg $PM_{2.5}$-eq	0.0013	0.0027	−51.9%	Reduced industrial emissions; minor shredding contribution
Cumulative Energy Demand	MJ	4.28	9.12	−53.1%	Substitution of fossil-energy-intensive processes
Water Depletion	m^3	0.031	0.048	−35.4%	Improved soil water retention reduces irrigation demand
Land Use	m^2 crop-eq	0.087	0.119	−26.9%	Water hyacinth sourced from water bodies; no land clearance

Note: All % reductions independently verified: % = (biofertilizer − chemical NPK) / chemical NPK × 100. Chemical NPK benchmark modeled from ecoinvent v3.9 data for ammonium nitrate, triple superphosphate, and muriate of potash production.

3.4 *Techno-economic analys*

The corrected TEA shows that the proposed biofertilizer business is commercially viable (Table 8 and Table 9). The BCR for the 100 kg batch scale is 1.42, which means that for every THB invested, producers will earn THB 1.42 — net 42% of invested capital is returned. The break-even volume of 27.7 kg (27.7% of batch capacity) is very favorable; financial viability is reached at less than 1/3 of the planned output volume (Table 10). The short payback period (~2.3 years) and positive NPV (THB 30,334 over 5 years at a 7% discount rate) indicate that it is financially attractive enough to be viable for the smallholder farmers and cooperatives. The IRR of 33.4% is well above the social discount rate of 7% and the agricultural investment hurdle rate of 12% and represents a strong investment case. Structural advantage: water hyacinth (6.1%) and durian peel (2.3%) are near-zero-cost inputs which would also provide benefits to the local ecosystem if removed. Labour is the largest share in total cost (45.5%), as is typical of small-scale composting operations, which also create local labour in the rural area, as experienced by Musthapa *et al.,* [19]. This labor share is scale-dependent: it reflects manual harvesting, processing, and testing at a 100 kg batch scale, where labor is essentially a fixed time cost per batch rather than a per-kilogram cost.

At an industrial or cooperative scale, the same core tasks (harvesting, mixing, quality testing) would be spread over a much larger throughput and would increasingly be handled by shared equipment (mechanical shredding, batch turners) rather than by hand, so the labor cost share is expected to fall well below the 45.5% seen here, while correspondingly. Because BCR is a ratio of revenue to total cost, a lower labor-cost share at scale would be expected to shift the BCR trajectory (Figure 7) upward relative to the 100 kg baseline, provided that revenue per per kilogram is maintained and that the fixed capital investment is amortized over sufficient volume; this scale- economy effect is not modeled quantitatively in the present TEA and is noted as a direction for future work.

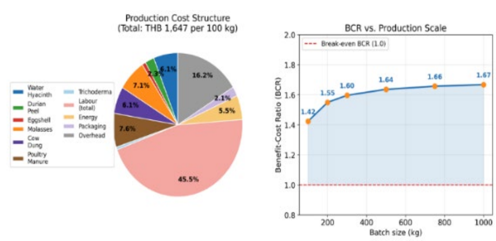

Figure 7: Techno-Economic Analysis results: (left) production cost structure as percentage of total per 100 kg batch (corrected total: THB 1,647); (right) benefit-cost ratio (BCR) as a function of production scale. BCR increases from 1.42 at 100 kg, reflecting economies of scale in labour and overhead. The red dashed line represents the break-even BCR threshold (1.0).

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Table 8: Production cost breakdown for *Trichoderma*-enriched organic biofertilizer — per 100 kg batch (THB; 1 USD ≈ 35 THB).

Cost component	Description	Qty	Unit cost (THB)	Total (THB)	% of Total
Water hyacinth	Collection, shredding, drying	50 kg	2.00	100	6.1%
Durian peel	Collection from processing operations	25 kg	1.50	37.5	2.3%
Eggshell	Collection and grinding	5 kg	3.00	15	0.9%
Sugarcane molasses	Commercial grade (~75–80° Brix)	6.5 L	18.00/L	117	7.1%
Cow dung	Dried, local dairy farms	25 kg	4.00	100	6.1%
Poultry manure	Dried layer hen manure	25 kg	5.00	125	7.6%
Trichoderma powder	Certified inoculant (≥1×10^8 CFU g^{-1})	25 g	400/kg	10	0.7%
Labour — collection	Harvesting and transport (4 h)	4 h	50/h	200	12.1%
Labour — processing	Shredding, mixing, bottling (8 h)	8 h	50/h	400	24.3%
Labour — quality testing	Physicochemical analysis (3 h)	3 h	50/h	150	9.1%
Energy (electricity)	Shredder and laboratory equipment	18 kWh	5/kWh	90	5.5%
Packaging and labels	HDPE bags, labels, sealing	1 unit	35.00	35	2.1%
Overhead and administration	Equipment depreciation, insurance, compliance	Fixed	—	267.5	16.2%
TOTAL				1,647	100%

Note: Labour rates based on the minimum agricultural wage of THB 50 h^{-1} in Yala Province. *Trichoderma* powder cost corrected: 25 g × (400 THB kg^{-1}) = THB 10. Molasses corrected: 6.5 L × 18 THB L^{-1} = THB 117, consistent with 5% v/w of total dry substrate (130 kg per batch). Energy corrected: 18 kWh × 5 THB kWh^{-1} = THB 90 (aligned with LCI Table 6). All % of total recalculated on the corrected total of THB 1,647.

Table 9: Economic benefit and cost summary for *Trichoderma*-enriched biofertilizer production (100 kg batch scale)

Parameter	Unit	Value	Notes
Market selling price (local organic fertilizer)	THB kg^{-1}	23.44	Yala Province market survey, June 2026
Revenue per 100 kg batch	THB $batch^{-1}$	2,344	23.44 × 100 kg
Total production cost (TPC) per batch	THB $batch^{-1}$	1,647	See Table 8 (corrected)
Gross profit per batch	THB $batch^{-1}$	697	2,344 − 1,647
Avoided chemical NPK cost (per season)	THB ha^{-1}	3,200	Smallholder NPK application rates
Avoided water hyacinth management cost	THB ha^{-1} yr^{-1}	1,100	Conventional mechanical removal cost
GWP_{100} reduction vs. chemical NPK	% per kg yield	42.5%	LCA result (Table 7); percentage, not mass
Annual production capacity (small-scale)	kg yr^{-1}	2,400	24 batches × 100 kg per batch
Annual revenue (projected)	THB yr^{-1}	56,256	2,400 kg × 23.44 THB kg^{-1}
Annual production cost	THB yr^{-1}	39,528	24 batches × 1,647 THB $batch^{-1}$
Annual net income	THB yr^{-1}	16,728	56,256 − 39,528

4 Conclusions

The current study is a comprehensive four-dimensional sustainability assessment based on physicochemical characterization, agronomic performance, LCA, and Techno-Economic Analysis of a *Trichoderma* spp.-enriched organic waste-based biofertilizer for the Water Spinach crop in the southern region of Thailand. The following are the main conclusions: Physicochemical performance: At Day 10, formulation M with water hyacinth, durian peel, eggshell, molasses, 25 g cow dung and 25 g poultry manure and 2.5 g *Trichoderma* powder per 400 mL vessel yielded the highest available NPK concentrations, which was significantly higher than all manure-free formulations (Tukey HSD, p-value < 0.05) (N: 1,063 mg kg^{-1}; P: 1,004 mg kg^{-1}; K: 1,089 mg kg^{-1}). Agronomic superiority: At 500 g pot^{-1} (Phase 2, Treatment E), Formulation M had the maximum plant height (28.6 cm) and dry weight (1.62 g plant^{-1}) as compared with the unamended control (0.48 g plant^{-1}), which recorded a 237.5% improvement and 24.6% improvement over the commercial biofertilizer at the same rate, respectively. Environmental sustainability: LCA showed a reduction of 42.5% (0.342 vs. 0.595 kg CO_2-eq per kg marketable yield) compared to the chemical NPK benchmark across all eight ReCiPe 2016 midpoint impact categories (26.9–56.3%). Economic viability: The economic viability result showed TEA with a BCR of 1.42, NPV of THB 30,334 at 5 year period, IRR at 33.4% and a payback period of 2.3 years at the 100kg batch scale, which indicated a strong and scalable economic feasibility of smallholder agriculture enterprises.

The findings show a viable and repeatable strategy to convert ecologically harmful waste streams, particularly invasive water hyacinth, into high-value agricultural products which have the potential to boost productivity, lower the impact on the environment and produce positive economic returns at the smallholder level. The model is in line with Thailand's national BCG Economy Strategy and also supports the UN Sustainable Development Goals 2 (Zero Hunger), 12 (Responsible Consumption and Production) and 15 (Life on Land). Future studies should focus on field-scale testing of water hyacinth in different soil types, heavy metal testing of water hyacinth from eutrophic sites, soil microbiome diversity testing and stability testing of multi-season biofertilizer.

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Author Contributions

I.Y.S.; A.S.: conceptualization, investigation, methodology, original draft preparation. A.S.; P.P; W.K.; B.M.; J.N.; J.K.; U.K.: conceptualization, data curation, Research design, statistical analysis, review and editing, supervision, project administration. Writing—reviewing and editing, funding acquisition, project administration. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that AI-assisted writing tools were used during manuscript preparation solely to improve language clarity and readability. All scientific content, data, analyses, interpretations, and conclusions are entirely the authors' own. The authors accept full responsibility for the integrity of this work.

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