

Optimizing Input Materials to Enhance Egg-Based Fertilizer Quality and Assessing Its Performance in Malabar Spinach (*Basella alba*) Cultivation

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Abstracts

This study explored a sustainable approach to agricultural waste management by converting spoiled eggs into high-quality liquid fertilizers. Egg-based fertilizers (EFs) produced from different input compositions were characterized for their physicochemical properties, and the most suitable EF was further evaluated for its effects on the growth, physiological performance, and yield of Malabar spinach (*Basella alba*). Three treatments were prepared: EF1 (spoiled eggs without chicks), EF2 (spoiled eggs with chicks), and EF3 (a 1:1 mixture of both). EF1 contained the highest levels of total nitrogen (6.09 g L^{-1}), total and available phosphorus (1.05 and $0.80 \text{ g L}^{-1} \text{ P}_2\text{O}_5$, respectively), and total calcium ($3.79 \text{ g L}^{-1} \text{ CaO}$), with no detectable *Escherichia coli* or *Salmonella*. EF3 exhibited the highest potassium content (1.16 g L^{-1} total and 1.04 g L^{-1} available K_2O). In a field trial, EF1 was applied at 1.0%, 1.5%, and 2.0%, and compared with a commercial organic fish fertilizer and an unfertilized control. Applications of EF1 at 1.5% significantly enhanced shoot length, leaf dimensions, SPAD, leaf area index (LAI), and yield (6.3 and $11.9 \text{ tons ha}^{-1}$ at 21 and 37 days after planting, respectively). Yield showed strong positive correlations with leaf length ($r = 0.96^{**}$), leaf width ($r = 0.92^{**}$), SPAD ($r = 0.84^{**}$), and LAI ($r = 0.90^{**}$), supporting the observed treatment effects. Nitrate concentrations in all the treatments remained below 150 mg kg^{-1} , within safe limits for leafy vegetables. Overall, EF1 at 1.5% represents a safe, effective, and sustainable fertilization option for Malabar spinach production.

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Keywords

Egg fertilizer, malabar spinach, spoiled eggs, yield

Introduction

Poultry is the fastest-growing sector in agriculture, particularly in developing countries, and is projected to continue expanding

globally as demand for meat and eggs increases with population growth, rising incomes, and urbanization (Mottet & Tempio, 2017). Global egg production increased from 73.9 million metric tons in 2016 to 82.17 million metric tons in 2019 (Awogbemi *et al.*, 2022). In Vietnam, egg production reached approximately 473,660 tons in 2020 (Vu *et al.*, 2022).

Along with the rapid expansion, generated waste from the poultry industry has also increased. Solid hatchery waste typically includes empty shells, infertile eggs, dead embryos, late hatchlings, and decaying tissues (Glatz *et al.*, 2011). Conventional disposal methods for solid hatchery waste include landfilling, composting, rendering, and incineration (Das *et al.*, 2002). However, landfilling has been restricted or banned in several countries due to limited space and environmental concerns (Kim & Kim, 2010). Direct applications of animal production waste to soil can cause nitrogen loss, eutrophication, pathogen spread, and greenhouse gas emissions (Williams *et al.*, 1999). Recycling these wastes into value-added products can reduce disposal costs, mitigate environmental impacts, and generate additional income for poultry farms (Musa & Ahmed, 2020).

Egg-based fertilizers (EFs) can be produced in a solid or liquid form. Eggshells, composed of approximately 94% calcium carbonate along with trace amounts of magnesium, phosphorus, and organic matter (Johnson & Liu, 2019), can be converted into powdered or liquid fertilizers via simple treatments (Awogbemi *et al.*, 2022). Their applications have improved crop growth in species such as groundnut (*Arachis hypogaea* L.) (Vu *et al.*, 2022) and sweet basil (*Ocimum basilicum* L.) (Wijaya & Teo, 2019). Hatchery waste contains high moisture levels (43-71%) and a substantial protein content. After drying, it typically comprises 33.1% crude protein, 29.0% ether extract, 12.1% crude fiber, 21.5% ash, and 28.8 MJ kg⁻¹ gross energy (Glatz *et al.*, 2011). The nutrient composition of "spoiled eggs" suggests their potential as organic fertilizers, though research on their use remains limited compared to eggshells. The high moisture contents of dead-in-shell eggs (41.51%) and

unfertilized eggs (21.09%) require careful drying to prevent spoilage during fertilizer production (Lipdo *et al.*, 2024). Moisture control is also essential to limit microbial growth and deterioration in eggshell-based materials (Olawale *et al.*, 2021). Among the types of EFs, liquid formulations are generally preferred for easier application, uniform distribution, and greater nutrient availability (Indrarosa, 2018).

This study aimed to evaluate the quality of liquid fertilizers prepared from different inputs of spoiled eggs from poultry hatchery waste and assess their effects on the growth and yield of Malabar spinach (*Basella alba*), a leafy vegetable rich in essential nutrients (USDA, 2019), and widely cultivated in Vietnam. The findings could contribute to improving hatchery waste management, and supporting the sustainable and environmentally responsible development of the poultry industry.

Materials and Methods

Research site

The experiments were conducted during the spring-summer season (February-June 2025) at the research farm of the Faculty of Agronomy, Vietnam National University of Agriculture (VNUA) (21°00' N, 105°55' E), with Experiment 1 performed in a net house and Experiment 2 conducted in the field. The experimental soil was classified as alluvial (IUSS Working Group WRB, 2015). The initial soil characteristics were as follows: 7.82 pH (H₂O); 1.91% total organic matter; 0.11% total nitrogen (N); 0.29% total phosphorus (P₂O₅); 2.10% total potassium (K₂O); 104.06 mg 100 g⁻¹ of available phosphorus (P₂O₅); and 9.6 mg 100 g⁻¹ of available potassium (K₂O).

Materials

Spoiled eggs were collected from a commercial poultry hatchery in Thuong Tin district, Hanoi, washed with tap water, and classified into two groups based on the visible developmental stage: (1) infertile eggs or eggs with early embryonic mortality, hereafter referred to as "spoiled eggs without chicks"; and (2) late-stage embryonic failure or shell-bound

eggs containing fully formed chick remains, referred to as "spoiled eggs with chicks".

The *Basella alba* variety used, MTNP11, was supplied by the Tan Nong Phat Production and Trading Co., Ltd. It is noted for disease resistance, uniform growth, sweet tender flavor, large thick round leaves, and dark green coloration.

The microbial inoculant Emuniv (Applied Microbiology JSC) contains *Trichoderma viride*, *Bacillus subtilis*, *Streptomyces murinus*, and *Lactobacillus plantarum* ($>10^8$ CFU g^{-1}), and is commonly used to enhance organic matter decomposition and suppress harmful microorganisms.

Experimental design and sampling parameters

Experiment 1: Assessment of egg-based fertilizer quality from different input materials

A one-factor experiment was conducted to evaluate the chemical and microbiological characteristics of EFs derived from different egg sources. Three types of EFs were tested:

EF1: 100% spoiled eggs without chicks

EF2: 100% spoiled eggs with chicks

EF3: A 1:1 mixture of spoiled eggs with and without chicks

Each treatment had three replicates. In each replicate, 3kg of spoiled eggs were mixed with 10L of water, 50g of Emuniv inoculant, and 0.5L of molasses. The ratio was based on commonly practiced formulations obtained through consultations with organic vegetable farmers experienced in fermenting spoiled eggs for fertilizer production. The mixture was homogenized to break the eggs and facilitate decomposition, then stored in sealed containers and stirred once or twice daily.

After two months, a distinct liquid phase formed above the sediment of eggshells and residues. The liquids, termed the EFs, were filtered, and the liquid fractions from the three replicates of each treatment were homogenized and pooled into one composite sample for analysis of total N, total P_2O_5 , total K_2O , total calcium (CaO), available P_2O_5 , available K_2O , and the presence of *Escherichia coli* and *Salmonella* spp., according to the following

standards: TCVN 8557:2010, TCVN 8563:2010, TCVN 8662:2010, TCVN 9284:2012, TCVN 8559:2010, TCVN 8559:2010, TCVN 6187-2:2020, and TCVN 10780-1:2017, respectively. Analyses were performed at the Laboratory of Soil and Fertilizer Quality Analysis, Faculty of Natural Resources and Environment, VNUA.

Experiment 2: Effects of egg-based fertilizer concentrations on the growth and yield of Malabar spinach (B. alba)

Based on the results of experiment 1, the EF with the most favorable nutrient composition was selected for application in *B. alba* cultivation. A one-factor experiment was established using a randomized complete block design (RCBD) with three replicates. Five treatments were evaluated: T1: No fertilizer; T2: Commercial organic fish fertilizer applied at 0.5% (v/v), following the manufacturer's recommendations; T3: EF applied at 1.0% (v/v); T4: EF applied at 1.5% (v/v); and T5: EF applied at 2.0% (v/v). Seeds were sown in nursery boxes and transplanted at the 3-4 leaf stage, with a spacing of 25cm \times 25cm. Each experimental plot had an area of 3 m² (3m \times 1m). The fertilizers were applied starting at seven days after planting (DAP) and subsequently every five days until one week prior to harvest. All plots received a basal application of cow manure at a rate of 10 tons ha⁻¹. For pest control, a biological product (BIO-B) was applied weekly during the first two weeks, following the manufacturer's instructions.

Growth parameters, namely shoot length (cm), number of leaves per main stem, and leaf size (length and width, cm), were recorded weekly from five randomly selected plants per plot. Leaf length was measured from the petiole base to the leaf tip, and width was measured at the widest point perpendicular to the midrib.

Malabar spinach was harvested twice, at 21 and 37 DAP. At each harvest, above ground biomass was collected, leaving a 5-cm stem base with 2-3 remaining leaves. Five representative plants per plot were sampled for physiological assessments. SPAD values were measured using a handheld SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) on three fully expanded leaves. Leaf area index

(LAI; m² of leaf area per m² of ground area) was calculated using the formula: $LAI = (A_1 \times \text{number of plants per m}^2) / A_2 \times 100$; where A_1 is the total fresh leaf weight of a single plant (g), and A_2 is the fresh weight of 1dm² of leaves (g). The plants were then oven-dried at 80°C until a constant weight was reached to calculate the dry matter content (%). Yield (t ha⁻¹) was determined as the total fresh plant weight in each experimental plot. Nitrate content (NO₃⁻, mg kg⁻¹) was measured using the SOEKS NUC 019-01.

Statistical analysis

For experiment 2, data were analyzed using Microsoft Excel and MINITAB 16. One-way analysis of variance (ANOVA) was performed to evaluate the treatment effects, and mean differences were compared using Tukey's honest significant difference (HSD) test at a significance level of $P < 0.05$. Pearson correlation analysis was conducted to assess linear relationships among the measured parameters at $P < 0.05$ and $P < 0.01$. The data are presented as mean \pm standard deviation (SD).

Results and Discussion

The quality of egg-based fertilizers from different input materials

The quality of organic fertilizers largely depends on the composition and chemical characteristics of the raw substrates, as each contributes distinct nutrients and organic compounds (Muscolo *et al.*, 2018; Thu & Loan,

2024). The pH of the EFs ranged from 6.27 to 6.46 (**Table 1**), indicating a mildly acidic to near-neutral reaction, which is favorable for nutrient availability. EF2 recorded the highest pH, likely due to intensified ammonification during chick-tissue decomposition, which releases NH₃ and elevates the pH, a mechanism reported for animal-derived composts (Bernal *et al.*, 2009).

Regarding the nutrient content, EF1 exhibited the highest total N concentration (6.09 g L⁻¹), likely due to its simpler organic composition, which decomposes more gradually and limits N loss. EF2 had the lowest N level (5.60 g L⁻¹), which is consistent with intensified microbial decomposition of chick tissues and the higher pH promoting ammonia volatilization (Yang *et al.*, 2019). These findings align with Tombarkiewicz (2022), who reported declining macronutrients (N, C, S) as embryonic development progresses. Although dead-in-shell eggs may contain higher initial nutrient levels (Lipdo *et al.*, 2024), our findings suggest that nutrient richness at the outset does not guarantee higher nutrient retention after composting.

Phosphorus differences among the treatments also reflected embryonic development. In EF1, P remained largely in the yolk and albumen, while in EF2 and EF3, part of this P had been mobilized into embryonic tissues (Hopcroft *et al.*, 2019), reducing the residual P available for release during composting and resulting in lower total and available P₂O₅ concentrations.

Table 1. The quality of egg-based fertilizers from different input materials

Parameters	Unit	EF1	EF2	EF3
pH		6.30	6.46	6.27
Total N	g L ⁻¹	6.09	5.60	5.81
Total P ₂ O ₅	g L ⁻¹	1.05	0.57	0.75
Total K ₂ O	g L ⁻¹	1.00	1.08	1.16
Total CaO	g L ⁻¹	3.79	1.48	2.45
Available P ₂ O ₅	g L ⁻¹	0.80	0.54	0.63
Available K ₂ O	g L ⁻¹	0.86	0.98	1.04
<i>E. coli</i>	CFU mL ⁻¹	nd*	2	nd
<i>Salmonella</i>	MPN 100mL ⁻¹	nd	nd	nd

Note: *nd: not detected; EF1: 100% spoiled eggs without chicks; EF2: 100% spoiled eggs with chicks; EF3: 1:1 mixture of both types.

Potassium followed a contrasting trend. EF3 contained the highest levels of total and available K₂O (1.16 g L⁻¹ and 1.04 g L⁻¹, respectively), followed by EF2, while EF1 had the lowest values. This trend reflects the high cytoplasmic localization and rapid release of K⁺ during tissue decomposition (Andrews *et al.*, 2021), along with dry-matter loss concentrating soluble minerals (Zhen *et al.*, 2021). Fermentation-based liquid fertilizers derived from eggshell mixtures demonstrated similar behaviors (Yerizam *et al.*, 2022). In our study, EF3 likely provided more favorable moisture and decomposition conditions for K⁺ solubilization than EF2, despite EF2's higher tissue content.

In EF1, where no embryogenesis occurred, most of the calcium remained in the eggshell as soluble CaCO₃, resulting in the highest CaO concentration (3.79 g L⁻¹). In EF2 and EF3, embryonic development had converted a portion of the shell-derived Ca into less soluble hydroxyapatite (Halgrain *et al.*, 2022), limiting its transfer to the liquid phase and yielding lower CaO levels (1.48 and 2.45 g L⁻¹, respectively).

Microbial analysis showed that *E. coli* was undetectable in EF1 and EF3, while EF2 contained a low level (2 CFU mL⁻¹). *Salmonella* was absent in all the treatments, confirming that composting effectively reduced pathogenic risks.

Effects of egg-based fertilizer concentrations on the growth and yield of Malabar spinach

Based on its nutrient profile, EF1 (derived from spoiled eggs without chicks) was selected for evaluation in Malabar spinach (*Basella alba*) cultivation, as its high N, P₂O₅ and CaO contents match the nutritional needs for vegetative growth and leaf development.

Effects of egg-based fertilizer concentrations on growth parameters

No significant differences in shoot length were observed among the treatments at 7 and 14 DAP (**Table 2**), likely due to root establishment and the delayed nutrient release characteristic of organic fertilizers. By 21 DAP, plants treated with EF1 at 1.5% (T4) showed the longest shoots (24.2cm), significantly surpassing the others.

The remaining treatments produced shorter shoots (20.0-21.9cm), with no significant differences among them.

These results suggest that the 1.5% concentration provided an optimal nutrient balance, particularly of N, P, and Ca. Higher application rates (T5) likely induced osmotic stress or nutrient imbalances, while lower rates (T3) may have been nutritionally inadequate. Similar findings were reported by Pratiwi *et al.* (2023) in hydroponic mustard greens, where excessive doses of egg-based fertilizer impaired plant height due to phosphorus-induced nutrient imbalances. Moreover, high ion concentrations can cause osmotic stress, limiting water uptake and plant growth (Deinlein *et al.*, 2014), supporting the reduced performance at elevated rates.

Although the differences were not significant, T4 had the highest number of leaves (5.8, 7.8, and 10.7 leaves), while T5 had the lowest (4.9, 6.8, and 9.3 leaves) (**Table 3**). Reduced numbers of leaves at high concentrations may have been the result of disrupted Ca and Mg uptake due to nutrient excess (Pratiwi *et al.*, 2023).

Leaf size was positively influenced by the T4 treatment. Leaf length did not differ among the treatments at 7 DAP but became significantly greater in T3 and T4 (7.3 and 7.4cm, respectively) at 14 DAP compared with T1 and T5 (**Table 4**). By 21 DAP, T4 showed the longest leaves (13.1cm), surpassing the others. This enhancement may result from improved nutrient availability, particularly calcium from the eggshell-derived fertilizer, which promotes cell expansion (Hepler, 2005).

Leaf width showed a similar trend (**Table 5**). No significant differences occurred at 7 DAP, but by 14 DAP, T3 and T4 had wider leaves (6.4 and 6.3cm, respectively) than T1 (5.5cm). At 21 DAP, T4 maintained the widest leaves (11.1cm), while T1 recorded the narrowest (8.8cm). Treatments T2, T3, and T5 produced leaf widths comparable to T4. These findings confirm the positive effect of EF1 at moderate concentrations in enhancing leaf expansion, ultimately contributing to increased biomass accumulation and marketable yield.

Table 2. Effect of egg-based fertilizer concentrations on shoot length (cm)

Treatment	7DAP	14DAP	21DAP
T1	8.7 ^a ± 0.2	11.6 ^a ± 0.2	20.0 ^b ± 0.4
T2	8.4 ^a ± 0.7	11.8 ^a ± 0.5	20.4 ^b ± 1.1
T3	8.3 ^a ± 1.1	11.3 ^a ± 1.0	21.3 ^b ± 0.6
T4	8.1 ^a ± 0.1	12.1 ^a ± 0.1	24.2 ^a ± 0.6
T5	8.2 ^a ± 0.1	11.1 ^a ± 0.7	21.9 ^b ± 0.6

Note: Values within a column followed by different letters differ significantly at $P < 0.05$. DAP: days after planting; T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Table 3. Effect of egg-based fertilizer concentrations on the number of leaves per main stem

Treatment	7DAP	14DAP	21DAP
T1	5.7 ^a ± 0.6	7.3 ^a ± 0.6	10.0 ^a ± 0.5
T2	5.1 ^a ± 0.2	7.3 ^a ± 0.2	9.5 ^a ± 0.7
T3	5.2 ^a ± 0.2	7.7 ^a ± 0.6	10.0 ^a ± 0.7
T4	5.8 ^a ± 0.3	7.8 ^a ± 0.6	10.7 ^a ± 0.8
T5	4.9 ^a ± 0.5	6.8 ^a ± 0.6	9.3 ^a ± 0.4

Note: Values within a column followed by different letters differ significantly at $P < 0.05$. DAP: days after planting; T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Previous studies have also reported the positive effects of eggshell-based fertilizers on leaf number (Kim *et al.*, 2020) and plant height (Wong *et al.*, 2023). Karne *et al.* (2023) found a 30% increase in legume plant height under eggshell fertilizer compared to animal manure, reinforcing the growth-promoting potential of such inputs.

Effects of egg-based fertilizer concentrations on physiological parameters

SPAD values, representing the chlorophyll content and plant N status (Fathi, 2022), were highest in T4 at both 21 and 37 DAP (41.6 and 43.6, respectively). T1 consistently recorded the lowest SPAD values (35.7 and 39.2), while T2, T3, and T5 showed intermediate levels (**Figure 1**). These trends reflect the role of EF1 in enhancing N availability and photosynthetic potential. However, no further improvement was observed at the highest EF concentrations, indicating a nutrient saturation threshold beyond which chlorophyll synthesis was not enhanced and may have even been inhibited (Pratiwi *et al.*, 2023).

At 21 DAP, the LAI values were highest in T3 and T4 (0.97 and 1.01 $\text{m}^2 \text{m}^{-2}$, respectively), and significantly exceeded T5 (0.80 $\text{m}^2 \text{m}^{-2}$), T2 (0.76 $\text{m}^2 \text{m}^{-2}$), and T1 (0.51 $\text{m}^2 \text{m}^{-2}$) (**Figure 2**). By 37 DAP, LAI continued to increase, with T4 maintaining the highest value (1.58 $\text{m}^2 \text{m}^{-2}$), followed closely by T5 (1.52 $\text{m}^2 \text{m}^{-2}$). These results suggest that the EF1 application at 1.5% effectively supported canopy expansion. While T2 improved LAI relative to T1, its performance remained lower than the EF1-treated groups, highlighting the latter's superior nutrient support. Over-fertilization, however, may risk nutrient imbalance and osmotic stress that limit canopy development (Szabó *et al.*, 2022).

Dry matter content (DMC) at 21 DAP ranged from 5.27% (T5) to 5.74% (T4), with no significant differences among the treatments (**Figure 3**). By 37 DAP, the highest DMC was recorded under T5 (5.22%), followed closely by T3 (5.15%), T2 (5.05%), and T4 (5.05%), but none of the treatments differed significantly from each other. The unfertilized control (T1, 4.57%) remained significantly lower than all the fertilized treatments.

Table 4. Effect of egg-based fertilizer concentrations on leaf length (cm)

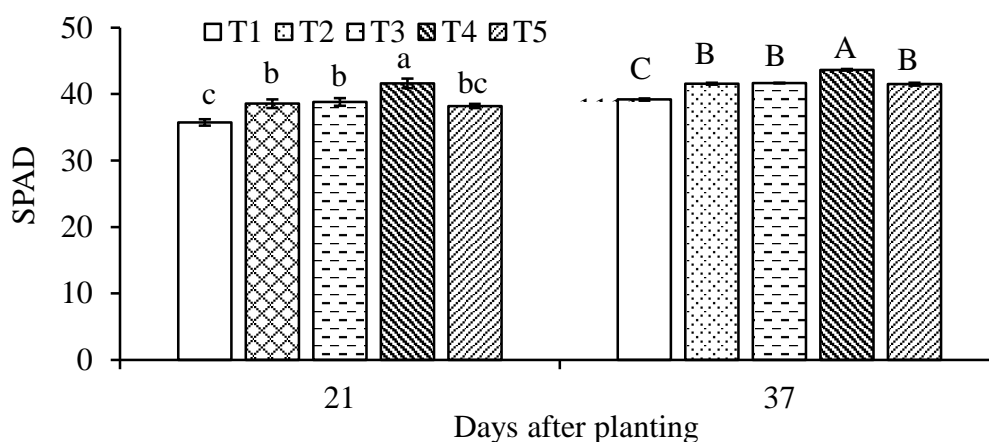
Treatment	7DAP	14DAP	21DAP
T1	5.2 ^a ± 0.4	6.6 ^b ± 0.2	10.7 ^c ± 0.4
T2	5.4 ^a ± 0.1	6.9 ^{ab} ± 0.2	12.8 ^{ab} ± 0.1
T3	5.5 ^a ± 0.1	7.3 ^a ± 0.2	12.6 ^{ab} ± 0.4
T4	5.3 ^a ± 0.4	7.4 ^a ± 0.1	13.1 ^a ± 0.4
T5	5.1 ^a ± 0.2	6.7 ^b ± 0.2	11.9 ^b ± 0.3

Note: Values within a column followed by different letters differ significantly at $P < 0.05$. DAP: days after planting; T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Table 5. Effect of egg-based fertilizer concentrations on leaf width (cm)

Treatment	7DAP	14DAP	21DAP
T1	4.3 ^a ± 0.4	5.5 ^b ± 0.1	8.8 ^b ± 0.1
T2	4.7 ^a ± 0.3	5.9 ^{ab} ± 0.1	10.9 ^a ± 0.1
T3	4.5 ^a ± 0.2	6.4 ^a ± 0.3	10.9 ^a ± 0.4
T4	4.5 ^a ± 0.5	6.3 ^a ± 0.1	11.1 ^a ± 0.1
T5	4.6 ^a ± 0.4	5.8 ^{ab} ± 0.4	10.5 ^a ± 0.4

Note: Values within a column followed by different letters differ significantly at $P < 0.05$. DAP: days after planting; T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.



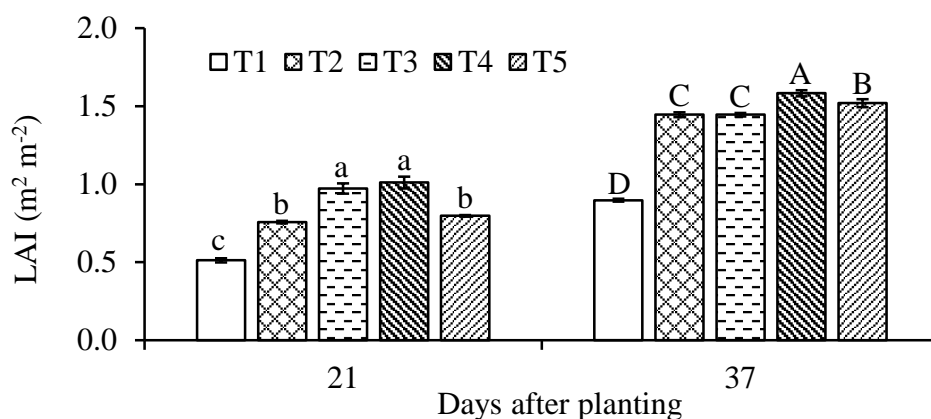
Note: Lowercase and uppercase letters indicate significant differences among treatments at 21 DAP and 37 DAP, respectively, at $P < 0.05$. Error bars represent standard deviation (SD). T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Figure 1. Effect of egg-based fertilizer concentrations on SPAD

DMC declined across all the treatments from 21DAP to 37DAP. This decrease may have reflected developmental shifts as *B. alba* matured: expansion in tissue water content and dilution by increased fresh biomass can reduce DMC per unit fresh weight despite higher total biomass. In many herbaceous plants, rising water content with leaf expansion and cell elongation similarly reduces DMC during later growth stages (Cai et al., 2023; Levinsh, 2023).

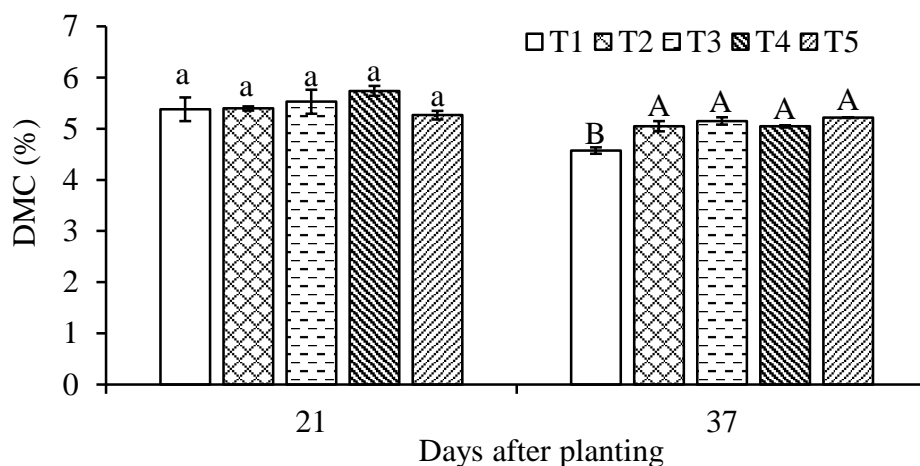
Effects of egg-based fertilizer concentrations on yield and nitrate content

At 21 DAP, T4 resulted in the highest yield (6.3 t ha⁻¹), significantly outperforming the others (Figure 4). Both fish fertilizer (T2) and EF1 at 1.0% (T3) showed intermediate but statistically similar yields (5.5-5.6 t ha⁻¹). Notably, T5 (2.0% EF) yielded only 4.4 t ha⁻¹, indicating that high EF concentrations may inhibit yield performance during early growth



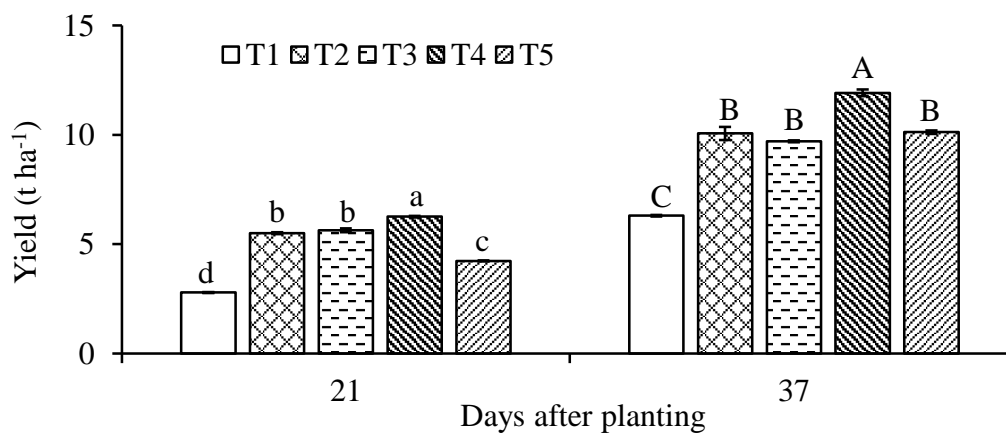
Note: Lowercase and uppercase letters indicate significant differences among treatments at 21 DAP and 37 DAP, respectively, at $P < 0.05$. Error bars represent standard deviation (SD). T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Figure 2. Effect of egg-based fertilizer concentrations on leaf area index (LAI)



Note: Lowercase and uppercase letters indicate significant differences among treatments at 21 DAP and 37 DAP, respectively, at $P < 0.05$. Error bars represent standard deviation (SD). T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Figure 3. Effect of egg-based fertilizer concentrations on dry matter content (DMC)



Note: Lowercase and uppercase letters indicate significant differences among treatments at 21 DAP and 37 DAP, respectively, at $P < 0.05$. Error bars represent standard deviation (SD). T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Figure 4. Effect of egg-based fertilizer concentrations on yield

stages. At 37 DAP, yield had increased across all the treatments, indicating substantial biomass accumulation over time. The highest yield was again observed in T4 (11.9 tons ha⁻¹), which was significantly greater than all the others. T2, T3, and T5 achieved statistically similar yields (9.7-10.1 tons ha⁻¹). These results highlight the consistent superiority of EF1 at 1.5% across the growth duration, suggesting it provides an optimal nutrient balance for maximizing productivity in *B. alba*.

The superior yield observed in T4 was strongly supported by its advantages in the vegetative and physiological traits, including the highest shoot length, leaf size, SPAD values, and LAI. In contrast, although T5 exhibited relatively high SPAD and LAI values, it produced shorter shoots, fewer leaves, and smaller leaf size than T4, particularly at early growth stages. This indicates that an excessive nutrient concentration may have caused osmotic stress or nutrient imbalances, limiting growth despite high physiological activity. Additionally, while the DMC in T5 remained high, it could not compensate for the early-stage growth constraints, resulting in only moderate yield.

Vegetables are the main source of dietary nitrate and nitrite, contributing about 80-92% of daily intake. Leafy vegetables are particularly susceptible to nitrate (NO₃⁻) accumulation due to their high N uptake capacity. Excessive NO₃⁻ levels in vegetables can inhibit cellular respiration and pose health risks, including certain cancers (Cintya *et al.*, 2018). Therefore, regulating the NO₃⁻ content in vegetable production is crucial for food safety.

As shown in **Table 6**, the NO₃⁻ content in *B. alba* ranged from 107.2 mg kg⁻¹ (T1) to 141.4 mg kg⁻¹ (T2) at 21 DAP, and from 113.0 mg kg⁻¹ (T1)

to 146.7 mg kg⁻¹ (T2) at 37 DAP. All the fertilized treatments (T2-T5) resulted in significantly higher NO₃⁻ concentrations than the unfertilized control (T1), but no significant differences were observed among the fertilized groups. These findings indicate that both EF1 and commercial organic fish fertilizer increased the NO₃⁻ accumulation as a result of enhanced N availability. Importantly, the NO₃⁻ concentrations in all the treatments remained well below the maximum allowable limit of 300 mg kg⁻¹ for leafy vegetables, as established by the European Commission (EC, 2002). This confirms that EF1, even at the highest tested concentration, does not present a food safety concern in terms of NO₃⁻ content.

Relationships among growth, physiology, and yield

The Pearson correlation matrix (**Table 7**) showed statistically significant relationships among the growth, physiological parameters, and yield traits. Yield was highly correlated with leaf length ($r = 0.96^{**}$), leaf width ($r = 0.92^{**}$), LAI ($r = 0.90^{**}$), and SPAD ($r = 0.84^{**}$). These results suggest that greater leaf expansion and higher chlorophyll content are key determinants of biomass accumulation and yield in *B. alba*. Shoot length also showed a moderate but significant correlation with yield ($r = 0.60^*$), while the number of leaves per main stem had a weaker, non-significant association ($r = 0.26^{ns}$).

Among the traits, leaf length and leaf width were strongly interrelated ($r = 0.91^{**}$) and both were closely associated with LAI and SPAD, further highlighting their central role in canopy development and light interception. SPAD readings, indicative of chlorophyll and N status, were also positively correlated with shoot length

Table 6. Effect of egg-based fertilizer concentrations on nitrate content (mg kg⁻¹)

Treatment	21DAP	37DAP
T1	107.2 ^b ± 5.3	113.0 ^b ± 4.6
T2	141.4 ^a ± 1.5	146.7 ^a ± 5.5
T3	129.2 ^a ± 4.8	137.3 ^a ± 3.8
T4	136.3 ^a ± 4.2	143.7 ^a ± 2.9
T5	140.0 ^a ± 4.6	144.3 ^a ± 5.0

Note: Values within a column followed by different letters differ significantly at $P < 0.05$. DAP: days after planting; T1: no fertilizer; T2: commercial fish fertilizer; T3: EF1 at 1.0%; T4: EF1 at 1.5%; T5: EF1 at 2.0%.

Table 7. Pearson correlation coefficients among growth, physiology parameters, and yield

	Shoot length	Number of leaves	Leaf length	Leaf width	SPAD	LAI	DMC	Yield
Shoot length	1							
Number of leaves	0.61 [*]	1						
Leaf length	0.54 [*]	0.15 ^{ns}	1					
Leaf width	0.54 [*]	0.10 ^{ns}	0.91 ^{**}	1				
SPAD	0.84 ^{**}	0.46 ^{ns}	0.72 ^{**}	0.77 ^{**}	1			
LAI	0.70 ^{**}	0.23 ^{ns}	0.86 ^{**}	0.86 ^{**}	0.79 ^{**}	1		
DMC	0.43 ^{ns}	0.46 ^{ns}	0.30 ^{ns}	0.35 ^{ns}	0.52 [*]	0.37 ^{ns}	1	
Yield	0.60 [*]	0.26 ^{ns}	0.96 ^{**}	0.92 ^{**}	0.84 ^{**}	0.90 ^{**}	0.42 ^{ns}	1

Note: **, Correlation is significant at the 0.01 level (2-tailed); *, Correlation is significant at the 0.05 level (2-tailed); ns, non-significant. SPAD: soil plant analysis development; LAI: leaf area index; DMC: dry matter content.

($r = 0.84^{**}$), and LAI ($r = 0.79^{**}$), supporting their utility as physiological indicators of vigor. DMC, however, showed no significant relationship with yield or the other parameters, suggesting that while useful for characterizing tissue composition, it may not directly predict fresh biomass production. Correlation analysis reinforced the links among leaf development, chlorophyll status, and yield, helping to clarify the physiological basis of the observed treatment effects.

Conclusions

The quality of the EFs varied according to the input composition, with EF1 (spoiled eggs without chicks) retaining the highest levels of N, P₂O₅, and CaO. When applied to *B. alba*, EF1 at 1.5% produced the greatest improvements in vegetative growth, physiological performance, and yield, outperforming both higher and lower EF concentrations and the commercial organic fish fertilizer. Nitrate concentrations in all the fertilized treatments remained well below regulatory limits, indicating safe application rates. Overall, EF1 at 1.5% is a promising and safe fertilization option while supporting the sustainable reuse of spoiled eggs in agricultural systems.

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