

Conversion of spoiled eggs into high-quality fertilizer using microbial inoculants and their effects on growth and yield of water spinach (*Ipomoea aquatica*)

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Abstract. Converting agricultural waste into value-added products is a sustainable strategy to reduce environmental pollution and enhances resource efficiency. This study evaluated the effectiveness of three microbial inoculants—EMZEO, EMUNIV, and EMIC—in composting spoiled eggs into high-quality fertilizers and assessed their effects on the growth and yield of water spinach (*Ipomoea aquatica*). Egg fertilizers produced using these inoculants (EFs) were compared with a commercial Organic Fish fertilizer in a randomized complete block design with three replications. Nutrient analysis showed that EF-EMUNIV had the highest levels of nitrogen (1.25%), phosphorus (0.94%), potassium (6.13%), and calcium (1.35%), followed by EF-EMIC (1.23%, 0.75%, 5.92%, 1.25%) and EF-EMZEO (1.20%, 0.63%, 5.52%, 0.75%). Microbiological tests confirmed that all fertilizers were free from *Escherichia coli* and *Salmonella* spp., indicating their safety for agricultural use. EF-EMUNIV significantly improved plant growth indicators such as shoot length, leaf number, and SPAD value, and produced the highest yield (7.74 t ha⁻¹), comparable to Organic Fish (7.41 t ha⁻¹) and EF-EMIC (7.23 t ha⁻¹). Nitrate concentrations in all treatments ranged from 120.3 to 149.7 mg kg⁻¹, remaining within safe limits for leafy vegetables. Pearson correlation analysis revealed strong associations between yield and branch number ($r = 0.895^{**}$), SPAD ($r = 0.789^{**}$), leaf number ($r = 0.748^{**}$), and leaf area index ($r = 0.701^{**}$). The findings highlight the potential of EMUNIV as the most effective inoculant for converting spoiled eggs into safe and efficient fertilizers for leafy vegetable production.

Keywords: Egg fertilizer, microbial inoculant, quality, water spinach, yield

1 Introduction

The rapid growth of the global population has led to increasing demands for food and fodder, which in turn has resulted in the generation of vast quantities of agricultural waste biomass—estimated to reach billions of tons annually [1]. In many developing countries, landfilling and composting are the most common methods for managing agricultural waste, collectively accounting for approximately 90–96% of total waste disposal [2]. However, the use of landfills has been restricted or banned in several countries

due to concerns over limited landfill space, groundwater and soil contamination, and a growing emphasis on waste recycling [3].

Composting, defined as the microbial decomposition and stabilization of organic matter, has emerged as an effective and sustainable waste management strategy. It transforms organic waste into valuable products such as organic fertilizers that supply essential plant nutrients and improve soil fertility [4, 5]. Furthermore, composting has been shown to mitigate environmental degradation and greenhouse gases emissions, making it an environmentally friendly alternative

to traditional waste disposal methods [2, 6]. Due to its simplicity, cost-effectiveness, and environmental benefits, composting is now considered a preferred approach for managing agricultural waste [7, 8].

During composting, organic matter is decomposed primarily through the metabolic activities of bacteria, fungi, and other microorganisms. To accelerate this process, specific functional microbial strains have been introduced into compost piles to enhance biodegradation. Reference [9] demonstrated that the addition of 1% nitrogen-transforming bacteria at the onset of pig manure composting significantly reduced nitrogen losses and improved compost maturation. Similarly, ref. [10] reported that inoculating ammonia-oxidizing bacteria into composted chicken manure and straw modified the microbial community structure, reduced ammonia volatilization, and minimized nitrogen loss. Reference [11] further showed that combining microbial inoculation with regular turning of compost piles improved compost quality and reduced processing time to 30–36 days. In addition, the incorporation of microbial strains has been associated with the extension of the thermophilic phase, increased microbial diversity, and elevated levels of humic substances, such as humic and fulvic acids, thereby enhancing the degree of compost humification [12]. Collectively, the incorporation of microbial inoculants into the composting process improves overall efficiency, accelerates the decomposition of recalcitrant organic compounds, facilitates the production of nutrient-rich organic fertilizers, and contributes to odor mitigation.

In Vietnam, the poultry industry has experienced rapid growth, with egg production reaching approximately 473,660 tons in 2020 [13]. While poultry manure has been widely studied

and utilized as a composted fertilizer, other by-products, such as unhatched or spoiled eggs resulting from failed incubation, remain underutilized despite their potential as a source of nutrient-rich organic fertilizer. Water spinach (*I. aquatica*) is a widely cultivated leafy vegetable in Vietnam. It has a short growth cycle, high nutrient requirements, and a rapid response to fertilizer application [14], making it a sensitive and practical bio-indicator for evaluating the effectiveness of organic fertilizers. This study aims to identify an appropriate microbial inoculant for the composting of spoiled eggs and to evaluate the quality of the resulting egg fertilizers (EF) based on their effects on the growth and yield of water spinach. These findings may contribute to the diversification of organic fertilizer sources for agricultural production and support the advancement of sustainable farming practices.

2 Material and method

2.1 Study site and research materials

The study was conducted in the Spring–Summer season (from March to August) of 2024 at the research farm of the Faculty of Agronomy, Vietnam National University of Agriculture (21°0' N, 105°55' E). This region's climate is tropical, and the experimental soil was classified as alluvial.

Three commercial microbial inoculants were used in Experiment 1:

EMZEO (Duc Binh Biotechnology Co., Ltd.) is a spore-based inoculant containing *Bacillus* spp., *Lactobacillus* spp., and *Saccharomyces* spp. ($>10^8$ CFU g⁻¹). It produces diverse enzymes capable of degrading proteins, starch, cellulose, chitin, and lipids. EMZEO is commonly used for composting agricultural waste, odor control, and pathogen suppression.

EMUNIV (Applied Microbiology Joint Stock Company) includes *Trichoderma viride*,

Bacillus subtilis, *Streptomyces murinus*, and *Lactobacillus plantarum* ($>10^8$ CFU g⁻¹), known for their strong enzymatic activity and production of bioactive compounds. It is widely applied in composting and livestock waste treatment due to its capacity to accelerate decomposition and suppress harmful microbes.

EMIC (Microtechnology and Environment Joint Stock Company) consists of *Bacillus* spp., *Lactobacillus* spp., *Saccharomyces* spp., and other active strains ($>10^8$ CFU g⁻¹). It is designed for efficient decomposition of organic waste and manure, reducing odors and enhancing compost stability through rapid humification.

The water spinach used in Experiment 2 was the GSA 609 variety, supplied by Hoang Nong Seed Co., Ltd. (Vietnam). This variety is well adapted to a wide range of soil types and is characterized by medium-sized stems, lanceolate leaves, vibrant green foliage, and a crisp, sweet taste.

2.2 Experimental design and sampling parameters

Experiment 1 – Effect of microbial inoculants on the quality of egg fertilizer

A one-factor experiment with treatments of different microbial inoculants, including EMZEO, EMUNIV, and EMIC, was conducted in a greenhouse. The microbial biomass was mixed with molasses and water, then incubated for two days to activate microbial activity. Spoiled eggs were collected from hatcheries, rinsed with water, and transferred into plastic containers. The eggs were stirred to break the shells, facilitating decomposition and reducing soaking time. The prepared microbial solution was added to the containers, mixed thoroughly, and the containers were sealed. The mixture was stirred once or twice daily.

After two months of composting, a distinct liquid layer was observed above the settled solids consisting of eggshells and residual egg components. The liquid fraction, referred to as egg fertilizer (EF), was filtered and analyzed for total nitrogen (TN), total phosphorus (TP), total potassium (TK), total calcium (TCa), and the concentrations of *E. coli* and *Salmonella* spp., according to the following standards: TCVN 8518:2018, TCVN 5815:2018, TCVN 8562:2010, TCVN 9284:2018, TCVN 6187-2:2020, and TCVN 10780-1:2017, respectively.

Experiment 2 – Effects of egg fertilizers composted with different microbial inoculants on the growth and yield of water spinach (*I. aquatica*)

A one-factor experiment was implemented using a Randomized Complete Block Design (RCBD) with three replications. The experimental fertilizer treatments consisted of three types of egg fertilizers from Experiment 1 (EF-EMZEO, EF-EMUNIV, and EF-EMIC), and Organic Fish, a commercially available fertilizer, was used as the control. Water spinach seeds were directly sown in the field with a spacing of 20 cm × 20 cm. Fertilizers were first applied at the three-true-leaf stage and subsequently applied every seven days until one week before harvest. Cow manure was uniformly applied as a basal fertilizer at a rate of 10 tons ha⁻¹ across all treatments. The cultivation and management of water spinach followed organic farming principles.

Five plants were randomly selected from each experimental plot at five-day intervals to measure shoot length (cm), number of leaves (leaves plant⁻¹), and number of branches (branches plant⁻¹). The number of branches was recorded after the first harvest.

Water spinach was harvested three times—at 30, 50, and 70 days after sowing (DAS). The entire shoot biomass was collected, leaving a 2 cm

stem base. At each harvest, five representative plants per plot were sampled to evaluate physiological parameters. SPAD values were measured using a handheld SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) at three points 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 1 dm² 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 (NO₃⁻) content (mg kg⁻¹) was measured using the SOEKS NUC 019-01 device at 50 and 70 DAS.

2.3 Statistical analysis

In experiment 2, data processing and statistical analysis were performed using Microsoft Excel and MINITAB 16 software. Analysis of variance (one-way ANOVA) was used for analyzing the effects of experimental factor. Differences between treatments were identified according to Tukey's honest significant difference (HSD) test with $p = 0.05$.

3 Results and discussions

3.1 Nutrient composition of the egg fertilizer

Table 1 presents the macronutrient composition of the three egg fertilizers. Among them, EF-EMUNIV exhibited the highest concentrations of all major nutrients analyzed. Total nitrogen (TN) content ranged from 1.20% in EF-EMZEO to 1.25% in EF-EMUNIV, placing all egg fertilizers within the expected range for composted animal-based organic amendments (1–2%) [15]. Despite being lower than synthetic fertilizers, this organic

N is known to contribute to sustained soil fertility through gradual mineralization.

Phosphorus (P) content followed a similar trend, with EF-EMUNIV containing the highest concentration (0.94%), significantly greater than EF-EMIC (0.75%) and EF-EMZEO (0.63%). Total potassium (TK) levels were also highest in EF-EMUNIV (6.13%), with EF-EMIC (5.92%) and EF-EMZEO (5.52%) exhibiting slightly lower but still considerable concentrations. These elevated K values are noteworthy, as K is crucial for physiological functions in plants, including osmotic regulation, enzyme activation, and stress tolerance. Previous studies have reported comparable K levels in composted agricultural residues, such as nutshells, fruit husks, and row-crop residues, ranging from 1 % to over 7 % [16].

Total calcium (TCa) content was substantially higher in EF-EMUNIV (1.35%) and EF-EMIC (1.25%) compared to EF-EMZEO (0.75%). The elevated Ca content reflects the presence of ground eggshells, a rich source of calcium carbonate. Reference [17] demonstrated that eggshell amendments can enhance P availability and reduce acidity in sandy loam and sandy clay loam soils, underscoring the potential agronomic value of these fertilizers.

The enhanced nutrient content observed in EF-EMUNIV may be attributed to the synergistic activity of its constituent microorganisms. *S. murinus* and *T. viride*, both present in EMUNIV, are known to produce potent extracellular enzymes and bioactive metabolites that accelerate the decomposition of complex organic materials such as proteins and lipids. A study investigating the role of *Streptomyces-Bacillus* inoculants (SBI) in carbon (C) and nitrogen (N) transformation during co-composting of cow manure and corn straw reported significant increases in total N content (+47%) and nitrate and nitrite reductase activities (+60% and +219%, respectively) [18].

Moreover, *Streptomyces* species can solubilize inorganic P by secreting organic acids and siderophores, thereby enhancing P mineralization [19]. In addition, *L. plantarum* improves composting performance through acid production and enzyme stimulation, which promotes faster compost maturation and improved nutrient retention [20]. Collectively, these mechanisms likely contributed to the superior nutrient release and stabilization observed in EF-EMUNIV.

Microbiological analysis confirmed the absence of *E. coli* and *Salmonella* spp. in all fertilizer samples, indicating that the composting process was effective in ensuring microbial safety. The hygienic quality of the products supports their suitability for organic agricultural use, where inputs must meet stringent biosafety standards.

Table 1. Nutrient composition of the egg fertilizer

| Parameters | Unit | EF-EMZEO | EF-EMUNIV | EF-EMIC |
|------------------------|------------|----------|-----------|---------|
| TN | % | 1.20 | 1.25 | 1.23 |
| TP | % | 0.63 | 0.94 | 0.75 |
| TK | % | 5.52 | 6.13 | 5.92 |
| TCa | % | 0.75 | 1.35 | 1.25 |
| <i>E. coli</i> | CFU/ml | nd* | nd | nd |
| <i>Salmonella</i> spp. | MPN/100 ml | nd | nd | nd |

*nd: not detected

3.2 Effects of egg fertilizers on the growth and yield of water spinach

Effects of egg fertilizers on growth parameters

Table 2 indicates that shoot length of water spinach increased steadily across all treatments over time, although the rate and magnitude of growth varied with fertilizer type. In the early growth phase (20–30 DAS), EF-EMUNIV exhibited the most pronounced acceleration, increasing from 19.2 cm to 31.6 cm (+12.4 cm); while EF-EMZEO was the slowest (increased from 18.8 cm to 27.1 cm). Despite these trends, statistical analysis at 30 DAS revealed no significant differences among treatments at

$\alpha = 0.05$. Following the first harvest, shoot length continued to increase, albeit more slowly. At 50 DAS, EF-EMUNIV (32.2 cm) was significantly taller than EF-EMZEO (27.6 cm), with EF-EMIC (29.5 cm) and Organic Fish (31.4 cm) exhibiting intermediate values. By 70 DAS, EF-EMUNIV again yielded the tallest shoots (32.7 cm), significantly exceeding EF-EMZEO (29.1 cm), while EF-EMIC (30.7 cm) and Organic Fish (31.2 cm) remained statistically similar to EF-EMUNIV. These findings confirm a sustained enhancement of shoot elongation by EF-EMUNIV across the entire growth period.

Table 2. Effect of egg fertilizers on shoot length (Unit: cm)

| Treatments | 20 DAS | 25 DAS | 30 DAS | 40 DAS | 45 DAS | 50 DAS | 60 DAS | 65 DAS | 70 DAS |
|--------------|--------|--------|-------------------|--------|--------|--------------------|--------|--------|--------------------|
| EF-EMZEO | 18.8 | 23.0 | 27.1 ^a | 14.8 | 21.6 | 27.6 ^b | 16.0 | 23.2 | 29.1 ^b |
| EF-EMUNIV | 19.2 | 24.3 | 31.6 ^a | 17.7 | 25.5 | 32.2 ^a | 18.1 | 27.0 | 32.7 ^a |
| EF-EMIC | 18.9 | 23.9 | 28.4 ^a | 15.9 | 23.0 | 29.5 ^{ab} | 17.5 | 24.3 | 30.7 ^{ab} |
| Organic Fish | 19.6 | 24.5 | 28.9 ^a | 16.8 | 24.0 | 31.4 ^a | 18.0 | 25.4 | 31.2 ^{ab} |

Values within a column followed by the different letter indicate significant differences at 0.05 level by Tukey's HSD test.

The extent of leaf development also varied depending on the fertilizer type (Table 3). During the early to mid growth phase (20–50 DAS), no significant differences were detected among treatments, although EF-EMUNIV tended to produce more leaves following the first harvest, indicating its emerging influence on leaf proliferation. At 70 DAS, EF-EMUNIV clearly outperformed the other treatments, producing the highest number of leaves (34.0 leaves), which was significantly greater than Organic Fish (31.4 leaves), EF-EMIC (30.9 leaves) and EF-EMZEO (28.4 leaves).

Table 4 reveals that the number of branches began to develop after the first harvest. While the differences were not statistically significant, EF-EMUNIV tended to produce more branches by 50 DAS, with an average of 3.1 branches. From 60 DAS onward, branch development progressed

steadily across all treatments. At 70 DAS, EF-EMUNIV sustained its leading effect, resulting in a significantly higher number of branches (4.7) compared to EF-EMZEO (4.0), while EF-EMIC (4.4) and Organic Fish (4.5) were statistically similar to EF-EMUNIV. These findings suggest that EF-EMUNIV and EF-EMIC consistently promoted superior shoot branching, potentially due to improved nutrient availability or hormonal stimulation enhancing axillary bud outgrowth. Conversely, although EF-EMZEO demonstrated a gradual increase in branch number over time, it ultimately produced significantly fewer branches at 70 DAS than the other treatments. The increase in branch number also explain the accelerated leaf production observed after the first harvest (Table 3), as each new branch serves as a site for additional leaf development, contributing to the overall vegetative growth.

Table 3. Effect of egg fertilizers on number of leaves (Unit: leaves plant⁻¹)

| Treatments | 20 DAS | 25 DAS | 30 DAS | 40 DAS | 45 DAS | 50 DAS | 60 DAS | 65 DAS | 70 DAS |
|--------------|--------|--------|------------------|--------|--------|-------------------|--------|--------|-------------------|
| EF-EMZEO | 5.5 | 7.1 | 9.4 ^a | 10.4 | 15.6 | 22.6 ^a | 15.5 | 21.4 | 28.4 ^c |
| EF-EMUNIV | 5.3 | 7.5 | 9.2 ^a | 10.9 | 16.1 | 24.5 ^a | 17.9 | 25.7 | 34.0 ^a |
| EF-EMIC | 5.6 | 7.7 | 9.9 ^a | 9.3 | 13.4 | 20.9 ^a | 17.0 | 23.2 | 30.9 ^b |
| Organic Fish | 5.9 | 7.8 | 9.7 ^a | 9.6 | 15.6 | 21.9 ^a | 16.5 | 22.5 | 31.4 ^b |

Values within a column followed by the different letter indicate significant differences at 0.05 level by Tukey's HSD test.

Table 4. Effect of egg fertilizers on number of branches (Unit: branches plant⁻¹)

| Treatments | 40 DAS | 45 DAS | 50 DAS | 60 DAS | 65 DAS | 70 DAS |
|--------------|--------|--------|------------------|--------|--------|------------------|
| EF-EMZEO | 2.1 | 2.2 | 2.7 ^a | 2.9 | 3.5 | 4.0 ^b |
| EF-EMUNIV | 1.9 | 2.2 | 3.1 ^a | 3.1 | 4.1 | 4.7 ^a |
| EF-EMIC | 2.0 | 2.0 | 2.4 ^a | 2.9 | 3.7 | 4.4 ^a |
| Organic Fish | 2.1 | 2.5 | 2.9 ^a | 2.8 | 3.6 | 4.5 ^a |

Values within a column followed by the different letter indicate significant differences at 0.05 level by Tukey’s HSD test.

Overall, EF-EMUNIV and EF-EMIC demonstrated notably greater effects on vegetative growth parameters of water spinach compared to EF-EMZEO, a finding consistent with the higher macronutrient concentrations (N, P, K, Ca) shown in Table 1. Nitrogen (N) plays a central role in promoting vegetative growth by stimulating leaf expansion, chlorophyll synthesis, and enhancing cell division and elongation [21]. Phosphorus (P) contributes to root development, energy transfer through ATP production, and supports early plant vigor as well as the initiation of lateral branching [22]. Additionally, the elevated calcium (Ca) content supported cell wall integrity and structural development, thereby facilitating stronger stem and branch formation [23]. This is reflected in the increased number of branches observed in both EF-EMUNIV and EF-EMIC compared to EF-EMZEO.

The synergistic combination of high levels of N, P, K, and Ca, particularly in EF-EMUNIV, created a nutrient-rich environment that fostered key physiological processes associated with shoot elongation, leaf proliferation, and axillary bud development. Consequently, EF-EMUNIV resulted in statistically significant enhancements in all three vegetative growth parameters - shoot length, leaf number, and branch number - particularly during the late stages of the experimental period.

Effects of egg fertilizers on physiological parameters

The SPAD index, which reflects relative chlorophyll content, was only marginally affected by fertilizer type at 30 and 70 DAS (Fig.1). However, at 50 DAS, significant differences emerged among treatments. EF-EMUNIV recorded the highest SPAD value (40.9), which was statistically comparable to that of the Organic Fish (40.5), and markedly higher than those observed in EF-EMIC (38.4) and EF-EMZEO (36.9). This result suggests that EF-EMUNIV may have enhanced chlorophyll accumulation or retention during the mid-growth stage, potentially due to its higher N availability, an essential factor for chlorophyll biosynthesis and photosynthetic efficiency.

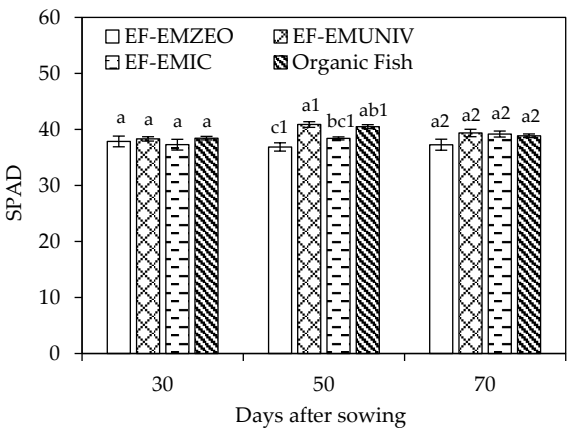


Fig. 1. Effect of egg fertilizers on SPAD

LAI increased consistently across all three sampling intervals. Although EF-EMUNIV exhibited the lowest LAI at the first harvest (0.13 $\text{m}^2 \text{m}^{-2}$), its values rose sharply in the subsequent harvest to 0.56 $\text{m}^2 \text{m}^{-2}$ and 0.87 $\text{m}^2 \text{m}^{-2}$, respectively (Fig.2). This improvement is likely attributable to the accelerated development of branches and leaves observed in this treatment. By 70 DAS, the LAI of EF-EMUNIV was statistically comparable to those recorded for EF-EMIC (0.87 $\text{m}^2 \text{m}^{-2}$) and Organic Fish (0.78 $\text{m}^2 \text{m}^{-2}$).

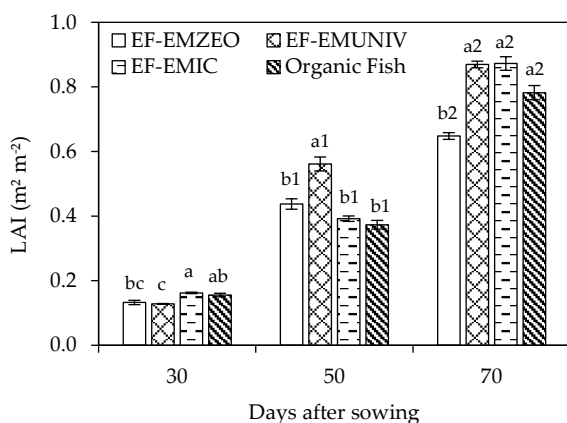


Fig. 2. Effect of egg fertilizers on leaf area index (LAI)

The enriched nutrients contents in EF-EMUNIV and EF-EMIC may have promoted cell division and leaf expansion, leading to the observed increases in LAI. Previous research has shown that sufficient N availability accelerates cell division, shortens the cell cycle, and enhances cell elongation, thereby contributing directly to greater leaf expansion in *Lolium perenne* [24]. Additionally, Ca has been reported to activate expansins, enzymes, and other cellular growth mechanisms that facilitate both cell and leaf expansion [25, 26]. Furthermore, adequate phosphorus (P) is essential for canopy development and leaf area expansion in various plant species [22].

Figure 3 indicates that the fertilizer types did not significantly influence dry matter content during the early to mid growth stages of water

spinach, although EF-EMUNIV tended to produce the highest values. This finding aligns with the previous results [27], which no significant effect of nitrogen and magnesium application on dry matter accumulation in two maize hybrids at the 5–6 leaf stage was observed.

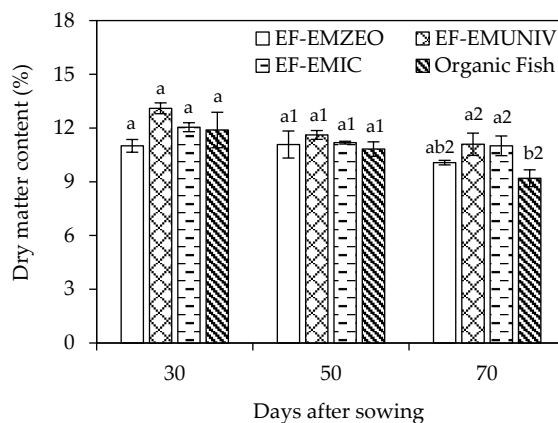


Fig. 3. Effect of egg fertilizers on dry matter content

At 70 DAS, however, higher dry matter contents were recorded in EF-EMUNIV (11.09%) and EF-EMIC (11.01%), both of which were statistically comparable to EF-EMZEO (10.07%) but significantly greater than that in Organic Fish (9.20%).

Effects of egg fertilizers on yield and nitrate content

Figure 4 compares the yields of water spinach under different fertilizer types at 30, 50, and 70 DAS. At 30 DAS, yields ranged from 0.86 to 0.89 t ha^{-1} , with no statistically significant differences among treatments. At 50 and 70 DAS, similar trends were observed, with EF-EMUNIV producing the highest yields (2.98 and 3.88 t ha^{-1} , respectively), which were significantly greater than those of EF-EMZEO (2.68 and 3.05 t ha^{-1} , respectively) and statistically comparable to those of EF-EMIC and Organic Fish. Notably, yields in the second and third harvests were substantially higher than in the first, suggesting that the beneficial effects of organic fertilizers on water

spinach yield are not immediate but become increasingly evident during the mid to late vegetative stages—critical periods for biomass accumulation.

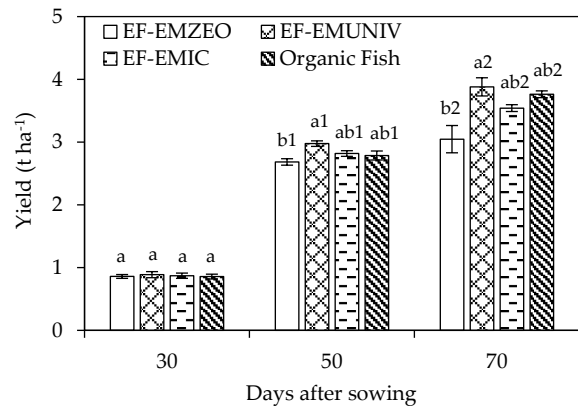


Fig. 4. Effect of egg fertilizers on yield

The progressive yield increases indicate that EF fertilizers provide a sustained release of nutrients, supporting continuous canopy expansion and biomass production. These cumulative physiological benefits ultimately result in significantly higher yields during the later stages of crop development. Furthermore, the absence of significant differences in growth and yield between EF-EMUNIV, EF-EMIC, and Organic Fish underscores the potential of fertilizers derived from spoiled eggs as effective organic nutrient sources for enhancing water spinach productivity through improved soil health and prolonged nutrient availability.

Vegetables are the primary source of nitrates and nitrites in the human diet, accounting for approximately 80–92% of average daily intake. Leafy vegetables, in particular, are prone to accumulating excessive amounts of NO_3^- . When NO_3^- concentrations exceed permissible levels, they can inhibit cellular respiration, negatively impact human health, and contribute to the development of diseases such as cancer [28]. Therefore, controlling NO_3^- content in vegetable production is crucial.

Table 5. Effect of egg fertilizers on nitrate (NO_3^-) content (Unit: mg kg^{-1})

| Treatments | 50 DAS | 70 DAS |
|--------------|--------------------|--------------------|
| EF-EMZEO | 125.0 ^a | 130.7 ^a |
| EF-EMUNIV | 128.3 ^a | 130.7 ^a |
| EF-EMIC | 132.3 ^a | 149.7 ^a |
| Organic Fish | 120.3 ^a | 127.0 ^a |

Values within a column followed by the different letter indicate significant differences at 0.05 level by Tukey’s HSD test.

As shown in Table 5, NO_3^- content in water spinach tended to increase over time under all fertilizer types. Although EF-EMIC consistently recorded the highest NO_3^- concentrations at both 50 and 70 DAS (132.3 and 149.7 mg kg^{-1} , respectively), the differences among treatments were not statistically significant ($p > 0.05$). The relatively higher NO_3^- levels observed in EF-EMIC and EF-EMUNIV may be attributed to their higher N content. Nevertheless, NO_3^- accumulation across all treatments remained well below the maximum allowable limit for fresh vegetables and fruits (300 mg kg^{-1}), indicating their safety for consumption.

Relationship among growth, physiology and yield

The Pearson correlation matrix (Table 6) showed statistically significant relationships among growth, physiology parameters and yield traits. The number of branches exhibited the strongest correlation with yield ($r = 0.895$, $p < 0.01$), indicating that branching is a key determinant of productivity. SPAD value also showed a high correlation with yield ($r = 0.789$, $p < 0.01$), followed by the number of leaves ($r = 0.748$, $p < 0.01$), and LAI ($r = 0.701$, $p < 0.01$) suggesting that photosynthetic capacity and leaf development contribute substantially to yield formation. Plant height ($r = 0.587$, $p < 0.05$) was also positively correlated with yield, albeit to a lesser extent.

Significant interrelationships were also found among vegetative growth traits. Notably, number of leaves was strongly correlated with number of branches ($r = 0.801$, $p < 0.01$) and LAI ($r = 0.801$, $p < 0.01$), while plant length was positively associated with both leaves ($r = 0.730$, $p < 0.01$) and branches ($r = 0.799$, $p < 0.01$). These

results underscore the coordinated development of structural growth attributes. SPAD values correlated significantly with branches ($r = 0.715$, $p < 0.01$) and LAI ($r = 0.571$, $p < 0.05$), indicating that chlorophyll content may be linked with both shoot development and canopy size.

Table 6. Pearson correlation coefficients among growth, physiology parameters and yield

| | Length | Leaves | Branches | SPAD | LAI | Dy matter | Yield |
|-----------|---------|---------|----------|---------|---------|-----------|-------|
| Length | 1 | | | | | | |
| Leaves | 0.730** | 1 | | | | | |
| Branches | 0.799** | 0.801** | 1 | | | | |
| SPAD | 0.524 | 0.534 | 0.715** | 1 | | | |
| LAI | 0.585* | 0.801** | 0.736** | 0.571* | 1 | | |
| Dy matter | 0.241 | 0.223 | 0.209 | 0.130 | 0.432 | 1 | |
| Yield | 0.587* | 0.748** | 0.895** | 0.789** | 0.701** | 0.134 | 1 |

**, Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

On the other hand, dry matter content did not exhibit statistically significant correlations with any of the growth or yield parameters, highlighting its limited predictive value for yield under the studied conditions. The weak correlation with yield ($r = 0.134$, $p > 0.05$) suggests that, although dry matter accumulation is important, it may not directly reflect productivity in this context.

Collectively, these findings highlight the central role of branching, leaf traits, and chlorophyll content in influencing yield performance, providing valuable insights for selection criteria in crop improvement and agronomic management strategies.

4 Conclusion

This study demonstrates that composting spoiled eggs using microbial inoculants can produce safe,

nutrient-rich fertilizers suitable for leafy vegetable cultivation. Among the treatments, EF-EMUNIV exhibited the highest levels of essential nutrients (N: 1.25%, P: 0.94%, K: 6.13%, Ca: 1.35%) and consistently promoted superior growth performance and yield in water spinach, comparable to a commercial Organic Fish fertilizer. EF-EMIC also showed promising results in both nutrient content and crop productivity. All fertilizers were free from *E. coli* and *Salmonella* spp., and nitrate concentrations remained within safe regulatory limits (120.3–149.7 mg kg⁻¹), ensuring food safety.

The positive correlations observed between yield and key plant traits—such as branch number, SPAD value, leaf number, and LAI—highlight the importance of structural and physiological responses to fertilizer application. Overall, EMUNIV was identified as the most effective inoculant for converting spoiled eggs

into high-quality fertilizer, offering a sustainable solution for improving yield and nutrient management in vegetable production systems.

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References

1. Arora J, Ramawat KG, Mérillon JM. Disposal of agricultural waste and its effects on the environment, production of useful metabolites and energy: potential and challenges. In: Ramawat K, Mérillon JM, Arora J. (Eds). *Agricultural waste: Environmental impact, useful metabolites and energy production*. Sustainable Development and Biodiversity. 2023; 31. Singapore: Springer.
2. Nguyen V-T, Le T-H, Bui X-T, Nguyen T-N, Vo T-D-H, Lin C, et al. Effects of C/N ratios and turning frequencies on the composting process of food waste and dry leaves. *Bioresource Technology Reports*. 2020;11:100527.
3. Kim M-H, Kim J-W. Comparison through a LCA evaluation analysis of food waste disposal options from the perspective of global warming and resource recovery. *Science of The Total Environment*. 2010;408(19):3998-4006.
4. Kucbel M, Raclavská H, Růžicková J, Švédová B, Sassmanová V, Drozdová J, et al. Properties of composts from household food waste produced in automatic composters. *Journal of Environmental Management*. 2019;236:657-66.
5. Al-Tawarah B, Alasasfa MA, Mahadeen AY. Efficacy of compost and vermicompost on growth, yield and nutrients content of common beans crop (*Phaseolus vulgaris* L.). *Journal of Ecological Engineering*. 2024;25:215-226.
6. Waqas M, Hashim S, Humphries UW, Ahmad S, Noor R, Shoaib M, et al. Composting Processes for Agricultural Waste Management: A Comprehensive Review. 2023;11(3):731.
7. Manea EE, Bumbac C, Dinu LR, Bumbac M, Nicolescu CM. Composting as a sustainable solution for organic solid waste management: Current practices and potential improvements. *Sustainability*. 2024;16(15):6329.
8. Aylaj M, Adani F. The evolution of compost phytotoxicity during municipal waste and poultry manure composting. *Journal of Ecological Engineering*. 2023;24:281-293.
9. Jiang J, Liu X, Huang Y, Huang H. Inoculation with nitrogen turnover bacterial agent appropriately increasing nitrogen and promoting maturity in pig manure composting. *Waste Management*. 2015;39:78-85.
10. Zhang L, Sun X, Tian Y, Gong X. Effects of inoculation with ammonia-oxidizing bacteria on composting of chicken manure and wheat straw. *Bioresource Technology*. 2016;213:54-62.
11. Manu MK, Kumar R, Garg A. Decentralized composting of household wet biodegradable waste in plastic drums: Effect of waste turning, microbial inoculum and bulking agent on product quality. *Journal of Cleaner Production*. 2019;226: 233-241.
12. Wan J, Li Y, Zhang M, Wang Q, Wang Y. Effects of microbial inoculants on organic matter degradation and humification during composting of pig manure. *Bioresource Technology*. 2020;301:122730.
13. Vu N-T, Dinh T-H, Le T-T-C, Vu T-T-H, Nguyen T-T, Pham T-A, et al. Eggshell powder as calcium source on growth and yield of groundnut (*Arachis hypogaea* L.). *Plant Production Science*. 2022;25(4):413-20.
14. Noora NM, Ropia NAM, Cheng K, Leong HY. Effects of organic, inorganic and compound fertilizer on growth and quality of water spinach (*Ipomoea aquatica*) under polyculture condition. *Journal of Agrobiotechnology*. 2022;13:1-12.
15. Bernal MP, Alburquerque JA, Moral R. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology*. 2009;100:5444-5453.
16. Andrews EM, Kassama S, Smith EE, Brown PH, Khalsa SDS. A review of potassium-rich crop residues used as organic matter amendments in tree crop agroecosystems. *Agriculture*. 2021;11:580.
17. Sebonela LK, Elephant DE, Sithole NJ. Eggshells improve soil pH and P availability in sandy loam and sandy clay loamy soil. *Agronomy*. 2024;14:2539.

18. Zhou Z, Shi X, Bhople P, Jiang J, Chater CCC, Yang S, et al. Enhancing C and N turnover, functional bacteria abundance, and the efficiency of biowaste conversion using *Streptomyces-Bacillus* inoculation. *Journal of Environmental Management*. 2024;358:120895.
19. Chouyia FE, Ventorino V, Pepe O. Diversity, mechanisms and beneficial features of phosphate-solubilizing *Streptomyces* in sustainable agriculture: A review. *Frontiers in Plant Science*. 2022;13:1035358.
20. Li W, Liu Y, Hou Q, Huang W, Zheng H, Gao X, et al. *Lactobacillus plantarum* improves the efficiency of sheep manure composting and the quality of the final product. *Bioresource Technology*. 2020;297:122456.
21. Ötvös K, Marconi M, Vega A, O'Brien J, Johnson A, Abualia R, et al. Modulation of plant root growth by nitrogen source-defined regulation of polar auxin transport. *The EMBO Journal*. 2021;40:1-21.
22. Fathi A, Mehдиниya Afra J. Plant growth and development in relation to phosphorus: A review. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture*. 2023;80:1-7.
23. Hepler PK. Calcium: A central regulator of plant growth and development. *The Plant Cell*. 2005;17:2142-2155.
24. Kavanová M, Lattanzi FA, Schnyder H. Nitrogen deficiency inhibits leaf blade growth in *Lolium perenne* by increasing cell cycle duration and decreasing mitotic and post-mitotic growth rates. *Plant, Cell & Environment*. 2008;31:727-737.
25. McQueen-Mason S, Durachko DM, Cosgrove DJ. Two endogenous proteins that induce cell wall extension in plants. *The Plant Cell*. 1992;4:1425-1433.
26. Cosgrove DJ. Loosening of plant cell walls by expansins. *Nature*. 2000;407:321-326.
27. Szulc P, Bocianowski J. Effects of application of different nitrogen fertilizer forms and magnesium on dynamics of dry matter accumulation in two maize (*Zea mays* L.) hybrids in their early growth stages. *Polish Journal of Agronomy*. 2012;11:65-80.
28. Cintya H, Silalahi J, Putra EDL, Siburian R. The influence of fertilizer on nitrate, nitrite and vitamin C contents in vegetables. *Oriental Journal of Chemistry*. 2018;34:2614-2621.