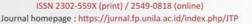


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Evaluating *Lumbricus rubellus* Performance and Vermicompost Quality in Cow Manure–Mealworm Waste Mixtures

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ABSTRACT

This study examined the effect of adding mealworm (Tenebrio molitor) frass to cow manure media on the productivity of Lumbricus rubellus and the quality of the vermicompost. A completely randomized design was employed with six treatments (T0-T5), consisting of increasing proportions of frass: T0 (100% cow manure), T1 (95.5), T2 (90.10), T3 (85.15), T4 (80.20), and T5 (75.25). The T2 treatment yielded the highest earthworm biomass gain (4.95 g), cocoon production (87.94 cocoons), and media reduction (198.83 g), all statistically significant (p<0.05). Vermicompost from each treatment was assessed through a growth trial using Ipomoea reptans (water spinach) under eight media combinations (U0-U7): U0 (100% soil), U1 (soil + NPK), and U2-U7 (soil + vermicompost from T0-T5, respectively). Among these, U7 (incorporating vermicompost from T5) demonstrated the highest nutrient content (C-organic 41.51%, N 1.99%, P_2O_5 1.99%, K_2O 1.47%) and significantly enhanced plant growth (p<0.05). These findings indicate that moderate frass supplementation (10%) optimizes earthworm productivity, while higher inclusion levels (25%) improve vermicompost agronomic value. Thus, moderate frass addition is recommended to optimize vermicompost production and worm performance.

1. INTRODUCTION

Earthworms are among the most essential soil macrofauna, contributing significantly to ecological balance and soil fertility. Their biological activity facilitates organic matter decomposition, soil structure improvement, nutrient cycling, and enhanced carbon flow, all vital for plant growth (Yadav et al., 2021). Through the process of vermicomposting, earthworms convert organic waste into nutrient-rich humus, thereby increasing the bioavailability of essential nutrients for plants. One indicator of fertile and moist soil is the active presence of earthworms. Among the 4,500 known earthworm species within the phylum Annelida, only a small number—such as *Lumbricus rubellus*—have been successfully cultivated on a large scale (Molnár et al., 2021). This species is favored for its adaptability to a variety of organic substrates and its ability to produce high-quality vermicompost.

Cow manure has traditionally been used as the primary substrate in vermiculture due to its availability and moderate nutrient content. However, its relatively low protein level—approximately 8.3% (Kadam et al., 2024; Utami et al., 2024)—may not optimally support earthworm growth and reproduction. Studies have shown that *L. rubellus* requires a protein range of 9–15% to achieve optimal biomass and cocoon production (Darmawan et al., 2023). Consequently, efforts to enhance the nutritional composition of the substrate are crucial for improving both earthworm performance and vermicompost quality.

Insect farming by-products, such as frass (feces and exuviae) from the mealworm *Tenebrio molitor*—commonly known in Indonesia as ulat Hongkong—are increasingly recognized as potential organic waste resources. Mealworm

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frass contains 10–12% protein (Watson *et al.*, 2021), making it a promising supplement to conventional vermiculture media. However, its potential use as an earthworm growth substrate remains underutilized. Moreover, the high ammonia content in insect waste can be detrimental to earthworms. Therefore, prior to application, the frass must undergo fermentation using Effective Microorganisms (EM4) and organic materials to reduce ammonia levels and enhance its nutritional quality (Iresha *et al.*, 2023).

This study investigates the effects of incorporating mealworm frass into cow manure-based media on the productivity of *Lumbricus rubellus* and the quality of the resulting vermicompost. The experimental design applies a range of frass proportions and evaluates key parameters such as worm biomass gain, cocoon production, media decomposition rate, and nutrient profile of the compost. Additionally, a planting trial using water spinach (*Ipomoea reptans*) assesses the agronomic benefits of the vermicompost.

Previous studies have used animal waste or plant residues in various combinations for vermicomposting. Bashir *et al.* (2021) utilized cow dung and vegetable waste, while Utami *et al.* (2024) evaluated cow dung combined with cricket droppings. Although both studies aimed to optimize earthworm growth and compost quality, the focus was not on mealworm waste, which differs significantly in composition and texture. Furthermore, while Rehman *et al.* (2023) provided a comprehensive literature review on the role of vermicompost in sustainable agriculture, their work did not involve empirical testing of novel substrates.

Despite the growing interest in insect-based organic materials, there remains a lack of empirical studies on the specific impact of mealworm frass on vermicompost systems. In particular, the effect of this substrate on the biological performance of *Lumbricus rubellus* and the nutrient composition of the compost has not been adequately explored. This study aims to fill that gap by systematically examining earthworm productivity and vermicompost quality across different cow manure and mealworm frass mixtures.

The novelty of this research lies in its integrated experimental approach that combines waste management, vermitechnology, and sustainable agriculture. By directly measuring both biological (earthworm biomass, cocoon count) and agronomic (nutrient content, plant growth response) outcomes, the study provides a comprehensive assessment of mealworm frass as a bioresource. The dual focus on optimizing the decomposition process and improving compost quality makes this study relevant for advancing circular economy practices in organic farming.

While the use of cow manure and various plant or insect-based waste products has been explored in prior vermicomposting studies, the specific use of mealworm frass remains under-researched. This study proposes that combining cow manure with mealworm waste can enhance substrate nutritional balance and improve vermicomposting efficiency. The outcomes are expected to contribute to both scientific understanding and practical applications in waste recycling, vermiculture development, and sustainable agricultural practices.

The expected benefit of this research is to provide an alternative solution for managing insect-based organic waste, particularly mealworm frass, to enhance the efficiency of earthworm cultivation and the quality of the resulting vermicompost. By demonstrating that a mixture containing mealworm frass can improve worm biomass, cocoon production, and compost nutrient content, this study can serve as a reference for farmers, organic agriculture practitioners, and vermiculture communities in formulating more nutritious and eco-friendly growth media. Furthermore, the findings are expected to strengthen circular agriculture practices, reduce insect farming waste, and support food security by providing high-quality biofertilizers for various horticultural crops such as water spinach.

2. MATERIALS AND METHODS

2.1. Tools and Materials

The tools used in this study included stationery, a digital scale (0.01 g precision), plastic rearing containers (38 × 30 × 12 cm), gloves, mini shovel, sprayer, digital thermo-hygrometer, soil meter, tarpaulin, sealed barrels, and polybags. Materials included 900 g of *Lumbricus rubellus* (50 g per unit), cow dung, pre-fermented mealworm (*Tenebrio molitor*) frass, EM4, soil, water spinach seeds (*Ipomoea reptans*), NPK 16-16-16 fertilizer, and water.

2.2. Procedures

2.2.1 Preparation of Media and Earthworms

Two types of media were used: cow dung and mealworm frass. Cow dung was air-dried to reduce ammonia. Mealworm frass was fermented for 7 days using 20 kg frass, 1 L of EM4, 1 L of molasses, 1 kg of fine bran, and 100 L of water. The resulting substrate was fine-textured, orange-colored, and odorless. Each of the 18 plastic containers was perforated and cleaned, then filled with 2 kg of media mixture according to treatment detailed as the following:

| Treatm | Treatment for composting process | | Treatment for compost application for planting | | | |
|--------|-----------------------------------|------|--|--|--|--|
| Code | Description | Code | Description | | | |
| T0 | 100% cow dung | U0 | 100% soil | | | |
| T1 | 95% cow dung + 5% mealworm frass | U1 | 100% soil + NPK fertilizer | | | |
| T2 | 90% cow dung + 10% mealworm frass | U2 | 50% soil + 50% vermicompost T0 | | | |
| T3 | 85% cow dung + 15% mealworm frass | U3 | 50% soil + 50% vermicompost T1 | | | |
| T4 | 80% cow dung + 20% mealworm frass | U4 | 50% soil + 50% vermicompost T2 | | | |
| T5 | 75% cow dung + 25% mealworm frass | U5 | 50% soil + 50% vermicompost T3 | | | |
| | | U6 | 50% soil + 50% vermicompost T4 | | | |
| | | U7 | 50% soil + 50% vermicompost T5 | | | |

2.2.2. Earthworm Maintenance and Vermicompost Harvesting

Earthworms (50 g) were introduced into each container. Moisture was maintained daily by spraying 100 mL of water. Weekly measurements for six weeks included weight gain, pH, temperature, humidity, population, cocoon count, and media shrinkage. At week six, vermicompost was harvested and sun-dried.

2.2.3 Vermicompost Plant Testing

Vermicompost was tested chemically for C-organic, N, P₂O₅, and K₂O. Growth tests were conducted on *I. reptans* using 13 treatments (4 replications, 28-day growth period). Measurements included leaf count, stem height, leaf dimensions, and root/stem/leaf weights and lengths. The quality of vermicompost was tested using the following 8 treatment combinations as detailed in Table 1.

2.3. Data Analysis

A Completely Randomized Design (CRD) was applied with six treatments (T0–T5) and three replications. Data were analyzed using analysis of variance (ANOVA) at a 95% confidence level. If significant differences were found, Tukey's HSD test was conducted (Steel *et al.*, 1997):

$$Y_{ij} = \mu + A_i + \varepsilon_{ij} \tag{1}$$

where: Y_{ij} is the observed value of treatment i and replication j, μ is overall mean, A_i is treatment effect, and ε_{ij} is random error.

3. RESULTS AND DISCUSSION

3.1. Performance of Lumbricus rubellus

3.1.1. Condition of Lumbricus rubellus Rearing Media

The rearing media provided to earthworms significantly affects their productivity and performance. The better the quality of the media, the higher the productivity of the earthworms. Data on the condition of the *Lumbricus rubellus* rearing media are presented in Table 1. The physical conditions of the rearing media—particularly pH, relative humidity (rH), and temperature—showed statistically significant differences among all treatments (P<0.05), yet remained within the optimal range for earthworm activity: pH 6.0–7.2 (Zulkarnain *et al.*, 2019), rH 60–80% (Wolkoff *et al.*, 2021), and temperature 15–31°C (Kartini, 2018), as shown in Table 1. This confirms the suitability of all tested media for supporting

the cultivation of *Lumbricus rubellus*. The pH values ranged from 6.50 in T5 to 6.57 in T0. The slight decrease in pH with increasing mealworm frass content may be attributed to the characteristics of post-fermentation frass, which, despite undergoing fermentation, still exhibits mild acidity—a trend also noted by Asmawati *et al.* (2020), in their study on organic substrates. However, this acidity does not exceed the earthworms' tolerance threshold, indicating sufficient microbial buffering and the stabilizing effect of cow dung.

Table 1. Average pH, relative humidity, and temperature of Lumbricus rubellus rearing media

| Treatment | pН | Relative Humidity (%) | Temperature (°C) |
|-----------|------------------------|--------------------------|------------------------|
| T0 | 6.57±0.00 ^a | 70.33±0.00 ^a | 26.62±0.04ab |
| T1 | 6.56 ± 0.02^{ab} | 69.80±0.23 ^b | 26.62 ± 0.04^{ab} |
| T2 | 6.53 ± 0.00^{ab} | 69.58 ± 0.10^{bc} | 26.58 ± 0.04^{abc} |
| Т3 | 6.52 ± 0.02^{ab} | 69.16 ± 0.15^{cd} | 26.64 ± 0.08^{ab} |
| T4 | 6.52 ± 0.02^{ab} | $69.02\pm0.10^{\rm cd}$ | 26.71 ± 0.10^{a} |
| T5 | 6.50 ± 0.00^{ab} | $68.87 \pm 0.00^{\rm e}$ | 26.73 ± 0.00^{a} |
| Optimal | 6.0 - 7.2* | 60 - 80** | 15 – 31*** |

Description: Numbers followed by different letters within the same column indicate significant differences (P<0.05); T0 = 100% cow manure; T1 = 95% cow manure + 5% mealworm manure; T2 = 90% cow manure + 10% mealworm manure; T3 = 85% cow manure + 15% mealworm manure; T4 = 80% cow manure + 20% mealworm manure; T5 = 75% cow manure + 25% mealworm manure; *=Zulkarnain et al. (2019); **= Wolkoff et al. (2021); ***= Kartini, (2018).

Relative humidity decreased from 70.33% in T0 to 68.87% in T5. Media with higher frass content (T4–T5) tended to have a more granular texture, thereby reducing water retention. This aligns with the findings of Mupambwa & Mnkeni (2018), who noted that coarser media lead to faster moisture loss, negatively impacting the stability of the microenvironment. Although the rH differences were statistically significant, all values remained within the physiological comfort zone of *L. rubellus*, indicating no harmful effects on worm hydration or gas exchange. Temperature slightly increased with higher frass proportions, peaking at 26.73°C in T5. Mealworm frass is known for its high nitrogen and protein content (Chia *et al.*, 2024; Hassanein *et al.*, 2025), which likely stimulates microbial activity and thereby increases thermal output. This is consistent with Mashur *et al.* (2021), who emphasized that nutrient-rich media enhance microbial metabolism, indirectly raising substrate temperature.

Interestingly, although T5 exhibited higher temperature and lower rH, both remained within the optimal physiological range. These environmental variations were not extreme enough to inhibit earthworm activity. On the contrary, they highlight how mealworm frass influences media dynamics—an effect that can be strategically utilized in controlled vermicomposting systems. Therefore, the observed differences in media conditions are causally related to the chemical and physical properties of mealworm frass. These findings underscore the importance of substrate composition not only in shaping environmental parameters but also in influencing downstream biological outcomes, as further discussed in the following sections.

3.1.2. Performance of Lumbricus rubellus Earthworms

The productivity of *Lumbricus rubellus* earthworms can be evaluated through weight gain, cocoon production, population growth, and reduction of the rearing medium. The average productivity of *Lumbricus rubellus* during the study is presented in Table 2.

a. Weight Gain of Lumbricus rubellus

The addition of mealworm frass at varying proportions significantly affected the weight gain of *Lumbricus rubellus* (p<0.05). Treatment T2 (90% cow dung + 10% frass) showed the highest gain (4.95 g), while T5 (75% cow dung + 25% frass) had the lowest (1.11 g). The superior performance in T2 is likely due to an optimal balance of protein (approx. 11%) and a fine, loose medium texture, both facilitating nutrient absorption and aeration. This aligns with findings by Utami *et al.* (2024) and Cui *et al.* (2024), who emphasized that loose media enhance worm respiration and nutrient uptake. Conversely, the coarse, sandy texture in T5 likely hindered mobility and water retention, triggering mucus production and respiratory stress, thereby suppressing growth (Utami *et al.*, 2024).

Table 2. Average weight gain, number of cocoons, population size, and medium reduction over six weeks of observation.

| Treatment | Variable | | | | | | |
|-----------|----------------------|---------------------|---------------------|---------------------------|--|--|--|
| Treatment | EWG (g) | NC (units) | EP (individuals) | MS (g) | | | |
| Т0 | 3.57 ± 0.40^{b} | 74.39 ± 3.22^{ab} | 37.60±3.51a | 182.00±11.46 ^a | | | |
| T1 | 2.82 ± 0.53^{b} | 77.61 ± 10.90^a | 33.39 ± 1.73^{a} | 178.83 ± 11.46^{a} | | | |
| T2 | 4.95 ± 0.95^{a} | 87.94±7.68a | 32.17 ± 10.01^{a} | 198.83±38.61a | | | |
| T3 | 1.67 ± 0.15^{c} | 55.56±12.18bc | 6.22 ± 3.84^{b} | 116.67±8.20 ^b | | | |
| T4 | 1.49 ± 0.31^{cd} | 42.33 ± 24.01^{d} | 4.72 ± 9.10^{b} | 119.83±6.93 ^b | | | |
| T5 | 1.11 ± 0.66^{cd} | 18.61±8.95° | 2.61 ± 4.57^{b} | 129.17 ± 18.82^{b} | | | |

Note: To show significant differences (p<0.05), the data are shown in the same column with numbers followed by various letters. Earthworm population (EP), number of cocoons (NC), weight growth (EWG), and media shrinkage (MS) are among the characteristics that are detected. The treatments applied were as follows: T0 consisted of 100% cow manure; T1 contained 95% cow manure and 5% mealworm frass; T2 had 90% cow manure and 10% mealworm frass; T3 included 85% cow manure and 15% mealworm frass; T4 was made up of 80% cow manure and 20% mealworm frass; and T5 comprised 75% cow manure and 25% mealworm frass.

b. Cocoon Production Quantity

Cocoon production differed significantly among treatments (p<0.05), ranging from 18.61 (T5) to 87.94 (T2). The high output in T2 may be attributed to a favorable C:N ratio and moderate protein content (~11%), which support gametogenesis and oviposition. Darmawan *et al.* (2023), similarly found that protein levels between 10–12% enhanced reproductive performance in annelids. In contrast, T5's lower cocoon count may result from excessive protein and poor media texture, which negatively affected worm vitality and reproductive behavior. This reinforces that not only nutrient content but also media structure influences reproductive success.

c. Population Growth of Lumbricus rubellus

Earthworm population growth ranged from 2.61 (T5) to 37.60 (T0). Though T0 showed the highest mean, it was not significantly different from T1 and T2. These treatments shared loose media textures conducive to cocoon hatching, as noted by Rahman & Hajam (2024). T5, with a coarse texture and possibly excessive protein, reduced cocoon viability and hatching success. Earthworms require 9–15% protein for optimal growth and reproduction (Darmawan *et al.*, 2023), and exceeding this may disrupt microbial stability or cause toxic buildup. Hence, moderate frass levels (T2) maintain a balance between fertility and survivability, and higher concentrations compromise cocoon development and hatchability.

d. Media Shrinkage in Lumbricus rubellus Cultivation

Media shrinkage, defined as the difference between the initial and final weight of the medium, serves as an important proxy for assessing earthworm activity and substrate decomposition. As shown in Figure 1, significant differences (p<0.05) in medium reduction were observed among treatments. Treatment T2 (90% cow dung + 10% mealworm frass) showed the highest medium shrinkage (198.83 g), which correlates with its superior performance in earthworm biomass gain and cocoon production. This suggests that T2 achieved an optimal nutritional balance—particularly in protein levels and texture—enhancing feed consumption and metabolic activity. Utami *et al.* (2024) reported that greater media reduction is often directly proportional to increased earthworm biomass due to higher ingestion and digestion rates. Conversely, T3–T5, which incorporated higher proportions of mealworm frass (15–25%), exhibited significantly lower shrinkage values (116.67–129.17 g). These results indicate reduced feeding activity, likely due to the coarse and sandy texture of the medium. Utami *et al.* (2024) similarly noted that sandy substrates hinder earthworm mobility and feeding, resulting in decreased organic matter processing. This is supported by Mashur *et al.* (2021), who found that physical media constraints—such as poor porosity or rough particle structure—can impair microbial and worm activity alike.

These findings emphasize a causal link, media shrinkage is not only a passive consequence of feeding but reflects the suitability of substrate characteristics for earthworm metabolism. Moderate frass inclusion improves nutritional content without compromising texture, whereas excess frass introduces structural constraints that suppress productivity. Hence, optimal media design should balance nutrient enrichment with physical comfort for the worms. Furthermore, similar trends have been observed by Kavvadias *et al.* (2024), who found that organic waste mixtures with overly fibrous or sandy components led to minimal media loss and poor vermicompost quality. Thus, media shrinkage can serve as both a biological and engineering indicator in optimizing vermiculture systems.

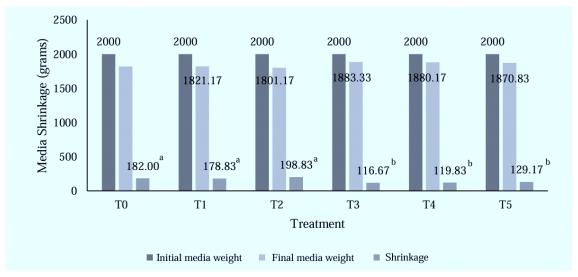


Figure 1. Average reduction in the maintenance medium of *Lumbricus rubellus* (Note: Bars with different letters indicate significant differences at p < 0.05 based on Tukey's HSD test)

3.2. Quality and Performance of Vermicompost

3.2.1 Environmental Conditions of the Planting Test

Environmental factors during the water spinach (*Ipomoea reptans*) growth trial were recorded three times daily (07:00, 12:00, 16:00), resulting in an average temperature of 29.4 °C and humidity of 79.47% (Table 3). These values fall within the optimal temperature range (25–30 °C) for water spinach development, as noted by Fadhlillah *et al.* (2019). Humidity peaked in the morning (98.79%) and declined at noon (68.48%), reflecting a typical diurnal pattern in tropical climates. While water spinach tolerates humidity levels as low as 60% (FAO, 2021), both excessive and insufficient humidity may impede growth. These environmental conditions were stable and supportive of plant physiological activity, allowing for a reliable assessment of the planting media's effects on agronomic performance. Thus, external factors did not confound the results, validating the interpretation that growth differences were primarily caused by vermicompost nutrient variation.

Table 3. Environmental temperature and humidity during water spinach cultivation

| Parameter | | Aviamaga | | |
|------------------|---------|----------|-----------|---------|
| rarameter | Morning | Noon | Afternoon | Average |
| Temperature (°C) | 24.31 | 33.19 | 30.71 | 29.40 |
| Humidity (%) | 98.79 | 68.48 | 71.14 | 79.47 |

3.2.2. Vermicompost Quality

Macronutrient content is a critical indicator of vermicompost quality, directly affecting plant growth. Plants require sufficient levels of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) to achieve optimal physiological development. Table 4 shows that all vermicompost treatments (U2–U7) exceed the minimum thresholds for organic fertilizers per SNI 7763:2018—namely \geq 15% organic C, \geq 0.40% N, \geq 0.10% P_2O_5 , and \leq 25.50% K_2O —indicating their qualification as quality organic fertilizers.

Nutrient content in the vermicompost increased as the proportion of mealworm frass in the rearing medium rose. Treatment U7 (vermicompost from T5: 75% cow dung + 25% frass) yielded the highest levels: 41.51% organic C, 1.99% N, 1.99% P₂O₅, and 1.47% K₂O. Compared to the baseline cow dung (Table 5), frass contributed significantly higher nutrient concentrations (especially P and K), which supports its value as a compost enhancer. These results are consistent with Antoniadis *et al.* (2023) and Zunzunegui *et al.* (2024), who found that insect frass contains elevated levels of essential macronutrients.

Table 4. Macronutrient content of vermicompost

| Treatment | C-organic (%) | N (%) | C/N | P2O5 (%) | K ₂ O (%) |
|-------------------------------------|---------------|-------|-------|----------|----------------------|
| 100% soil (U0) | 1.6 | 0.16 | 10.00 | 1.36 | 0.33 |
| 50% soil + 50% vermicompost T0 (U2) | 41.13 | 1.52 | 27.06 | 1.05 | 0.72 |
| 50% soil + 50% vermicompost T1 (U3) | 40.55 | 1.66 | 24.43 | 1.29 | 0.91 |
| 50% soil + 50% vermicompost T2 (U4) | 40.48 | 1.74 | 23.26 | 1.44 | 1.01 |
| 50% soil + 50% vermicompost T3 (U5) | 41.04 | 1.82 | 22.55 | 1.76 | 1.22 |
| 50% soil + 50% vermicompost T4 (U6) | 41.41 | 1.96 | 21.13 | 1.91 | 1.4 |
| 50% soil + 50% vermicompost T5 (U7) | 41.51 | 1.99 | 20.86 | 1.99 | 1.47 |

Note: U1 was excluded from nutrient analysis as it used inorganic NPK fertilizer, whose nutrient content is commercially predefined and not derived from vermicomposting.

The observed trend is not coincidental but reflects a clear causal relationship, the inclusion of frass—rich in protein and minerals—improves microbial activity during decomposition, thereby enhancing nutrient mineralization. Utami et al. (2024) emphasized that vermicompost quality is not solely dependent on worm productivity, but also on substrate composition and microbial fermentation dynamics. This explains why treatments with lower worm biomass (e.g., T5) can still produce nutrient-dense vermicompost suitable for plant cultivation. In practical terms, treatment U7 not only exceeds SNI standards but also leads to significantly improved agronomic performance in water spinach, as shown in the next section. This confirms that high-nutrient vermicompost translates to higher bioavailability for plants. Therefore, even if excessive frass reduces worm productivity, it can still yield valuable compost, especially in systems focused on plant fertilization rather than vermiculture efficiency.

Table 5. Macronutrient Content of Cow Dung and Mealworm Frass

| Treatment | C-organic (%) | N (%) | C/N | $P_2O_5(\%)$ | K ₂ O (%) |
|--------------------|---------------|-------|-------|--------------|----------------------|
| 100% cow dung | 44.71 | 1.41 | 31.71 | 0.81 | 0.53 |
| 100% Mealworm dung | 53.1 | 1.89 | 28.10 | 2.5 | 1.95 |

3.2.3. Planting Test Performance

The use of planting media derived from vermicompost on water spinach (*Ipomoea reptans*) had a significant effect (p<0.05) on stem height, leaf width, leaf length, number of leaves, stem weight, leaf weight, root weight, and root length. The growth of water spinach plants was greatly influenced by the nutrient content present in the media. This is in line with Raksun *et al.* (2022), who stated that to obtain optimal plant growth, fertilization is necessary to increase the nutrient elements beneficial to plants. The productivity data of water spinach during the study is presented in Table 6.

In the agronomic test on water spinach (*Ipomoea reptans*), eight treatments were applied, labeled U0 to U7. Although only seven of these treatments (U2–U7) were derived from vermicompost produced using media T0–T5, treatment U1 was still included as a comparison because it used inorganic fertilizer (NPK), which is commonly applied commercially. Therefore, U1 was not included in Table 4, which focuses on the analysis of vermicompost nutrient content, but it remains relevant in Table 6 to assess plant growth responses compared to organic treatments.

Table 6. Agronomic Characteristics of Water Spinach Plants

| | Variable | | | | | | | |
|----------------|---------------------------|-----------------------|---------------------------|---------------------------------|----------------------|-----------------------|-----------------------|-------------------------|
| Treat- ment | Stem Height (mm) | Leaf Width (mm) | Leaf Length (mm) | Number of Leaves (leaves) | Stem Weight (g) | Leaf Weight (g) | Root Weight (g) | Root Length (mm) |
| U0 | 94.31 ± 25.24^{d} | 5.52 ± 1.18^{d} | 34.28 ± 8.82^{d} | 4.85 ± 1.45^{d} | 3.02±1.83° | 1.52±1.29e | 3.49 ± 2.40^{c} | 119.08±75.54° |
| U1 | 109.34 ± 31.95^{bcd} | 7.28 ± 1.53^{bcd} | 49.62 ± 18.46^{abc} | 5.75 ± 1.36^{cd} | 8.98 ± 1.79^{bc} | 7.42 ± 2.15^{cd} | 8.13 ± 5.37^{bc} | 223.80±83.30abc |
| U2 | 106.04 ± 14.99^{cd} | 6.06 ± 0.54^{cd} | 41.38±5.51 ^{bcd} | 5.79 ± 0.66^{cd} | 4.96 ± 1.60^{c} | 4.01 ± 1.57^{de} | 3.38 ± 0.83^{c} | 163.73 ± 66.97^{bc} |
| U3 | 99.67 ± 12.86^{d} | 5.30 ± 1.38^{d} | 36.70 ± 9.90^{cd} | 4.74 ± 1.40^{d} | $15.42\pm22.76c$ | 2.83 ± 1.94^{de} | 3.54 ± 2.27^{c} | 223.84±74.42abc |
| U4 | 124.7 ± 6.51^{bcd} | 7.79 ± 0.56^{bc} | 45.64 ± 0.95^{bcd} | 6.50 ± 0.40^{bcd} | 7.55 ± 1.37^{bc} | 5.76 ± 1.36^{cde} | 7.28 ± 1.73^{bc} | 280.93 ± 60.23^a |
| U5 | 141.95±14.61 ^b | 8.53±1.41° | $49.62\pm3,37^{abc}$ | 7.64 ± 0.66^{ab} | 11.07 ± 3.57^{ab} | 10.53±5.87bc | 10.94 ± 6.53^{ab} | 290.83±104.02a |
| U6 | 135.65±20.14bc | 9.30 ± 1.95^{b} | 51.46±3.72ab | 7.57 ± 1.07^{abc} | 13.23±3.15abc | 14.56±3.80ab | 9.25 ± 2.16^{abc} | 274.27 ± 56.64^{ab} |
| U7 | 173.40±29.79a | 11.35±1.70a | 60.14 ± 5.40^{a} | 9.10 ± 1.60^{a} | 19.72±5.92a | 17.78±6.25a | 14.55±4.55a | 329.11 ± 31.87^a |

An interesting finding emerged in treatments U5 to U7, which showed significantly higher growth performance in terms of stem height, number of leaves, leaf width and length, and fresh weight. Notably, the vermicompost used in these treatments came from media T3–T5, which contained high proportions (15–25%) of mealworm frass and previously demonstrated lower earthworm productivity (low biomass, fewer cocoons, and minimal media shrinkage). This phenomenon indicates that the high nutrient content of vermicompost derived from T3–T5, including organic carbon (C-organic), nitrogen (N), phosphorus (P₂O₅), and potassium (K₂O), still positively influenced plant growth, despite the reduced earthworm productivity during the composting process.

According to Liu et al. (2022) and Sani & Yong (2022), mealworm frass is rich in protein and macronutrients, making it a potentially high-quality compost input even if it is not optimal for earthworm growth. Moreover, Zhou et al. (2023), emphasized that high nitrogen levels significantly impact chlorophyll formation, photosynthesis, and vegetative plant growth, including leaf number and area. Utami et al. (2024) also explained that vermicompost quality does not solely depend on earthworm performance but is strongly influenced by the composition of the initial materials and fermentation conditions, which determine final nutrient availability. Thus, this finding confirms that the final nutrient quality of vermicompost can remain high even when the composting process encounters challenges such as coarse media or high frass content that are unfavorable for earthworms. This opens up opportunities to utilize higher proportions of frass as an organic fertilizer input, especially when the goal is to enhance plant growth rather than earthworm productivity.

a. Root Length

Roots are the most essential part of a plant because roots function as a tool to absorb water and minerals, which are then distributed throughout the plant. Soliman *et al.* (2022) said that roots function to absorb water, nutrients, and organic materials that plants use as stimulants for plant growth and development. After analyzing the root length, it was found that the lowest root length of the water spinach plant was in the U0 treatment, which was 119.08 mm, while the highest root was in the U7 treatment, which was 329.11 mm. The difference influences the root length in nutrients contained in the planting medium, especially phosphorus levels. The higher the nutrient content, the longer the plant roots. The longer the root size, the greater the possibility of nutrients being absorbed (Sun *et al.*, 2023). The high content of nitrogen and phosphorus stimulates root growth, increasing absorption capacity and speed. Deficiencies in plants can result in disturbed plant growth, such as small plant stems, yellow leaves, and roots that do not grow well, disrupting absorption (Vaishnav *et al.*, 2022; X. Wang *et al.*, 2023).

b. Increase in Stem Height of Water Spinach

After analysis in Table 6, it was found that vermicompost produced from the digestion of earthworms added with a mixture of cow dung and mealworm dung had a significant effect on plant height (p<0.05). This occurs because of differences in nutrients contained in the planting medium, which affects the height of the kale plant. The results of the study showed that the highest stems were produced in the U7 treatment, namely with the use of 75% cow dung + 25% mealworm manure vermicompost, while the lowest was in the U0 treatment of 94.31 mm. The less-than-optimal growth of plant height is due to the lack of nutrient availability, so plants cannot grow optimally. In addition, the lack of nutrients can also inhibit the process of cell division and elongation in plants. In line with Jaoudé *et al.* (2025) stated that the less than optimal growth of plant height that is not given compost is due to the low availability of nutrients in the soil so that the process of cell division and elongation at the tip of the plant is not optimal. Giving vermicompost to the soil can increase the existing nitrogen elements and stimulate plant height growth. The nitrogen content in the soil is 0.16%, while in U7 vermicompost, it is 1.99%. The difference in nitrogen content, which is relatively high, dramatically affects the growth of plant height.

c. Increase in Leaf Width and Length of Water Spinach

After analyzing Table 6, it was found that the average leaf width and length of water spinach differed significantly (p<0.05). The lowest average leaf width was found in treatment U3 at 5.30 mm, while the highest was in treatment U7 at 11.35 mm. The highest average leaf length was also recorded in treatment U7 at 60.14 mm, and the lowest in treatment U0 at 34.28 mm. These differences in average leaf width and length were influenced by variations in the nutrient content of the growing media. One of the essential nutrients used by plants for leaf development is nitrogen. Treatment U7 had

a nitrogen content of 1.99%, which contributed to the increased leaf width of the water spinach. Broader leaves in water spinach support the photosynthesis process; the wider the leaf, the more energy is produced through photosynthesis. Muhammad *et al.* (2022) also stated that nitrogen in the growing media plays a crucial role in the formation of chlorophyll in leaves. The higher the chlorophyll content, the more photosynthates will be produced.

d. Increase in the Number of Water Spinach Leaves

The number of leaves in water spinach is a crucial factor, as the leaves are one of the main parts of the plant that attract attention. Good leaves are characterized by optimal width and length and are free from pest damage. Analysis showed that the number of leaves differed significantly (p<0.05), with an average ranging from 4.74 to 9.10 leaves. The highest number of leaves was observed in treatment U7, and the lowest in treatment U3. The nutritional composition of the developing medium affects this variance. Treatment U7 produced the most leaves, most likely because the growing medium fit the demands of water spinach, therefore hastening the development of the plant. Furthermore more nitrogen (N), phosphorous (P), and potassium (K) U7 had than U3. Particularly nitrogen is essential for vegetative development—that is, for the creation of leaves, stems, and shoots. Barlóg et al. (2022) claim that plant development is influenced strongly by the suitable fertilizer dose. Increased plant output will result from more leaves per stem. Moreover, Zhou et al. (2023) noted that additional leaves might boost chlorophyll content, thereby influencing the photosynthesis process.

e. Root Length

One of the most important components of a plant is its roots, which collect nutrients and water then distribute all throughout the plant. Bayala & Prieto (2020) claims that root systems help the plant to collect water, nutrients, and organic matter—all of which are used for stimulation of development and growth. The smallest root length of water spinach was noted in treatment U0 at 119.08 mm based on the root length study; the longest was noted in treatment U7 at 329.11 mm. Variations in nutrient contents—especially phosphorous—in the growth medium affected the root length variance. The roots often grow longer the more nutrients are present. The possibility for nutrient absorption increases with length of the roots (Mishra et al., 2023). Root development is greatly stimulated by appropriate quantities of nitrogen and phosphorous, hence improving absorption capacity and speed of intake. Nutrient deficiencies can disrupt plant growth, leading to symptoms such as stunted stems, yellowing leaves, and poorly developed root systems, which hinder the plant's ability to absorb water and nutrients effectively (Ghimirey et al., 2024; Mishra et al., 2023).

f. Fresh Weight of Water Spinach Plants

The fresh weight of water spinach, derived from its stems, leaves, and roots, differed significantly among treatments (p<0.05), with treatment U7 yielding the highest weight. This outcome is consistent with agronomic parameters such as increased stem height, leaf size, and leaf count, all of which contribute to biomass accumulation. Yanes *et al.* (2020) and Wang *et al.* (2021) emphasize that greater leaf area enhances photosynthetic activity, thus increasing fresh weight. Das *et al.* (2022) further links optimal nutrient uptake with improved growth performance. In this study, U7's superior performance correlates with the high nutrient content of the applied vermicompost, especially nitrogen and phosphorus, both known to stimulate vegetative growth and chlorophyll synthesis. Moreover, the productivity of *Lumbricus rubellus* was highest in T2 (10% mealworm frass), yielding the greatest weight gain (4.95 g), cocoon count (87.94), and media reduction (198.83 g). These indicators reflect optimal nutrient availability and media structure—factors echoed by Utami *et al.* (2024), who found that loose, nutrient-rich media enhance worm growth and reproduction. This aligns with the principle that moderate protein levels facilitate microbial activity and substrate breakdown, thereby supporting worm metabolism.

Interestingly, while T2 optimized worm productivity, the vermicompost from T5 (used in U7) provided the most balanced macronutrient profile (41.51% C, 1.99% N, 1.99% P₂O₅, 1.47% K₂O), which exceeded the SNI 7763:2018 minimum standards. These findings suggest a trade-off between maximizing worm growth and producing nutrient-rich compost, yet both outcomes support improved plant development. This dual benefit reinforces the idea proposed by Rehman *et al.* (2023) and Bhunia *et al.* (2021) that vermicompost quality is influenced not just by nutrient input, but also by the biological efficiency of the decomposers. Compared to previous studies that used cricket frass (Utami *et al.*, 2024) or vegetable waste (Bashir *et al.*, 2021), this research uniquely evaluates both worm productivity and plant growth

outcomes using mealworm frass—a novel substrate with underexplored agronomic potential. The holistic approach adopted here demonstrates a stronger causal link between media composition, worm biology, compost quality, and plant response, providing a comprehensive model for sustainable organic fertilizer development. Ultimately, integrating 10% mealworm frass into earthworm rearing media enhances worm productivity while enabling the production of high-quality vermicompost that significantly boosts water spinach growth. These results contribute to organic waste valorization and support the transition toward environmentally sustainable agricultural practices.

4. CONCLUSION

Supplementing cow manure with 10% mealworm frass significantly improved the productivity of *Lumbricus rubellus*, as indicated by the highest weight gain (4.95 g), cocoon production (87.94 cocoons), and media reduction (198.83 g). Meanwhile, vermicompost derived from the 75% cow manure and 25% mealworm frass mixture (T5) demonstrated the best nutrient composition—41.51% organic carbon, 1.99% nitrogen, 1.99% phosphorus, and 1.47% potassium—meeting the SNI 7763:2018 standard. This vermicompost significantly enhanced the agronomic performance of water spinach (*Ipomoea reptans*), particularly in plant height, leaf development, and root growth.

To extend the applicability of these findings, future research should explore longer observation periods to assess the long-term stability and maturity of the vermicompost. It is also recommended to investigate a wider range of frass concentrations beyond the current 5–25% to identify the threshold at which productivity and compost quality are balanced. Additionally, trials on different horticultural crops and implementation at field scale are necessary to evaluate the generalizability and scalability of this method. Integrating economic and environmental impact assessments will also be crucial in supporting the adoption of this approach for sustainable agriculture and organic waste management.

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