

Optimizing vegetative propagation of black cincau (*Mesona palustris* bl): synergistic effects of growing media composition and foliar nutrient application

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Abstract

Mesona palustris BL. (black jelly grass) propagation requires an optimized cultivation system to maximize vegetative multiplication and establish vigorous propagules. This study investigated the singular and interactive effects of four growing media mixtures (M1: 100% Soil; M2: 50% Soil + 50% Goat Manure; M3: 50% Soil + 50% Compost; M4: 30% Soil + 35% Goat Manure + 35% Compost) and three foliar fertilizer types on the growth performance of stem cuttings over 60 days after planting. Results showed that the growing media significantly influenced all measured parameters ($p < 0.05$). The ternary mixture M4 provided the most comprehensive platform for aerial growth, resulting in maximum plant height (71.19 cm), leaf area (19.48 cm), shoot count (93.33 shoots/plant), and total dry weight (13.68 g). This superiority is attributed to the synergistic balance achieved in M4, which offers an optimal physical structure, sustained nutrient release, and enhanced microbial activity, promoting high cytokinin production and maximizing multiplication efficiency. Conversely, the highest root fresh weight (16.60 g) was achieved by M2, suggesting that root biomass prioritizes the high, readily available Phosphorus and organic matter density of goat manure over the structural benefits of compost. The findings establish that the M4 media combination is the most effective and sustainable strategy for producing high-quality, high-biomass *M. palustris* propagules, offering a direct contribution to efficient resource management in agricultural systems..

KEYWORDS

Mesona palustris; Vegetative propagation; Growing media; Organic amendment; Goat manure; Compost; Biomass partitioning

1. INTRODUCTION

Black grass jelly (*Mesona palustris*) often treated taxonomically as *Platostoma palustre* in several floristic databases) is a perennial herb in the Lamiaceae family widely cultivated in parts of East

and Southeast Asia for its stems and leaves, which are processed into grass jelly a traditional food ingredient increasingly positioned as a functional product (Deswati et al., 2024). Beyond its culinary value, *M. palustris* has attracted pharmacological

attention because it contains phenolic compounds and flavonoids associated with antioxidant and other bioactivities, supporting its broader use as a traditional health supplement (Rahmah et al., 2021). As demand for plant-based functional foods grows, developing efficient and scalable cultivation and propagation protocols becomes essential to ensure a reliable supply of uniform planting material and stable biomass production for this economically important crop (Khoi & Thom, 2024).

For commercial cultivation, vegetative propagation is generally favored because it preserves elite genotypes and enables rapid multiplication. In *M. palustris*, field-oriented references describe the species as readily propagated by stem cuttings when planted in well-prepared, loose soil, indicating that cutting propagation is feasible but still highly dependent on management conditions (Sanz Gallego et al., 2025). However, the success of cuttings is determined primarily by the establishment of adventitious roots and the recovery of shoot growth after excision (Hidayat et al., 2025). Physiologically, excision triggers wound responses and hormonal reprogramming—particularly auxin-related signaling—followed by root primordia initiation and emergence. These developmental steps require (i) adequate oxygen for respiration at the cutting base, (ii) favorable water relations to prevent desiccation, and (iii) sufficient carbon and nutrients to support new root and shoot meristem activity. When cuttings are still rootless, limited uptake from the substrate makes internal reserves and early photosynthetic recovery crucial for supplying carbohydrates to the rooting zone and sustaining growth.

Previous studies across horticultural cutting systems consistently show that the rooting environment (growing medium) is a major determinant of early establishment. An appropriate medium must simultaneously provide mechanical support, maintain sufficient water-holding capacity to avoid water stress, and preserve adequate air-filled porosity to prevent hypoxia at the cutting base

(Hidayat et al., 2025). Excessive water content and low air content in propagation substrates can delay adventitious root development and increase disease risk, whereas balanced water–air relations support faster rooting and healthier root systems. In practice, growers often improve substrate function by combining mineral soil with organic amendments (e.g., compost and manure) to enhance structure, moisture retention, and nutrient buffering, although the optimal proportion depends on local materials and container/rooting conditions (Khoi & Thom, 2024). Despite broad agreement that substrate physical properties shape rooting outcomes, evidence remains context-specific and species-dependent, particularly for medicinal/functional crops that are increasingly cultivated intensively. (Hidayat et al., 2025; Rahmah et al., 2021).

In parallel, foliar fertilization has been widely used to rapidly correct nutrient limitations or support growth during stages when root uptake is still constrained. The efficacy of foliar nutrition depends on leaf-surface properties and uptake pathways, which can involve penetration through the cuticle and, under certain conditions, stomatal or other epidermal routes (Hidayat et al., 2025; Khoi & Thom, 2024). Uptake efficiency varies with formulation, humidity, and leaf anatomy, but foliar application is generally recognized as a practical approach to deliver essential macro- and micronutrients quickly when soil/substrate uptake is suboptimal. From a physiological perspective, foliar N supply can accelerate canopy recovery and photosynthetic capacity, thereby strengthening carbohydrate source activity, while P and K contribute to energy metabolism and overall growth processes that indirectly support root formation and biomass accumulation. Consequently, foliar nutrition may influence not only shoot traits (height, leaf area, shoot proliferation) but also carbon allocation patterns that determine biomass partitioning between shoots and roots. We hypothesize that a specific combination of growing media composition (topsoil amended with compost and/or goat manure) and foliar NPK fertilizer typ

will produce a significant interaction, optimizing early establishment and biomass partitioning toward vigorous rooting and whole-plant growth. Therefore, this study evaluated the individual and interactive effects of several growing media mixtures and foliar fertilizer types on growth traits (plant height, leaf area, shoot number) and biomass accumulation (fresh and dry weights of shoots and roots) in *M. palustris* stem cuttings.

2. METHODS

2.1 Material and experimental site

The study was conducted from 20 January to 20 March 2020 (60 days after planting, DAP) in Krajan Hamlet, Watuagung Village, Dongko District, Trenggalek Regency, East Java, Indonesia. The planting material consisted of apical stem cuttings of black cincau (*Mesona palustris* Bl.; Lamiaceae) collected from healthy, mature mother plants.

The growing media components were locally sourced topsoil, well-decomposed goat manure, and mature compost. The topsoil was a friable mineral soil with a loam–sandy loam texture and near-neutral to slightly acidic reaction (typical pH $\approx$ 6.0–6.8), while the goat manure and compost were fully matured (dark brown, crumbly, odorless, and free from visible fresh residues), which generally indicates stable organic matter, low phytotoxicity, and moderate salinity suitable for nursery substrates. Before mixing, manure and compost were air-dried, screened (5 mm), and homogenized to ensure uniformity. The rationale for mixing mineral soil with organic amendments was to improve the substrate's functional properties for cutting establishment, including water-holding capacity, nutrient buffering, and air-filled porosity (oxygen availability) in the rooting zone.

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The rooting process was supported using Growtone (commercial auxin-based rooting

promoter, typically IBA-based). Foliar nutrition treatments used three commercial NPK foliar fertilizers: Gandasil D (20–15–15), Growmore (32–10–10), and Hyponex (20–20–20). Each experimental unit consisted of one 35 × 35 cm polybag. A hand sprayer equipped with a fine nozzle was used for foliar application. Fresh biomass was weighed using a digital balance (Sartorius), and dry biomass was determined using a drying oven. The experiment was conducted under nursery conditions designed to minimize desiccation during early rooting. Polybags were maintained under partial shade (approximately 40–50% light reduction using a shade net or equivalent protection) with good ventilation. Under these conditions, the microclimate was typically within the suitable range for herbaceous cutting establishment in the humid tropics (approximately 24–32 °C air temperature and 70–90% relative humidity), with moderate light levels that support leaf function while reducing excessive transpiration.

2.2 Experimental and procedure

The experiment was arranged as a 4 × 3 factorial Completely Randomized Design (CRD) with three replications, resulting in 12 treatment combinations and 36 experimental units (one polybag = one experimental unit; one cutting per polybag). The first factor was growing media composition (M) consisting of four levels: M1 (100% topsoil), M2 (50% topsoil : 50% decomposed goat manure), M3 (50% topsoil : 50% compost), and M4 (30% topsoil : 35% decomposed goat manure : 35% compost). The second factor was foliar fertilizer type (P) consisting of three commercial NPK formulations: P1 (Gandasil D, 20–15–15), P2 (Growmore, 32–10–10), and P3 (Hyponex, 20–20–20). To apply the growing media treatments, topsoil, decomposed goat manure, and compost were air-dried, screened (approximately 5 mm), and homogenized prior to mixing. Media mixtures were prepared according to the specified ratios (by volume) and thoroughly blended to ensure uniformity.

Apical stem cuttings (approximately 15 cm) were collected from healthy mother plants. The basal end was cut obliquely to increase the rooting surface area, and only four upper leaves were retained to maintain photosynthetic capacity while limiting transpiration. The basal 2–3 cm of each cutting was treated with Growtone (IBA-based rooting promoter) using a dip method for approximately 5 minutes. One cutting was planted at the center of each polybag at a depth of 5 cm, and all units were irrigated immediately after planting. Polybags were arranged in a uniform grid with equal spacing (approximately 30–40 cm between polybags) to reduce mutual shading and competition. To minimize positional/micro-environmental variability typical of polybag systems (e.g., edge effects and light gradients), all units were maintained under similar nursery conditions (partial shade and good ventilation), received the same irrigation schedule, and polybag positions were rotated systematically once per week throughout the experimental period.

Routine maintenance was applied uniformly across treatments and included manual watering and weeding. Foliar fertilizer treatments were applied as follows: each fertilizer was dissolved at 2 g L⁻¹ in clean water, mixed until fully dissolved, and sprayed using a hand sprayer with a fine nozzle. Spraying was carried out in the morning (approximately 07:00–09:00 local time) under calm weather to avoid drift (low wind) and to reduce evaporation. Foliar solution was applied at approximately 20–30 mL per plant per application, or until uniform wetting with slight incipient runoff, with emphasis on the abaxial (lower) leaf surface. To prevent cross-contamination among foliar fertilizer treatments, separate sprayers were used for each fertilizer type; when separate sprayers were not available, the sprayer tank and nozzle were thoroughly rinsed and flushed with clean water before preparing and applying the next fertilizer solution. Applications were conducted only when no rainfall occurred during spraying and for several hours afterward, and overhead irrigation was withheld for approximately

3–4 hours post-spraying to minimize wash-off. Foliar fertilization began one week after planting and was repeated every two weeks, for a total of three applications during the 60-day cultivation period.

2.2.1 Planting procedure

The components for each medium (M1–M4) were thoroughly homogenized according to the specified ratios and filled into 35 × 35 cm polybags. Media were brought to near field capacity by watering prior to planting. Healthy apical stem cuttings, approximately 15 cm in length, were harvested from mother plants. The basal cut was made obliquely to increase the rooting surface area. Four upper leaves were retained to maintain photosynthetic capacity, while lower leaves were removed to reduce transpiration and minimize decay. To enhance rooting, the basal end (≈2–3 cm) of each cutting was treated with Growtone using a dip method for approximately 5 minutes, then excess material was gently removed by tapping. One prepared cutting was planted into the center of each polybag to a depth of 5 cm. After planting, polybags were irrigated to field capacity and maintained under uniform nursery conditions.

2.2.2 Maintenance and fertilization

Routine maintenance included daily manual watering and mechanical weeding to eliminate competition. The foliar fertilizer treatments were prepared at a concentration of 2 g per liter of water. The fertilizer solution was applied using a hand sprayer, targeting the lower surface of the leaves to maximize nutrient absorption. Fertilization commenced one week after planting (Behera et al.) and was repeated every two weeks thereafter, resulting in a total of three applications throughout the 60-day cultivation period.

2.3 Growth and biomass measurement

Growth parameters were measured using both non-destructive and destructive methods. Observations were recorded bi-weekly, starting from 2 WAP until 8 WAP (60 DAP), and included; plant height (cm), number of leaves (leaves/plant),

and number of shoots (shoots/plant). At final harvest (60 DAP), destructive measurements were taken; fresh leaf weight (g/plant), dry leaf weight (g/plant), total fresh plant weight (g/plant) (including roots, stems, and leaves), total dry plant weight (g/plant) and fresh root weight (g/plant). Dry weight measurements were obtained by oven-drying the samples at 105°C for 2×24 hours until a constant weight was achieved.

2.4 Data analysis

All collected data were statistically analyzed using Analysis of Variance (ANOVA) to determine the significant effect of the individual factors (Growing Media and Foliar Fertilizer) and their interaction on the measured parameters. Treatment effects were tested at both the 1% and 5% probability levels. When the F-test indicated a significant difference ($p \leq 0.05$), mean separation was performed using the Least Significant Difference (LSD) post hoc test at the 5% confidence level to identify specific differences between treatment means.

All collected data were statistically analyzed using Analysis of Variance (ANOVA) to determine the significant effect of the individual factors (Growing Media and Foliar Fertilizer) and their interaction on the measured parameters

3. RESULT AND DISCUSSION

3.1 Growing media effects on plant height

Two-way ANOVA indicated that growing media composition (M) significantly affected plant height at 60 DAP ($p < 0.05$), whereas foliar fertilizer type (P) had no significant main effect ($p > 0.05$) and the $M \times P$ interaction was not significant ($p > 0.05$). When averaged across foliar fertilizer treatments, plant height was maximized by M4 (Topsoil + Goat

Manure + Compost, 30:35:35), reaching 71.19 cm at 60 DAP, which was statistically superior to the other media treatments ([Figure 1](#)). This result highlights the importance of optimizing substrate conditions to support early establishment and vigorous shoot elongation in *M. palustris* cuttings.

The superior performance of M4 is consistent with the functional advantage of a ternary mixture that likely provides a better balance between water availability and aeration compared with single- or binary-component substrates (Criscione et al., 2022). In cutting propagation, adequate air-filled porosity supports respiration at the cutting base and root growth, while stable moisture availability helps maintain leaf turgor and reduces desiccation during early establishment (Li et al., 2023). In addition, combining decomposed manure and compost with mineral soil can enhance nutrient buffering and supply, supporting sustained vegetative growth across the 60-day period.

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In contrast, the lower performance of M3 (Topsoil + Compost) suggests that structural improvement alone may not be sufficient for rapid early shoot elongation if short-term nutrient availability is comparatively lower than manure-amended mixes. Overall, the M4 mixture appears to integrate physical support and nutrient supply more effectively than the other media, thereby sustaining height increment through the observation period (Karavidas et al., 2022), demonstrating an optimized strategy for efficient propagule establishment.

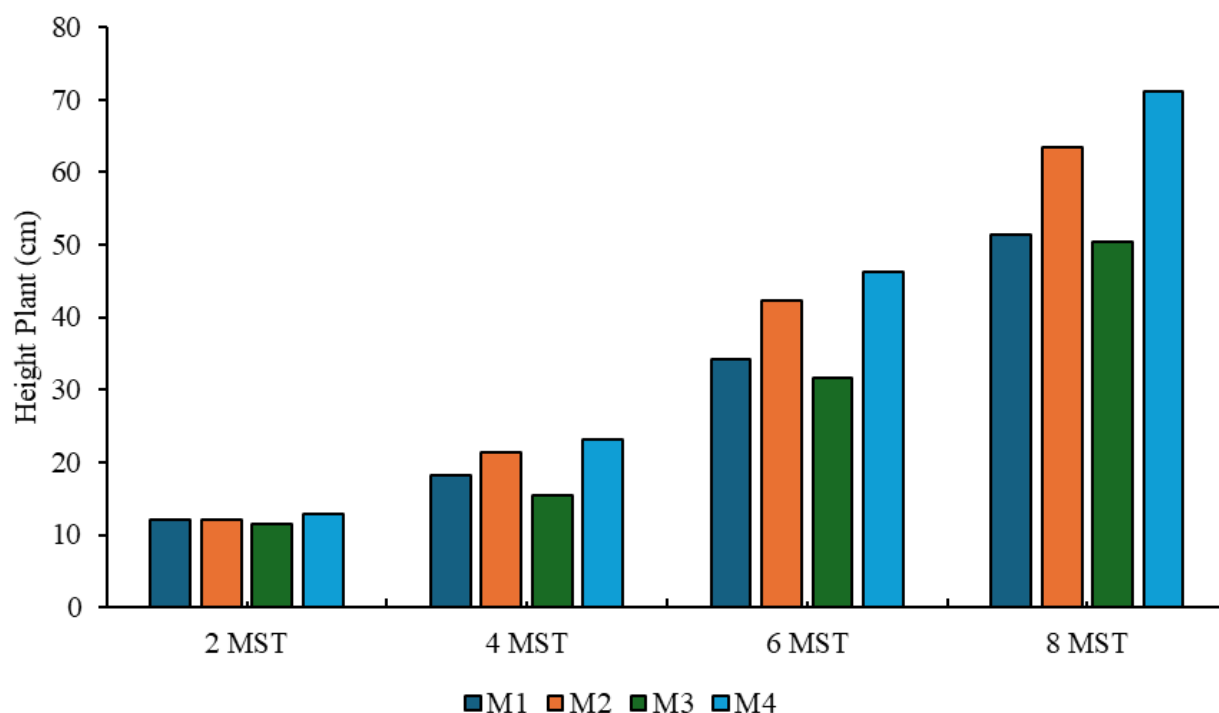


Figure 1. Effect of Growing Media on Plant Height (cm)

3.2 Growing media effects on leaf area

Foliar fertilizer (P) was not significant ($p > 0.05$) and the $M \times P$ interaction was not significant ($p > 0.05$). Leaf area is a key determinant of canopy photosynthetic capacity and is typically associated with biomass accumulation. At 60 DAP, M4 produced the largest leaf area (19.48 cm^2) and was significantly higher than the control M1 (11.86 cm^2) and other mixes (Figure 2).

The improvement in leaf area under M4 likely reflects more favorable rooting-zone conditions that support sustained water status and nutrient uptake. Leaf expansion depends strongly on cell turgor and adequate nitrogen supply to support chlorophyll formation and the development of photosynthetic tissues (Tian et al., 2022). Organic amendments can

contribute to improved moisture retention and nutrient buffering; thus, a combined manure–compost–soil medium may maintain more stable conditions for leaf growth than a medium with compost alone (Ali et al., 2023).

The relatively lower leaf area in M3 (Topsoil + Compost) especially compared with manure-containing treatments may reflect differences in short-term nutrient release patterns among organic amendments and potential transient N immobilization in high C:N materials during early establishment (Adekiya et al., 2020; Zhang et al., 2020). The M4 combination mitigated these risks by providing the necessary energy for rapid early growth (from manure) and the sustained environmental support (from compost and soil) required for full canopy development.

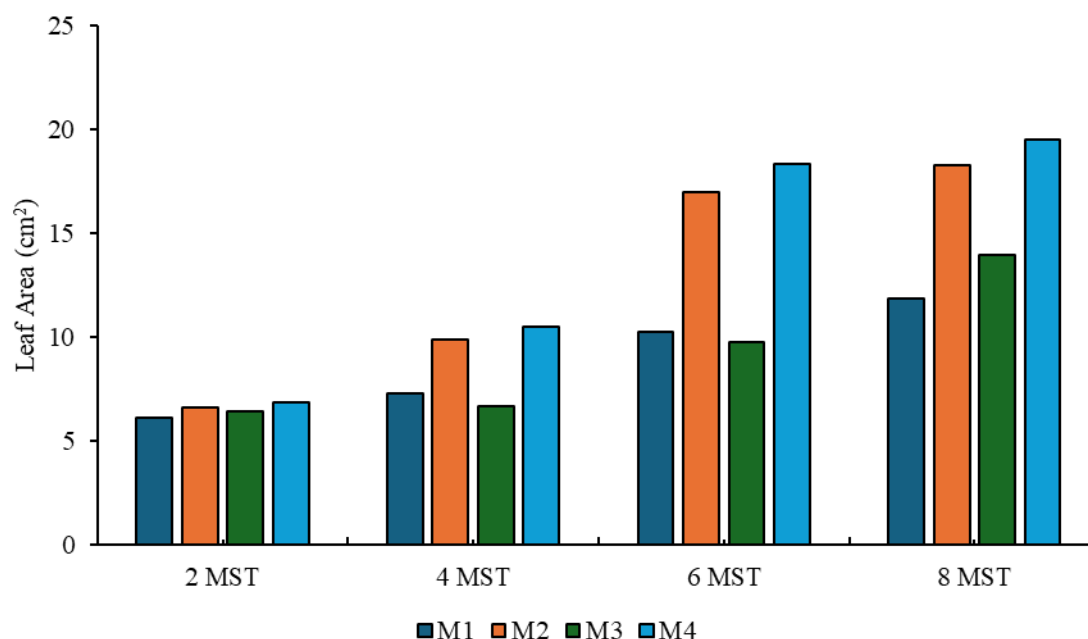


Figure 2. Effect of Growing Media on Leaf Area (cm²)

3.3 Growing media effects on shoot count

Shoot number is an important propagation trait because it reflects lateral bud activation and branching potential. At 60 DAP, M4 produced the highest shoot number (93.33 shoots plant⁻¹), significantly greater than the binary mix M2 (77.22) and the lowest-performing M3 (33.89) (Figure 3). The higher shoot production under M4 can be explained without over-specifying unmeasured mechanisms: a well-aerated and sufficiently moist rooting medium can promote more robust establishment, improving overall plant vigor and enabling more consistent development of axillary shoots. Sustained nutrient availability (particularly N and P) can support the formation of new meristematic tissues and the metabolic costs of repeated bud outgrowth (Ma et al., 2024).

Accordingly, any linkage between organic-amended media and hormone-mediated bud activation should be framed cautiously. Organic amendments can support microbial communities that may influence nutrient cycling and rhizosphere signaling, potentially contributing to improved growth, but confirming specific pathways would require direct microbial/hormone assessments (Arya et al., 2022; Bhattacharya, 2021). Furthermore, this structural integrity facilitated the continuous absorption of essential nutrients specifically Nitrogen (N) for protein synthesis in new meristematic tissues and Phosphorus (P) for the high metabolic energy (ATP) required for intense cell division which sustains the continuous emergence and growth of the multiple lateral buds (Singh et al., 2016).

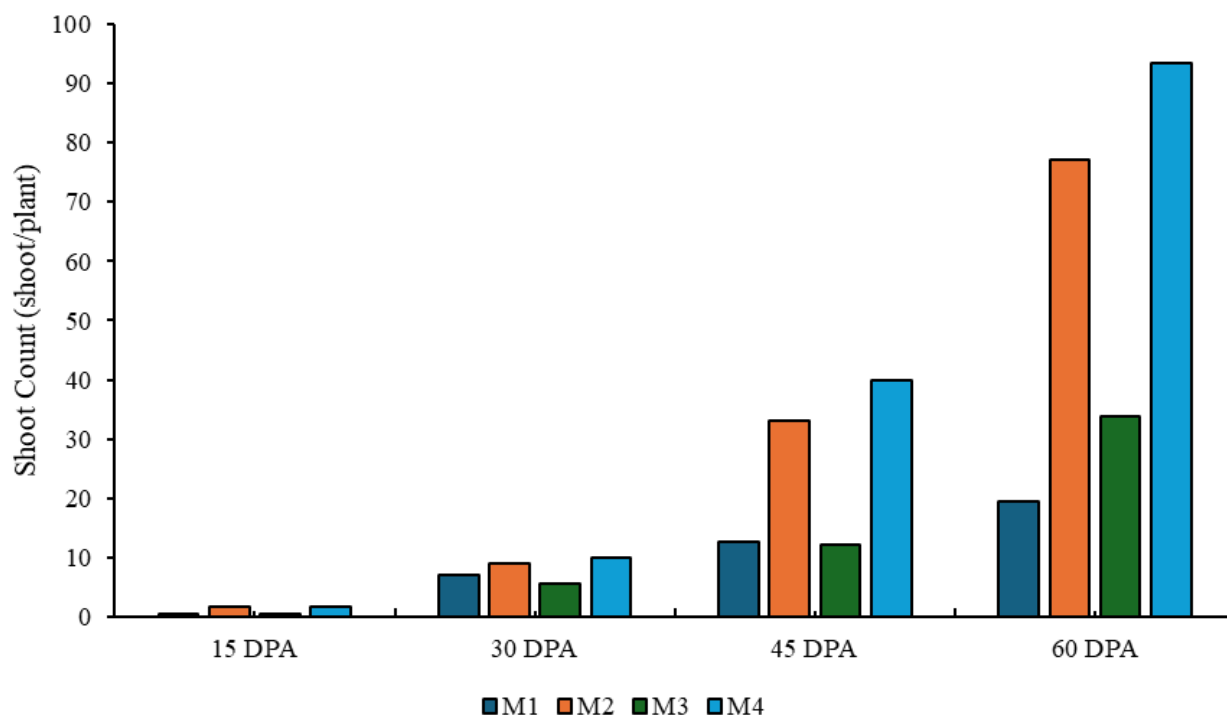


Figure 3. Effect of Growing Media on Shoot Count.

3.4 Growing media effect on plant

Foliar fertilizer (P) had no significant main effect ($p > 0.05$) and the $M \times P$ interaction was not significant ($p > 0.05$). At 60 DAP, M4 produced the highest total biomass, with mean total fresh weight of 106.73 g and total dry weight of 13.68 g (Figure 4). This pattern is consistent with the superior plant height, leaf area, and shoot number observed under M4, suggesting that the medium supported a more effective conversion of intercepted light into structural biomass. Higher dry weight generally indicates greater accumulation of structural carbohydrates and other organic compounds produced via photosynthesis, which is typically supported by larger canopy area and stronger plant vigor. In addition, K is known to be involved in enzyme activation and assimilate transport, and balanced nutrient availability can improve biomass accumulation and partitioning (Cao et al., 2025).

The comparatively low dry weight in M3 (5.01 g) further supports the view that compost-only

amendment may not provide the same early nutrient availability or rooting-zone function as media containing both manure and compost. When discussing microbial stimulation, it should remain evidence-aligned: organic blends may increase nutrient cycling through enhanced biological activity, but because this study did not quantify microbial activity, this mechanism should be presented as a plausible explanation rather than a confirmed cause (Hamnér et al., 2017). Furthermore, the structural stability of M4 ensures efficient water uptake, which is directly reflected in the high fresh weight value. Studies have confirmed that optimal organic media blends significantly enhance the harvestable biomass of vegetative crops by improving nutrient use efficiency and stimulating microbial activity that enhances nutrient cycling (Kumar et al., 2016). Thus, the optimized physical and chemical synergy achieved in M4 represents a highly efficient and sustainable strategy for maximizing *M. palustris* propagule production.

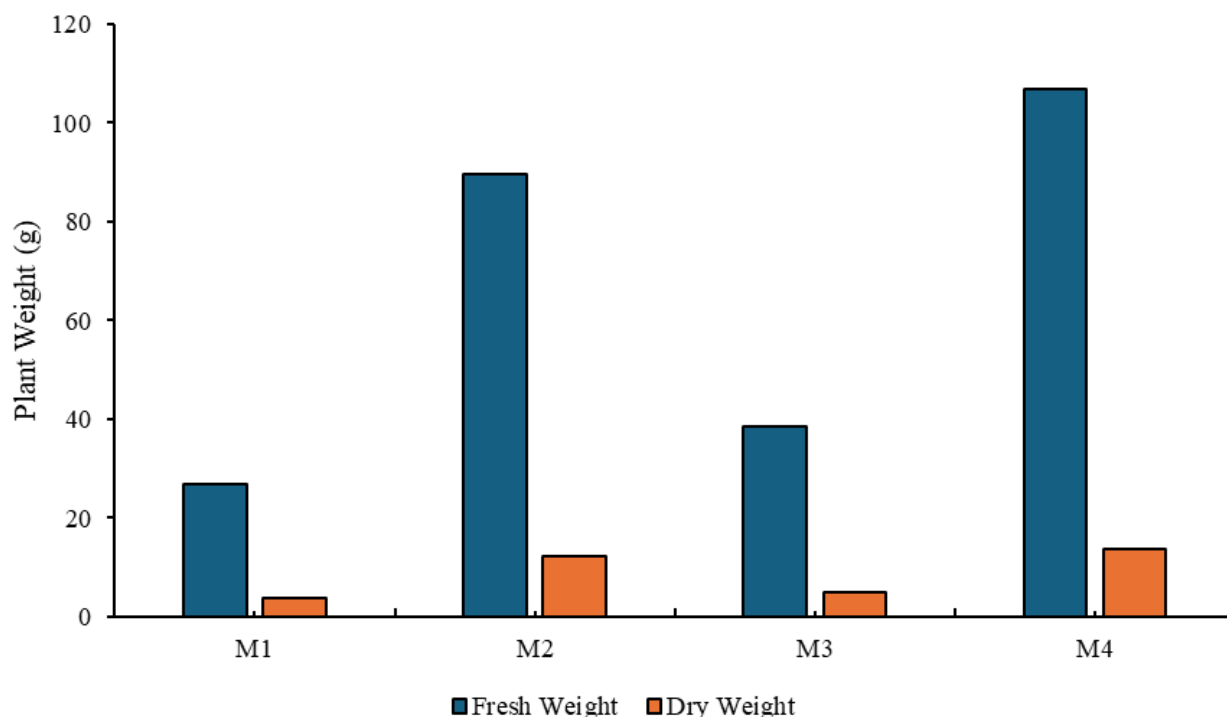


Figure 4. Effect of Growing Media on Plant: Fresh Weight and Dry Weight.

3.5 Growing media effect on root

Root fresh weight is a key indicator of rooting success and early field establishment potential. In contrast to the canopy traits (height, leaf area, shoot number) that were maximized by M4, the highest root fresh weight was achieved by M2 (50% Topsoil + 50% Goat Manure), reaching 16.60 g at 60 DAP, and was statistically superior or comparable to M4 (15.67 g) (Figure 5).

This contrasting response suggests that the substrate conditions that maximize canopy development are not always identical to those that maximize root fresh mass. Root growth is strongly influenced by local oxygen availability, moisture status, and nutrient availability (particularly P for energy metabolism and cell division), and manure-amended substrates can provide relatively rapid nutrient release during decomposition (Dhaliwal et al., 2019). Furthermore, the decomposition process of fresh organic manure may transiently produce or

harbor higher concentrations of natural auxins (or their precursors) and growth regulators crucial for rapid root biomass expansion compared to the more stabilized humic acids found in compost (Nardi et al., 2021; Rathor et al., 2023).

The slightly lower root fresh weight in M4 compared with M2 may reflect a trade-off in biomass allocation, where the stronger shoot growth under M4 increases assimilate demand aboveground, potentially reducing the proportion allocated to root fresh mass. Another explanation is a short-term nutrient dilution effect: adding compost can improve structure and buffering but may reduce the proportion of readily mineralizable nutrients compared with the 50:50 manure mix. Nevertheless, both M2 and M4 produced substantially higher root fresh weights than M1 and M3, indicating that organic amendment is important for reliable rooting and establishment in *M. palustris* cuttings (Rotowa et al., 2025).

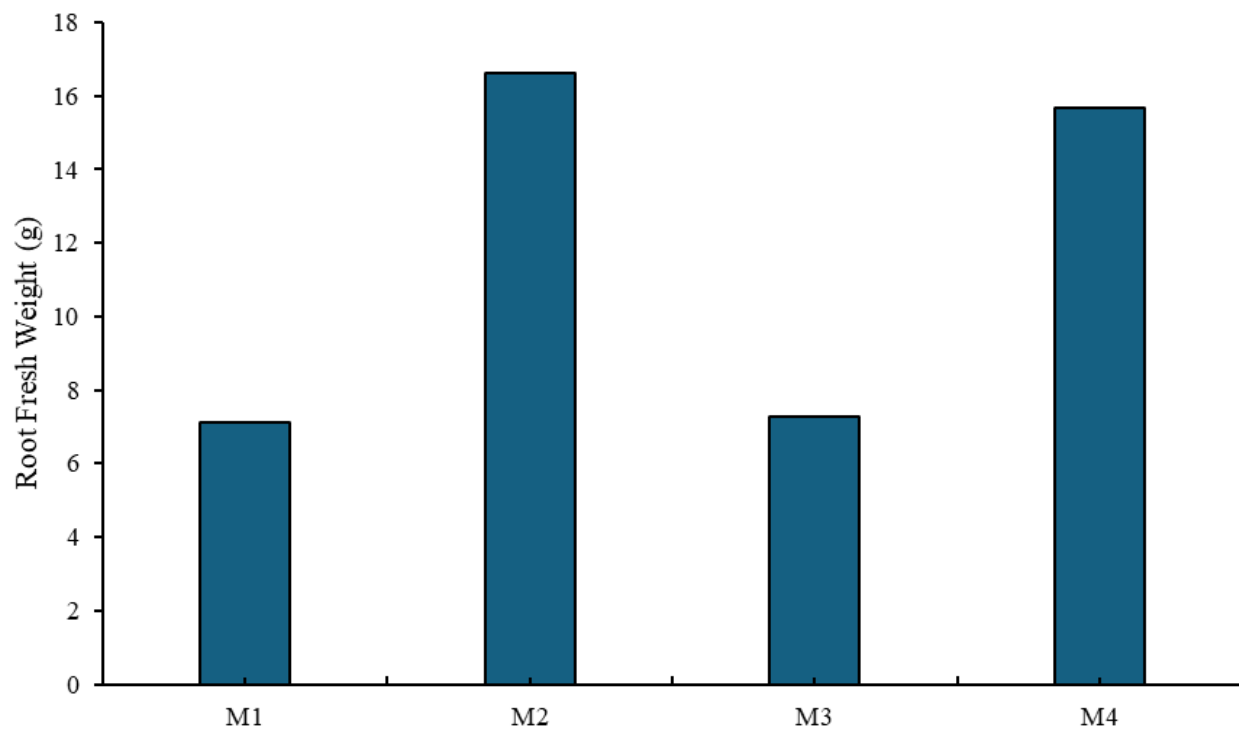


Figure 5. Effect of Growing Media on Root Fresh Weight

4. CONCLUSION

This study provides factorial evidence that growing media composition is the primary driver of early establishment, vegetative performance, and biomass accumulation of *Mesona palustris* stem cuttings during a 60-day nursery phase. Among the tested media, the ternary mixture M4 (30% topsoil + 35% decomposed goat manure + 35% compost) consistently produced the highest aboveground vigor (plant height, leaf area, and shoot number) and the greatest total dry biomass (13.68 g), indicating that a balanced combination of mineral soil and organic amendments can create a rooting environment that supports rapid canopy development and efficient propagule multiplication. In contrast, M2 (50% topsoil + 50% goat manure) produced the highest root fresh weight (16.60 g), suggesting that media conditions that maximize shoot proliferation may differ from those that maximize root fresh mass, which has practical implications for nursery objectives (rapid multiplication versus root-heavy transplants). Under

the fertilization schedule used in this study, the foliar NPK formulations did not provide a clear additional advantage over the strong media effect, implying that root-zone conditions dominated early growth responses in this propagation system. Therefore, we recommend M4 when the goal is to maximize overall plant vigor and shoot multiplication, while M2 may be considered when prioritizing root fresh mass for transplant robustness. Future studies should evaluate longer cultivation periods and post-transplant field establishment, while directly measuring substrate properties and physiological indicators to validate mechanisms and refine media fertilization strategies.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Muhammad Muharram (MM): Conceptualization, Methodology, Data Curation, Writing – Original Draft, and Corresponding Author. Eko Wahyu Rohmatulloh (EWR): Formal Analysis, Validation, and Writing – Review & Editing. Siti Alfiatul Amani (SAA): Investigation, Resources, and

Visualization. Tjatur Prijo Rahardjo (TPR): Data Interpretation, Project Administration, and Review. Edy Kustiani (EK): Literature Review, Editing, and Final Proofreading.

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ETHICS APPROVAL

No ethical approval was needed for this study.

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CONFLICT OF INTEREST

All authors state no conflict of interest.

DATA AVAILABILITY STATEMENT

The data available by request.

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