

Characteristics of Liquid Organic Fertilizer from Vegetable Waste Fermented with Various EM4 Concentrations and Its Effects on the Growth of Green Spinach (*Amaranthus hybridus* L.)

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ABSTRACT

*Green spinach (*Amaranthus hybridus* L.) is a high-value leafy vegetable commodity that requires optimal nutrient availability from the early stages of growth. Utilizing vegetable waste as liquid organic fertilizer (LOF) with EM4 and molasses bioactivators serves as an alternative solution for sustainable cultivation. This study aimed to analyze the physical characteristics of vegetable waste LOF under various EM4 and molasses concentrations, and to evaluate the effect of its application dosage on the vegetative growth and physiological components of green spinach. The research was conducted from February to June 2026 using a Factorial Randomized Block Design (RBD) with two factors and three replications. The first factor was the type of LOF based on EM4 and molasses concentrations (P1: 0 ml, P2: 20 ml, P3: 40 ml, P4: 60 ml). The second factor was the LOF application dosage (D1: 15 ml L⁻¹ dan D2: 30 ml L⁻¹). The results indicated that increasing EM4 and molasses concentrations altered the LOF color to dark brown and intensified the fermented tape-like aroma, while maintaining a stable temperature (33,29–34,08°C). Variations in the LOF type exerted no significant effect on any growth parameters of the spinach. Conversely, the application dosage factor had a significant independent effect during the early growth phase (21 days after planting / DAP). The 15 ml L⁻¹ (D1) produced a significantly higher number of leaves, leaf area (11,32 cm²), fresh weight (0.45 g), dry weight (0.035 g), Relative Growth Rate/RGR (0,74 g g⁻¹ day⁻¹), and Net Assimilation Rate/NAR (0,0075 g cm⁻² day⁻¹) compared to the 30 ml L⁻¹ dosage (D2).*

Keywords: Spinach; Liquid Organic Fertilizer; Molasses; EM4; Dosage.

INTRODUCTION

Green spinach (*Amaranthus hybridus* L.) is a leaf vegetable commodity of high economic value due to its relatively short harvest period, high productivity, and role as an essential source of vitamins, minerals, and antioxidants for the community. Demand for spinach continuously increases alongside growing fresh vegetable consumption, necessitating cultivation technologies capable of enhancing sustainable productivity (Rosyid et al., 2024).

As a short-duration vegetable crop, spinach requires sufficient nutrient availability from the onset of growth to ensure optimal leaf formation. Nitrogen is the most critical nutrient during the vegetative phase, playing a vital role in chlorophyll formation, protein synthesis, and cell division. Consequently, fertilization management stands out as a crucial factor in improving both the growth and yield of spinach crops (Karlitis et al., 2024).

The use of liquid organic fertilizer (LOF) offers an alternative to minimize dependence on inorganic fertilizers while boosting the efficiency of organic waste utilization. Market vegetable waste contains high organic matter that degrades easily, making it highly potential as a raw material for LOF. Fermentation using the EM4 bioactivator can accelerate the decomposition of organic matter, thereby increasing the availability of essential nutrients (Karyanto et al., 2022).

Although various studies have proven that LOF can enhance spinach growth, its effectiveness is heavily influenced by the quality of the produced fertilizer, fermentation duration, type of raw materials, and field application dosage. Different LOF dosages can trigger distinct growth responses, as they

correlate with the capacity of the plant root system to absorb water and nutrients at each growth stage (Azgara et al., 2024). Based on these conditions, this study aimed to analyze the characteristics of liquid organic fertilizer derived from vegetable waste under various EM4 and molasses concentrations, and to evaluate the impact of its application dosage on the growth of green spinach (*Amaranthus hybridus* L.).

METHOD

The research was conducted from February to June 2026 at the Experimental Field of Universitas KH. A. Wahab Hasbullah, Jombang. The primary raw materials for the LOF consisted of 8 kg of vegetable waste, 4 kg of banana pseudostem, 4 kg of rice bran, and 4 liters of rice-washing water. The solid components were finely chopped and placed into a modified anaerobic gallon reactor. The fermentation process was carried out anaerobically for 14 days. The trial utilized four levels of activator volume combinations (EM4 and molasses at a 1:1 ratio), namely: P1 : Without EM4 and molasses (control); P2 : 20 mL EM4 + 20 mL molasses; P3 : 40 mL EM4 + 40 mL molasses, & P4 : 60 mL EM4 + 60 mL molasses.

The field experiment was arranged in a Factorial Randomized Block Design (RBD) with two factors and three replications. The first factor was the Type of LOF (P1, P2, P3, P4), and the second factor was the LOF Application Dosage consisting of two levels: D1 (15 ml L⁻¹) and D2 (30 ml L⁻¹). The interaction of these two factors yielded 8 treatment combinations for the experimental units (P1D1, P2D1, P3D1, P4D1, P1D2, P2D2, P3D2, P4D2). The growing medium utilized 1 kg of homogenous soil per polybag, requiring a total of 144 plant pots. Spinach seeds were sown directly without nursery transplanting at a depth of ± 2 cm. LOF application was carried out by drenching the root zone once a week during the morning or evening.

The observed physical characteristics of the LOF included temperature, color, aroma, and solution condition. Vegetative growth parameters included plant height, leaf number, leaf area, fresh weight, and dry weight, which were periodically monitored from 14 to 42 days after planting (DAP). Physiological components were calculated based on the Relative Growth Rate (RGR) and Net Assimilation Rate (NAR). The data obtained were analyzed using ANOVA (Analysis of Variance) at a 5% significance level to determine treatment effects, followed by a post-hoc test if significant effects were detected.

RESULT AND DISCUSSION

Result

Observations on the physical characteristics of the vegetable waste LOF showed that the addition of EM4 and molasses induced changes in color, aroma, and the condition of the post-fermentation solution. Table 1 indicates that LOF without EM4 and molasses (P1) exhibited a faded green color with a vinegar-like and slight tape-like aroma. In contrast, increasing concentrations of EM4 and molasses turned the solution brown to dark brown, accompanied by an increasingly pungent tape-like aroma. The fermentation temperature across all treatments ranged between 33,29–34,08°C, indicating a relatively stable fermentation process. Furthermore, some treatments showed rice bran sediment at the bottom of the fermentation container, particularly in treatments P2 and P4.

Table 1 Physical Characteristics Observation of Vegetable Waste LOF

Treatment	Temperature (°C)	Color	Aroma	Solution Condition
P1: No EM4 and molasses (Control)	33.29	Faded green	Vinegar aroma, slight tape odor	Homogenous/well-mixed solution
P2: 20 mL EM4 + 20 mL molasses	34.08	Yellowish brown	Pungent tape aroma	Vegetable waste mixed with bran, some bran settled at the bottom
P3: 40 mL EM4 + 40 mL molasses	33.36	Brown	Highly pungent tape aroma	Homogenous/well-mixed solution
P4: 60 mL EM4 + 60 mL molasses	33.29	Dark brown	Pungent tape-like aroma	Vegetable waste slightly mixed, bran settled at the bottom

The results indicated no significant interaction between the type of LOF and LOF dosage on spinach plant height. Moreover, neither the different types of vegetable waste LOF nor the variations in LOF dosage exerted a significant effect on plant height across all observation periods (Table 2). This lack of variance suggests that all LOF formulations were able to supply nutrients in relatively equal amounts, hence failing to trigger different physiological responses regarding stem elongation. Additionally, plant height growth is heavily dictated by nitrogen availability, growing medium conditions, and genetic factors; thus, variations in EM4 and molasses compositions were insufficient to enhance nitrogen availability during the early vegetative phase.

Table 2 Spinach Plant Height Influenced by Vegetable Waste LOF Application

Treatment	Plant Height (cm) at Various Observation Times				
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
LOF Type					
P1: No EM4 and molasses (Control)	6,25	8,72	10,17	13,94	17,17
P2: 20 mL EM4 + 20 mL molasses	6,84	8,59	9,59	13,48	17,50
P3: 40 mL EM4 + 40 mL molasses	6,25	8,47	10,50	12,47	18,67
P4: 60 mL EM4 + 60 mL molasses	6,17	8,59	9,67	13,24	18,67
LSD	ns	ns	ns	ns	ns
LOF Dosage					
D1 : Dosage 15 ml L ⁻¹	6,29	8,88	9,96	12,58	18,30
D2 : Dosage 30 ml L ⁻¹	6,46	8,30	10,00	13,99	17,71
LSD	ns	ns	ns	ns	ns

Note: ns = non-significant, DAP = day after planting

The results showed no significant interaction between LOF type and LOF dosage on the leaf number of spinach plants. LOF type also did not significantly affect the leaf number at any observation age; however, differences in LOF dosage showed a significant effect at 21 DAP (Table 3). The application of the 30 ml L⁻¹ dosage yielded a higher number of leaves than the 15 ml L⁻¹ dosage. From 28 DAP until harvest, this difference was no longer significant.

Table 3 Spinach Leaf Number Influenced by Vegetable Waste LOF Application

Treatment	Leaf Number at Various Observation Times				
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
LOF Type					
P1: No EM4 and molasses (Control)	3,17	5,17	6,50	7,67	9,17
P2: 20 mL EM4 + 20 mL molasses	2,84	4,83	6,34	7,84	8,50
P3: 40 mL EM4 + 40 mL molasses	3,33	4,67	6,17	7,50	8,67
P4: 60 mL EM4 + 60 mL molasses	3,00	5,00	6,32	8,33	8,83
LSD	ns	ns	ns	ns	ns
LOF Dosage					
D1 : Dosage 15 ml L ⁻¹	3,08	3,09 b	3,10	3,11	3,12
D2 : Dosage 30 ml L ⁻¹	3,08	4,50 a	6,41	7,67	9,17
LSD	ns	0,51	ns	ns	ns

Note: Values followed by the same letter in the same column are not significantly different based on LSD test; ns = non-significant, DAP = day after planting

No significant interaction was observed between LOF type and LOF dosage regarding spinach leaf area. The LOF type also showed no significant effect on leaf area, but the LOF dosage demonstrated a significant influence at 21 DAP (Table 4). The 15 ml L⁻¹ dosage resulted in a larger leaf area compared to

the 30 ml L⁻¹ dosage. After the plants reached 28 DAP until harvest, both dosages demonstrated relatively similar leaf area development.

Table 4 Spinach Leaf Area Influenced by Vegetable Waste LOF Application

Treatment	Leaf Area (cm ²) at Various Observation Times				
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
LOF Type					
P1: No EM4 and molasses (Control)	4,11	10,51	19,35	38,75	38,76
P2: 20 mL EM4 + 20 mL molasses	3,66	9,19	19,19	35,85	41,75
P3: 40 mL EM4 + 40 mL molasses	4,08	8,51	20,61	28,83	42,35
P4: 60 mL EM4 + 60 mL molasses	3,39	9,69	21,05	36,62	42,84
LSD	ns	ns	ns	ns	ns
LOF Dosage					
D1 : Dosage 15 ml L ⁻¹	4,07	11,32 a	18,74	31,19	40,90
D2 : Dosage 30 ml L ⁻¹	3,55	7,62 b	21,35	38,83	41,95
LSD	ns	2,24	ns	ns	ns

Note: Values followed by the same letter in the same column are not significantly different based on LSD test; ns = non-significant, DAP = day after planting

The results revealed no significant interaction between LOF type and LOF dosage on the plant fresh weight. LOF type similarly did not significantly alter the fresh weight, yet the LOF dosage showed a significant effect at 21 DAP (Table 5). The 15 ml L⁻¹ dosage generated higher fresh weight compared to the 30 ml L⁻¹ dosage, whereas during subsequent observations, this difference faded away.

Table 5 Spinach Fresh Weight Influenced by Vegetable Waste LOF Application

Treatment	Fresh Weight (g) at Various Observation Times				
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
LOF Type					
P1: No EM4 and molasses (Control)	0,19	0,43	0,73	1,51	1,91
P2: 20 mL EM4 + 20 mL molasses	0,17	0,40	0,64	1,29	2,12
P3: 40 mL EM4 + 40 mL molasses	0,18	0,38	0,81	1,17	2,14
P4: 60 mL EM4 + 60 mL molasses	0,16	0,40	0,79	1,43	2,32
LSD	ns	ns	ns	ns	ns
LOF Dosage					
D1 : Dosage 15 ml L ⁻¹	0,18	0,45 a	0,78	1,15	2,17
D2 : Dosage 30 ml L ⁻¹	0,17	0,36 b	0,71	1,54	2,08
LSD	ns	0,07	ns	ns	ns

Note: Values followed by the same letter in the same column are not significantly different based on LSD test; ns = non-significant, DAP = day after planting

The analysis demonstrated that there was no significant interaction between LOF type and LOF dosage on the dry weight of spinach. LOF type also showed no significant influence on plant dry weight, but the LOF dosage factor exhibited a significant effect at 21 DAP (Table 6). The 15 ml L⁻¹ produced a higher dry weight than the 30 ml L⁻¹ dosage, while no significant difference was observed in later phases.

Table 6 Spinach Dry Weight Influenced by Vegetable Waste LOF Application

Treatment	Plant Dry Weight (g) at Various Observation Times				
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
LOF Type					

P1: No EM4 and molasses (Control)	0,00	0,03	0,15	0,28	0,38
P2: 20 mL EM4 + 20 mL molasses	0,00	0,02	0,16	0,37	0,45
P3: 40 mL EM4 + 40 mL molasses	0,00	0,00	0,21	0,26	0,40
P4: 60 mL EM4 + 60 mL molasses	0,00	0,02	0,16	0,29	0,42
LSD	ns	ns	ns	ns	ns
LOF Dosage					
D1 : Dosage 15 ml L ⁻¹	0,00	0,035 a	0,17	0,28	0,43
D2 : Dosage 30 ml L ⁻¹	0,00	0,001 b	0,17	0,32	0,39
LSD	ns	0,031	ns	ns	ns

Note: Values followed by the same letter in the same column are not significantly different based on LSD test; ns = non-significant, DAP = day after planting

There was no significant interaction between LOF type and LOF dosage on the relative growth rate (RGR) of spinach plants. Table 7 reflects that the LOF type did not induce any significant impact on the plant RGR; conversely, the LOF dosage introduced a significant impact during the 14–21 DAP interval, where the 15 ml L⁻¹ dosage produced higher RGR values than the 30 ml L⁻¹ dosage. Beyond this phase, no significant differences were detected.

Table 7 Relative Growth Rate (RGR) of Spinach Influenced by Vegetable Waste LOF Application

Treatment	Relative Growth Rate (g g ⁻¹ day ⁻¹) at Various Intervals			
	14-21 DAP	21-28 DAP	28-35 DAP	35-42 DAP
LOF Type				
P1: No EM4 and molasses (Control)	0,79	0,15	0,61	0,27
P2: 20 mL EM4 + 20 mL molasses	0,36	0,02	0,87	0,21
P3: 40 mL EM4 + 40 mL molasses	0,00	0,00	0,30	0,42
P4: 60 mL EM4 + 60 mL molasses	0,34	0,02	0,66	0,24
LSD	ns	ns	ns	ns
LOF Dosage				
D1 : Dosage 15 ml L ⁻¹	0,74 a	0,09	0,56	0,44
D2 : Dosage 30 ml L ⁻¹	0,01 b	0,06	0,66	0,13
LSD	0,61	ns	ns	ns

Note: Values followed by the same letter in the same column are not significantly different based on LSD test; ns = non-significant, DAP = day after planting

The results verified that no significant interaction occurred between LOF type and LOF dosage on the net assimilation rate (NAR) of spinach plants. Table 8 clarifies that the LOF type had no significant influence on the plant NAR; on the contrary, the LOF dosage offered a significant effect during the 14–21 DAP interval, with the 15 ml L⁻¹ dosage establishing a higher NAR value than the 30 ml L⁻¹ dosage. No significant differences were reported past this phase.

Table 8 Net Assimilation Rate (NAR) of Spinach Influenced by Vegetable Waste LOF Application

Treatment	Net Assimilation Rate (g cm ⁻² day ⁻¹) at Various Intervals			
	14-21 DAP	21-28 DAP	28-35 DAP	35-42 DAP
LOF Type				
P1: No EM4 and molasses (Control)	0,005	0,010	0,0055	0,0030
P2: 20 mL EM4 + 20 mL molasses	0,005	0,020	0,0080	0,0020
P3: 40 mL EM4 + 40 mL molasses	0,001	0,015	0,0040	0,0040
P4: 60 mL EM4 + 60 mL molasses	0,005	0,015	0,0060	0,0080

LSD	ns	ns	ns	ns
LOF Dosage				
D1 : Dosage 15 ml L ⁻¹	0,0075 a	0,0125	0,0048	0,0045
D2 : Dosage 30 ml L ⁻¹	0,0001 b	0,0175	0,0070	0,0040
LSD	0,0048	ns	ns	ns

Note: Values followed by the same letter in the same column are not significantly different based on LSD test; ns = non-significant, DAP = day after planting

Discussion

Overall, this study highlights that differences in LOF formulations based on EM4 and molasses concentrations could not significantly enhance spinach growth, whereas the application dosage generated a significant response during the early growth phase (21 DAP). This implies that the nutrient quality of the LOF produced across formulations was relatively uniform, making the quantity of applied fertilizer a more dominant factor than its fermentation composition.

The color shift of the liquid organic fertilizer from faded green to brown and dark brown indicates the ongoing decomposition of organic matter during fermentation. A brown color serves as an indicator that complex organic compounds have been degraded into simpler forms due to the activity of fermentative microorganisms. The addition of the EM4 bioactivator alongside molasses provides an energy source for microorganisms, thereby accelerating the breakdown of organic materials (Yanti et al., 2022).

The increasingly intense tape-like aroma in treatments with EM4 also implies that fermentation was actively progressing. During anaerobic fermentation, lactic acid bacteria, photosynthetic bacteria, and yeasts present in EM4 utilize simple sugars from molasses as a carbon source, thereby generating alcohol, organic acids, and various secondary metabolites that create the distinctive fermentation aroma (Mustikarini et al., 2022).

The relatively stable fermentation temperature within the 33–34°C range points out that microbial activity took place under optimum conditions. This temperature range supports the growth of decomposer microorganisms without triggering cell mortality from excessive heat, ensuring efficient mineralization of organic materials (Dewi & Kusnopranto, 2022).

At 21 DAP, the LOF dosage factor significantly influenced leaf area, fresh weight, dry weight, relative growth rate (RGR), and net assimilation rate (NAR). The D2 treatment (30 mL L⁻¹) triggered lower values compared to D1 (15 mL L⁻¹) across all these parameters. This condition implies that a LOF concentration of 30 mL L⁻¹ might not yet match the physiological capacity of spinach plants during the initial vegetative stage. At 21 DAP, the plant root system is still expanding actively, meaning its capacity to absorb water and nutrients has not peaked. According to Udiyana et al. (2023), applying liquid organic fertilizer at concentrations exceeding the requirements of young plants does not necessarily promote growth and can even suppress nutrient uptake efficiency due to increased solution concentration around the root zone.

The hampered water absorption in the D2 treatment was reflected in its lower leaf area (7,62 cm²) compared to the D1 treatment, which achieved 11,32 cm². Leaf area is a primary component that determines a plant's capacity to intercept solar radiation, heavily impacting photosynthetic activity. A larger leaf surface area captures more light energy via chlorophyll to generate photosynthate. Farhanah et al. (2022) reported that leaf area expansion in spinach is closely linked with heightened accumulation of photosynthetic products and plant biomass production. Similarly, Widayati et al. (2025) stated that applying liquid organic fertilizer at optimum concentrations can expand leaf area, leading to higher plant photosynthetic efficiency.

The smaller leaf area under the D2 treatment was subsequently followed by a low Net Assimilation Rate (NAR) value of 0,0001 g cm⁻² day⁻¹. NAR represents the plant's capability to accumulate dry matter through photosynthesis per unit of leaf area. According to Sitompul (2016), the NAR value is strongly influenced by active leaf area, chlorophyll content, and the leaves' ability to convert light energy into photosynthate. Therefore, a smaller leaf area diminishes the plant's capacity to produce photosynthate. The depressed NAR value in the D2 treatment then led to a decline in the Relative Growth Rate (RGR). The connection between NAR and RGR indicates that reduced photosynthesis slows down biomass accumulation. Consequently, both plant fresh weight and dry weight at 21 DAP were lower than those in the D1 treatment. Pratiwi et al. (2023) documented that boosting photosynthetic efficiency through

precise liquid organic fertilizer application will increase biomass formation, significantly enhancing the fresh and dry weights of spinach. Similar results were reported by Putri et al. (2023), showing that elevated photosynthetic activity via optimum organic nutrition correlates positively with vegetative growth and biomass accumulation in spinach

The application of (15 mL L⁻¹) is believed to have supplied nutrients in a more balanced proportion relative to the absorption capacity of young roots. Adequate availability of nitrogen, phosphorus, and potassium stimulates cell division, chlorophyll formation, and leaf expansion, which increased leaf area up to 11,32 cm². This larger leaf area directly led to higher NAR and RGR values, bringing about greater plant biomass accumulation, as reflected in increased fresh and dry weights. Udiyana et al. (2023) maintained that optimum liquid organic fertilizer concentrations can enhance nutrient uptake efficiency, promoting better vegetative growth in spinach than higher concentrations.

On the other hand, the significant effect of the dosage factor began to disappear at 28–42 DAP. This indicates that the plants had entered the late vegetative phase with a more developed root system, thereby enhancing water and nutrient absorption capacities. An expanded root system allows plants to utilize nutrients more efficiently, enabling plants in the D2 treatment to catch up with the growth of the D1. According to Taiz et al. (2018), root system development during the vegetative phase expands absorption capabilities for water and nutrients, meaning response discrepancies caused by early fertilization treatments tend to diminish. A similar phenomenon was reported by Farhanah et al. (2022), noting that the impact of liquid organic fertilizer on spinach is more pronounced during the early vegetative phase than close to harvest, as the plant's physiological system becomes fully developed in the final stages.

Conclusively, the results demonstrate that a dosage of 15 mL L⁻¹ is a more suitable concentration to support the early growth of spinach compared to a dosage of 30 mL L⁻¹. This outcome indicates that successful liquid organic fertilizer application depends not only on the quantity supplied but also on the alignment of concentration with the plant's growth stage. Applying fertilizer at the optimum dosage can advance nutrient uptake efficiency, expand the photosynthetic area, elevate NAR and RGR values, and ultimately generate higher plant biomass

CONCLUSIONS

The physical characterization of liquid organic fertilizer (LOF) from vegetable waste indicates that increasing EM4 and molasses concentrations during fermentation turned the solution browner, strengthened the fermented (tape-like) aroma, and maintained a stable fermentation temperature within the 33,29–34,08°C range, confirming a successful fermentation process. However, variations in EM4 and molasses concentrations exerted no significant effect on any growth parameters of green spinach (*Amaranthus hybridus* L.), including plant height, leaf number, leaf area, fresh weight, dry weight, relative growth rate (RGR), and net assimilation rate (NAR). Conversely, the LOF application dosage significantly influenced the early growth phase (21 DAP), particularly affecting leaf number, leaf area, fresh weight, dry weight, and relative growth rate. A dosage of 15 mL L⁻¹ resulted in a superior early growth response compared to 30 mL L⁻¹, presumably because this concentration was better aligned with the nutrient absorption capacity of the young plants' root systems.

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