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Influence of biofertilizer produced using drumstick (*Moringa oleifera* L.) unused parts on the growth performance of two leafy vegetables

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ABSTRACT

The non-edible parts of *Moringa oleifera,* such as stems, branches or leaf petioles, have often been discarded while the leaves are consumed as a vegetable or are used to produce organic fertilizer. This study aimed to determine the optimal conditions for producing *Moringa* organic fertilizer (MOF) from previously unused parts and to compare these fertilizers with cow manure and bio-organic fertilizer. Seventy kilograms of the unused *Moringa* parts were blended with fifty kilograms of manure, 0.2 kilogram of Trichoderma-based product and two kilograms of superphosphate. The mixture was incubated at different intervals, including 5, 7 or 9 weeks. Next, the effects of MOF on the growth, yield, ascorbic acid content and Brix of lettuce and mustard spinach were also determined and compared with other organic fertilizers (cow manure and bio-organic fertilizer). Results of the study revealed that 25 tons per ha of MOF were significantly superior to those treated with cow manure and bio-organic fertilizer in the case of vegetable yields. Further, 7 weeks of MOF incubation was found suitable to produce an optimal yield during the various incubation period. These results suggested that the *Moringa* non-edible parts can make organic fertilizer and enhance growth, yield, and leafy vegetable production.

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1 Introduction

Leafy vegetables such as lettuce (*Lactuca sativa* L.) and mustard spinach (*Brassicca juncea*) are important sources of nutrients, fibre, minerals and vitamins. Lettuce also contains the most common types of vitamins, such as E, A, C and B9 (Wang et al. 2013), and bioactive compounds, such as polyphenols, carotenoids and chlorophyll (Coria–Cayupán et al. 2009). Similarly, mustard spinach is a good source of vitamins (A, C, K, B1, B2, B6 and B9) and mineral nutrients (Van Wyk 2005). Furthermore, its oil is used in traditional medicine and cosmetics (Yu et al. 2003; Kumar et al. 2011). Recently, the consumption of leafy vegetables in Vietnam has decreased due to food safety issues (Ha et al. 2020). Further, large amounts of nitrate residues have also been found in vegetable samples (Dang et al. 2018). The excessive use of nitrogen fertilizer causes nitrate accumulation in soil, water and leafy vegetables, which poses a risk to human health (Ahmed et al. 2017; Zhao et al. 2019).

Using organic fertilizer helps to enrich soil fertility and soil organic matter, leading to enhanced carbon sequestration (Verma et al. 2019). When soil organic matter is low, vegetable yields decline even if sufficient nutrients are supplied via synthetic fertilizers (Bauer and Black 1994). Therefore, organic fertilizers are needed to achieve optimal vegetable yields. Although organic fertilizers can be produced from agricultural wastes, animal manure, and compost, the organic fertilizer supply is limited and does not meet the demand in organic farming.

Moringa oleifera, a species from the family Moringaceae, grows well in tropical and subtropical regions. It is a vegetable crop with vast nutritional benefits. Various parts of *Moringa* trees are found to be enriched with nutrients. However, this tree is considered underutilized due to the lack of awareness (Faizi et al. 1994; Padulosi et al. 2011). The extract products derived from *Moringa* leaves help promote crops' growth and yield (Culver et al. 2012; Matthew 2016; Chanthanousone et al. 2020). Supplement of *M. oleifera* residues as soil conditioner increases available nitrogen in sandy and calcareous soil and polluted soil (Taiwo et al. 2022) and enhances grain yields (Merwad and Khalil 2018). Composts are necessary to produce safe agricultural products on a large scale (Paulin and O'Malley 2008).

Except for the leaf, most *M. oleifera* parts are still unused and have been discarded as waste. These materials can be utilized to generate *Moringa* organic fertilizer. Previous studies indicated that applying *Moringa* foliar biofertilizer produced from non-edible parts promotes the growth, yield, ascorbic acid content and Brix of lettuce (Chanthanousone et al. 2022). This study aimed to evaluate the effect of the incubation period on the *Moringa* residual organic fertilizer and its impact on the growth of lettuce and mustard spinach in Thua Thien Hue province, Vietnam. Finally, *Moringa* residual organic fertilizer was compared to other organic fertilizers.

2 Materials and methods

2.1 Experimental site and plant materials

The experiments were conducted at the experimental field of the Institute of Biotechnology, Hue University (Thua Thien Hue province, Vietnam) from December 2020 to May 2021. During the study period, the daily temperature varied from 26 and 35°C. Plant materials included in the study are a lettuce variety (*Lactuca sativa* L.) obtained from Phu Nong Seeds Company and a mustard spinach variety (*Brassica juncea*) obtained from Ha Noi Xanh Company.

2.2 Moringa organic fertilizer (MOF) preparation

MOF was prepared from *Moringa* non-edible parts, including stems, branches and leaf petioles, at the Institute of Biotechnology, Hue University. The fertilizer was prepared with the following materials in the predetermined quantities, including 70 kilograms of ground moringa residues, 50 kilograms of manure, 0.2 kilograms of Tricho–compost (Trichoderma–based product) and 2.0 kilograms of superphosphate (Lam Thao Fertilizers and Chemicals JSC). First, *Moringa* residues were chopped into small parts and mixed with water and Tricho–compost until the mixture humidity reached 70%. For this, the mixture was fully covered by a dark plastic sheet. After three weeks (the mixture's temperature increased to 30–40^{o}C), water was supplemented, and the mixture was stirred and incubated for another 5, 7 or 9 weeks.

2.3 Nutrient contents of MOF following different incubation periods

In this experiment, MOF was incubated for 5 weeks (I1), 7 weeks (I2) and 9 weeks (I3). Physicochemical properties of the MOF included the percentages of N, P, available P, available K, organic matter and pH were investigated. For each incubation period, three samples were taken for physicochemical analyses.

2.4 Effect of MOF amounts on the growth, yield and quality of leafy vegetables

The field experiment was conducted from January to March 2021 with two planting times. The investigation was conducted in a completely randomized design (CRD) following four treatments with different amounts of MOF applied (15 (R1), 20 (R2), 25 (R3) and 30 (R4) tons per ha). The plot size of each treatment was 10 m^2. Before planting, the soil was ploughed, and MOF was applied as basal dressing. The seedlings at the 3–4 leaf stage were planted with a density of 33 plants per m^2.

2.5 Effect of various organic fertilizers on growth, yield and quality of leafy vegetables

The field experiment was carried out from March to May 2021 with two planting times to compare the effects of MOF and other organic fertilizers on the growth, yield and quality of leafy vegetables (lettuce and mustard spinach). The experiment was conducted in a completely randomized design (CRD) with four treatments: F1 (25 tons of MOF per ha), F2 (Cow manure), F3 (Bio-organic fertilizer) and control (without fertilization). The plot size of each treatment was 10 m^2. The seedlings at 3-4 leaf stage were planted with a density of 33 plants/$m^{2,}$ and all fertilizers were applied as basal dressing before planting.

2.6 Data collection and analysis

Growth time (day) was defined from sowing to harvesting. Growth parameters, including plant height (cm), canopy diameter (cm), number of leaves and leaf area index (leaf area/ground area), were observed. Plant height was measured from the ground to the highest point of the leaves. The leaf area index (LAI) is the sum of the leaf area from all plants divided by the ground area. Yield components included: (i) fresh mass per plant (g/plant) (including the stems, leaves and roots); (ii) theoretical yield (tons per ha) (average fresh mass/plant × plant density); (iii) actual yield (tons per ha) was also estimated (Chanthanousone et al. 2022). Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by Tukey's test, using the SPSS statistic 20.0 software (SPSS Inc., Chicago, IL, USA). Data represented significant differences as $p < 0.05$.

3 Results

3.1 Nutrient contents of MOF at different incubation periods

The results presented in Table 1 indicated that the nitrogen contents changed during the incubation period. MOF prepared with seven-week incubation had the highest nitrogen content (3.57%). On the other hand, phosphorus contents increased with the incubation period. While in the case of potassium content, it ranged from 20.63% (7 weeks) to 25.58% (5 weeks), while organic matter ranged from 6.58% (5 weeks) to 11.49% (7 weeks), but the differences were not significant. Further, the pH values for different incubation periods ranged from 5.88 (9 weeks) to 6.27 (5 weeks), suitable for planting vegetables.

Table 1 Effect of incubation periods on the quality of *Moringa* organic fertilizer (MOF)

Treatment	N (%)	P (%)	P_2O_5 (%)	K_2O (%)	Organic matter (%)	pH
I1	$0.82±0.01^{c}$	$2.02±0.19^{b}$	$4.62±2.05^{b}$	$25.58±4.41^{a}$	$6.58±1.42^{c}$	$6.27±0.03^{a}$
I2	$3.57±0.11^{a}$	$3.50±0.64^{a}$	$8.00±1.90^{a}$	$20.63±5.84^{b}$	$11.49±4.12^{a}$	$6.13±0.02^{a}$
I3	$2.29±0.17^{b}$	$3.76±1.39^{a}$	$8.61±2.42^{a}$	$26.24±4.63^{a}$	$8.12±0.75^{b}$	$5.88±0.17^{b}$
$LSD_{0.05}$	0.21	1.75	4.05	8.30	5.09	0.22

The means with similar lower-case letters within columns did not differ significantly at a 5% probability. I1: 5 weeks, I2: 7 weeks, I3: 9 weeks. LSD: Least significant difference.

Table 2 Effect of MOF amounts on the growth of lettuce

Treatment	Growth time (day)	Plant height (cm)	Number of leaves (leaves per plant)	Canopy diameter (cm)	Leaf area index
First planting					
R1	30	$19.2±1.83^{ab}$	$10.7±1.01^{ab}$	$28.3±1.66^{ab}$	$47.6±0.82^{ab}$
R2	29	$20.4±1.27^{a}$	$11.6±0.12^{a}$	$28.7±1.34^{a}$	$48.3±2.52^{a}$
R3	30	$19.5±1.17^{ab}$	$11.1±0.53^{ab}$	$26.7±0.61^{ab}$	$48.2±2.43^{a}$
R4	30	$17.3±2.01^{b}$	$9.6±1.28^{b}$	$25.3±0.42^{b}$	$45.3±1.15^{b}$
$LSD_{0.05}$		2.95	1.62	3.2	2.6
Second planting					
R1	28	$24.5±1.56^{b}$	$12.1±1.83^{b}$	$24.8±1.41^{b}$	$41.1±4.32^{c}$
R2	28	$25.6±0.64^{ab}$	$14.0±1.83^{a}$	$28.6±0.70^{a}$	$49.2±2.23^{a}$
R3	29	$26.7±0.92^{a}$	$14.4±1.83^{a}$	$29.2±0.57^{a}$	$50.9±3.71^{a}$
R4	28	$26.5±1.96^{ab}$	$13.6±1.83^{ab}$	$29.3±0.69^{a}$	$44.8±2.08^{b}$
$LSD_{0.05}$		2.2	2.9	3.7	6.3

The means with similar lower-case letters within columns did not differ significantly at a 5% probability. R1: 15 tons per ha, R2: 20 tons per ha, R3: 25 tons per ha, R4: 30 tons per ha. LSD: Least significant difference.

3.2 Effect of MOF on the growth, yield and quality of leafy vegetables

3.2.1 Lettuce

In the first planting, 15 to 25 tons of MOF per ha seemed to promote various plant growth parameters of lettuce, including plant height (19.2–20.4 cm), number of leaves (10.7–11.6), canopy diameter (26.7–28.7 cm) and leaf area index (47.6–48.3). In the second planting, the plant growth parameters were similar when MOF application varied from 20 to 30 tons per ha. The canopy diameter of lettuce was lower in 15 tons per ha treatment than the others. At both planting times, fresh mass, theoretical yield, and actual yield of lettuce grown with 25 tons of MOF per ha were significantly higher than those grown with 15 and 20 tons of MOF per ha (Table 2). Increasing the amount of MOF from 25 to 30 tons per ha did not affect the theoretical yield, actual yield, ascorbic acid content and Brix of lettuce. When 25 tons of MOF per ha were applied, lettuce yields peaked at 23.7 tons per ha and 25.6 tons/ha in the first and second planting times, respectively. These yields were higher than when 15 tons of MOF per ha were applied. Regarding ascorbic acid contents (Table 3), the values remained constant across treatments in the first planting, but in the second planting, the treatment with 15 tons of MOF per ha resulted in the lowest ascorbic content. Furthermore, the lowest amount of MOF (15 tons per ha) yielded the lowest values of fresh mass, yields and Brix in the second planting.

Table 3 Effect of MOF amounts on the yield and quality of lettuce

Treatment	Fresh mass (g per plant)	Theoretical yield (ton per ha)	Actual yield (ton per ha)	Ascorbic acid (%)	Brix (%)
First planting					
R1	100.3±6.66^{b}	26.7±0.63^{b}	19.0±1.67^{c}	2.767±0.11^{a}	4.93±0.31^{ab}
R2	101.7±4.23^{b}	27.0±1.78^{b}	20.3±2.01^{bc}	2.730±0.14^{a}	4.76±0.46^{ab}
R3	123.3±5.04^{a}	32.7±0.53^{a}	23.7±1.30^{a}	2.741±0.30^{a}	5.17±0.25^{a}
R4	125.4±6.50^{a}	33.0±1.34^{a}	22.7±1.71^{ab}	2.693±0.15^{a}	4.90±0.32^{a}
$LSD_{0.05}$	7.89	3.12	2.56	0.41	0.39
Second planting					
R1	99.9±2.01^{c}	25.7±0.54^{c}	20.8±0.42^{c}	2.607±0.11^{b}	4.40±0.26^{b}
R2	110.0±5.29^{bc}	29.3±1.42^{b}	22.9±1.10^{bc}	2.770±0.23^{ab}	4.76±0.33^{ab}
R3	122.7±4.73^{a}	31.7±0.67^{a}	25.6±0.98^{a}	2.863±0.05^{a}	5.10±0.36^{a}
R4	117.8±9.62^{b}	30.0±0.85^{ab}	24.5±2.00^{ab}	2.874±0.07^{a}	4.86±0.29^{ab}
$LSD_{0.05}$	12.0	2.1	2.5	0.2	0.4

The means with similar lower-case letters within columns did not differ significantly at a 5% probability. R1: 15 tons per ha, R2: 20 tons per ha, R3: 25 tons per ha, R4: 30 tons per ha. LSD: Least significant difference.

Table 4 Effect of MOF amounts on the growth of mustard spinach

Treatment	Growth time (day)	Plant height (cm)	Number of leaves (leaves/plant)	Canopy diameter (cm)	Leaf area index
First planting					
R1	31	23.2±1.36^{b}	11.0±0.64^{a}	25.8±1.51^{b}	39.8±1.79^{b}
R2	32	27.2±2.98^{ab}	11.9±1.63^{a}	26.9±0.64^{b}	43.5±1.13^{ab}
R3	33	28.8±2.65^{a}	12.1±0.99^{a}	29.3±2.09^{a}	44.6±0.86^{a}
R4	31	27.9±1.10^{ab}	11.3±0.91^{a}	28.1±1.33^{ab}	43.8±0.92^{ab}
$LSD_{0.05}$		2.50	1.80	3.34	4.03
Second planting					
R1	30	24.0±1.35^{c}	11.9±0.12^{b}	27.9±1.51^{a}	39.1±0.97^{b}
R2	32	26.6±1.04^{b}	12.5±0.50^{ab}	29.0±0.64^{a}	43.2±0.94^{a}
R3	31	29.6±0.50^{a}	13.3±0.84^{a}	29.4±1.39^{a}	43.3±0.97^{a}
R4	32	29.1±0.59^{a}	13.2±0.48^{a}	29.2±0.41^{a}	44.7±1.62^{a}
$LSD_{0.05}$		1.9	1.0	1.9	2.4

The means with similar lower-case letters within columns did not differ significantly at a 5% probability. R1: 15 tons per ha, R2: 20 tons per ha, R3: 25 tons per ha, R4: 30 tons per ha. LSD: Least significant difference.

3.2.2 Mustard spinach

The plant treated with 20 to 30 tons of MOF per ha showed a significant increase in plant height compared to those treated with 15 tons of MOF per ha (Table 4). The number of leaves did not change significantly according to MOF amounts in the first planting but was lower in those treated with 15 tons of MOF per ha in the second planting. The canopy diameter and LAI seemed to increase with the amount of MOF in the first planting, while in the second planting, no significant difference was observed in plants treated with 20 to 30 tons of MOF per ha. In addition, the yields were the highest when 25 tons of MOF per ha were used in both planting times (Table 5).

Mustard spinach grown with 25 tons of MOF per ha produced a higher yield (7 tons/ha) than those grown with 15 tons of MOF per ha (Table 5). The ascorbic acid content of mustard spinach grown with 20–25 tons of MOF per ha was significantly higher than those grown with 15 tons of MOF per ha. Brix of mustard spinach ranged from 3.5 to 4.5 in the first planting while it was reported from 3.9 to 5.4 in the second planting. Brix was higher when applying 25 and 30 tons/ha of MOF.

3.3 Effect of various organic fertilizers on the growth, yield and quality of leafy vegetables

3.3.1 Lettuce

Applying organic fertilizers, including MOF, cow manure and bio-organic fertilizer, enhanced the lettuce's performance compared to the control (Table 6). Applying organic fertilizers did not affect the

Table 5 Effect of MOF amounts on the yield and quality of mustard spinach

Treatment	Fresh mass (g/plant)	Theoretical yield (ton/ha)	Actual yield (ton/ha)	Ascorbic acid (%)	Brix (%)
First planting					
R1	111.0±4.17^{b}	29.7±1.21^{b}	19.3±0.54^{b}	4.1±0.66^{b}	3.5±0.32^{a}
R2	121.3±5.42^{b}	32.0±2.12^{b}	21.0±0.67^{b}	5.4±0.35^{a}	3.4±0.17^{a}
R3	149.3±8.15^{a}	39.3±0.69^{a}	25.7±0.47^{a}	5.7±0.44^{a}	4.5±0.51^{a}
R4	146.0±3.67^{a}	38.7±0.47^{a}	25.3±0.36^{a}	5.3±0.51^{a}	4.4±0.46^{a}
$LSD_{0.05}$	13.68	2.92	2.49	1.18	1.16
Second planting					
R1	108.7±2.89^{b}	28.7±0.96^{d}	18.7±0.50^{c}	4.5±0.36^{b}	3.9±0.33^{b}
R2	115.3±9.18^{b}	32.1±0.70^{c}	19.6±1.51^{c}	5.4±0.51^{a}	4.3±0.58^{b}
R3	146.1±4.78^{a}	38.0±0.81^{a}	25.7±0.94^{a}	5.7±0.57^{a}	5.4±0.16^{a}
R4	136.7±2.35^{a}	35.3±1.05^{b}	23.0±0.58^{b}	5.4±0.39^{ab}	5.2±0.29^{a}
$LSD_{0.05}$	11.7	1.9	2.2	0.9	0.6

The means with similar lower-case letters within columns did not differ significantly at a 5% probability. R1: 15 tons per ha, R2: 20 tons per ha, R3: 25 tons per ha, R4: 30 tons per ha. LSD: Least significant difference.

Table 6 Effect of various organic fertilizers on the growth of lettuce

Treatment	Growth time (day)	Plant height (cm)	Number of leaves (leaves /plant)	Canopy diameter (cm)	Leaf area index
First planting					
F1	31	26.4±1.21^{a}	13.1±0.31^{a}	23.5±3.52^{a}	43.4±0.77^{a}
F2	32	24.2±2.00^{b}	12.5±0.91^{a}	24.1±2.33^{a}	41.8±2.24^{a}
F3	31	25.3±3.03^{ab}	13.2±0.69^{a}	24.9±1.68^{a}	42.4±1.08^{a}
Control	33	22.1±2.08^{c}	11.7±1.02^{b}	24.2±1.94^{a}	31.5±4.44^{b}
$LSD_{0.05}$		1.87	1.71	6.26	2.83
Second planting					
F1	33	25.5±2.12^{a}	13.4±0.25^{a}	28.6±0.92^{a}	41.8±10.64^{a}
F2	32	24.2±2.07^{a}	12.3±0.86^{a}	25.3±1.47^{b}	41.1±0.97^{a}
F3	33	25.9±1.16^{a}	13.0±0.62^{a}	24.8±1.69^{bc}	40.3±1.54^{a}
Control	34	19.8±1.35^{b}	10.1±0.56^{b}	23.2±2.62^{c}	28.9±3.08^{b}
$LSD_{0.05}$		2.7	1.7	1.8	2.2

The means with similar lower-case letters within columns did not differ significantly at 5% probability; F1: Moringa organic fertilizer (MOF); F2: Cow manure; F3: Bio-organic fertilizer; Control: without fertilization; LSD: Least significant difference.

Table 7 Effect of various organic fertilizers on the yield and quality of lettuce

Treatment	Fresh mass (g/plant)	Theoretical yield (ton/ha)	Actual yield (ton/ha)	Ascorbic acid (%)	Brix (%)
First planting					
F1	150.0±3.05[a]	38.7±0.81[a]	25.6±1.22[a]	5.2±0.22[a]	5.0±0.43[a]
F2	133.7±2.57[b]	35.6±0.39[b]	23.1±0.76[b]	5.2±0.31[a]	4.7±0.49[ab]
F3	128.3±6.02[b]	33.5±2.11[b]	22.1±1.18[b]	5.3±0.16[a]	5.0±0.47[a]
Control	105.0±3.78[c]	28.0±1.18[c]	18.0±1.34[c]	4.3±0.56[b]	3.6±0.26[b]
$LSD_{0.05}$	12.31	2.30	1.40	0.6	0.6
Second planting					
F1	145.7±3.52[a]	37.4±0.53[a]	25.5±0.34[a]	5.6±0.30[a]	5.1±0.10[a]
F2	129.6±4.04[b]	34.0±0.59[b]	22.8±0.73[b]	5.7±0.23[a]	5.0±0.26[a]
F3	123.5±4.92[c]	33.5±1.67[b]	21.7±1.42[b]	5.7±0.29[a]	5.1±0.15[a]
Control	101.7±5.44[d]	26.2±1.26[c]	18.1±0.95[c]	4.7±0.27[b]	3.9±0.49[b]
$LSD_{0.05}$	5.99	2.12	1.55	0.3	0.2

The means with similar lower-case letters within columns did not differ significantly at 5% probability; F1: Moringa organic fertilizer (MOF); F2: Cow manure; F3: Bioorganic fertilizer; Control: without fertilization; LSD: Least significant difference

lettuce's number of leaves and canopy diameter in the first planting. However, the canopy diameter increased when MOF was applied in the second planting. The height of lettuce was also significantly higher when MOF was applied in the first planting, but this observation was not reproducible in the second planting. LAI was larger when organic fertilizers were applied at both planting times.

Similarly, fresh mass, theoretical yield and actual yield were higher in MOF treatment than in other treatments (Table 7). The fresh mass of lettuce treated with MOF was 150 g per plant in the first planting and 146 g per plant in the second planting. Lettuce grown with cow manure and bio-organic fertilizer exhibited lower fresh mass (134 and 130 g per plant for cow manure and 128 and 124 g for bio-organic fertilizer in the first and second planting seasons, respectively). The yield of lettuce grown with MOF was 7.4–7.6 tons per ha higher than control plants.

3.3.2 Mustard spinach

Like lettuce, organic fertilizers enhanced the growth of mustard spinach compared to the control (Table 8). In the first planting, there were no significant differences in plant height, number of leaves, canopy diameter and LAI between MOF and other organic fertilizers. However, in the second planting, plant height and LAI were the highest with the application of MOF (28.2 cm and 43.1, respectively).

Table 8 Effect of various organic fertilizers on the growth of mustard spinach

Treatment	Plant height (cm)	Number of leaves (leaves/plant)	Canopy diameter (cm)	Leaf area index
First planting				
F1	26.7±2.44[a]	12.2±0.42[a]	32.1±1.50[a]	42.8±3.28[a]
F2	27.1±1.55[a]	11.9±0.35[a]	33.0±0.95[a]	42.3±3.57[a]
F3	27.4±5.63[a]	12.0±0.30[a]	30.3±2.61[ab]	41.7±3.73[a]
Control	21.6±3.21[b]	11.6±0.87[a]	27.4±1.54[b]	32.0±4.52[b]
$LSD_{0.05}$	3.6	1.8	3.6	7.1
Second planting				
F1	28.2[a]±1.63	12.8[a]±0.69	33.3[a]±1.25	43.1[a]±0.96
F2	25.7[b]±1.06	12.5[a]±0.62	33.4[a]±1.06	40.3[b]±0.84
F3	27.2[ab]±0.53	13.1[a]±0.53	31.6[a]±4.60	40.1[b]±1.17
Control	22.5[c]±1.47	11.7[a]±0.93	27.1[b]±0.68	30.2[c]±2.06
$LSD_{0.05}$	1.7	1.5	1.9	2.3

The means with similar lower-case letters within columns did not differ significantly at 5% probability; F1: Moringa organic fertilizer (MOF); F2: Cow manure; F3: Bio-organic fertilizer; Control: without fertilization; LSD: Least significant difference.

Table 9 Effect of various organic fertilizers on the yield and quality of mustard spinach

Treatment	Fresh mass (g/plant)	Theoretical yield (ton/ha)	Actual yield (ton/ha)	Ascorbic acid (%)	Brix (%)
First planting					
F1	158.0±8.93[a]	38.7±0.38[a]	25.9±0.51[a]	5.7±0.38[a]	4.5±1.01[a]
F2	140.3±9.14[b]	37.3±1.55[a]	23.3±1.35[b]	5.6±0.56[a]	4.4±0.76[a]
F3	136.7±7.70[b]	37.0±1.97[a]	24.3±1.42[ab]	5.7±0.63[a]	4.5±0.95[a]
Control	111.3±7.26[c]	28.2±1.70[b]	18.4±0.98[c]	4.2±0.74[b]	3.6±2.14[b]
$LSD_{0.05}$	14.4	2.4	1.8	1.2	1.2
Second planting					
F1	155.0±6.39[a]	37.4±0.66[a]	26.8±0.66[a]	5.5±0.19[a]	5.9±0.28[a]
F2	138.1±4.55[b]	35.3±1.87[b]	23.9±1.24[b]	5.2±0.84[a]	4.7±0.74[b]
F3	130.3±8.95[b]	34.8±1.16[b]	24.1±1.28[ab]	5.3±0.58[a]	5.5±0.32[a]
Control	110.4±8.04[c]	27.3±1.81[c]	19.9±0.93[c]	4.4±0.60[b]	4.0±1.01[b]
$LSD_{0.05}$	9.4	1.9	1.1	0.7	0.7

The means with similar lower-case letters within columns did not differ significantly at 5% probability; F1: Moringa organic fertilizer (MOF); F2: Cow manure; F3: Bioorganic fertilizer; Control: without fertilization; LSD: Least significant difference.

At harvest time, fresh mass and yields of mustard spinach were significantly different across organic fertilizer treatments (Table 9). Mustard spinach treated with MOF had more fresh mass than other organic fertilizers during both planting times. The MOF treatment also produced 2.6 to 2.9 tons per ha (actual yield) more than the cow manure treatment. On the other hand, cow manure and bio-organic fertilizer treatments resulted in similar yields and quality of mustard spinach. The ascorbic acid contents were similar among the organic fertilizer treatments. Finally, the Brix of mustard spinach was significantly higher in the MOF and bio-organic fertilizer treatments compared to the other two in the second planting.

4 Discussion

Using plant materials to produce organic fertilizers is an area of active research. These fertilizers contain various amino acids, vitamins and growth regulators, which will help to improve plant growth and the quality of agricultural products even if plants grow under stress or in hydroponic systems (Nofal et al. 2020; Khan et al. 2021; Jagathy and Lavanya 2021; Upendri and Karunarathna 2021). Research on the production of bio-extract or organic fertilizer derived from *M. oleifera* has demonstrated their effects in enhancing the performance of crops (Culver et al. 2012; Matthew 2016; Merwad 2018; Chanthanousone et al. 2020). Works from Fahey (2005) and Chanthanousone et al. (2020) demonstrated that *Moringa* leaves should be utilized as food rather than to produce fertilizers due to their high nutritional values. This work aimed to produce organic fertilizer from *Moringa* non-edible parts like the stems, branches and leaf petioles. Here, a more detailed method for producing moringa organic fertilizer (MOF) derived from *Moringa* non-edible parts was described, and its usefulness for growing leafy vegetables was characterized.

The length of the incubation period changed the quality of MOF. Nitrogen content was the highest during incubation for seven weeks. Nitrogen is an essential element that determines crop yield. The contents of nitrogen and organic matter kept in soil and fertilizer help to promote plant growth. Thus, the incubation period produced with the higher nitrogen content should be considered optimal for MOF production. Furthermore, seven-week incubation yielded the highest amount of organic matter, although the difference was insignificant. The phosphorus and phosphorus pentoxide contents did not change much between seven and nine weeks of incubation. In another report, *Moringa*–fortified compost made with poultry manure and sawdust achieved a higher level of total nitrogen after an eight-week incubation period, resulting in a pH similar to those reported in this study (Taiwo et al. 2022). In summary, a seven-week incubation period was optimal for producing organic fertilizer from unused moringa parts.

MOF doses affected the performance of leafy vegetables grown in both planting times. Plant height, number of leaves, canopy diameter and leaf area index of lettuce and these parameters were recorded highest when 20–25 tons of MOF per ha were applied at the first planting, and these values were similar to the second planting between 20–30 tons of MOF per ha. Meanwhile, for mustard spinach, these parameters were not significantly different (between 20–30 tons of MOF per ha) at both planting times, except for plant height. With increasing amounts of MOF up to 25 tons per ha, fresh mass, theoretical yield and actual yield were increased in lettuce and mustard spinach. In a previous report, Akther et al. (2019) found that the yield of Indian spinach increased with increasing amounts of vermicompost, and it was higher than 35 tons per ha in the combination of fertilizer and insect netting. Also, increasing levels of organic fertilizer prepared from meat and bone greatly influenced the weight and size of *Brassicaceae* vegetables (Fracchiolla et al. 2020). Different amounts of MOF seemed not to change the ascorbic acid content in lettuce, although the lowest ascorbic acid content was observed in the 15 tons of MOF per ha treatment. The difference in Brix in both leafy vegetables was negligible across MOF amounts. The highest Brix at both planting times was recorded with the 25 tons of MOF per ha treatment.

Moringa organic fertilizer positively affected plant growth and lettuce and mustard spinach yield. Overall, the highest leaf area index, fresh mass, theoretical yield and actual yield were observed with the MOF treatment. The application of vegetable residues helps improve soil moisture content, water holding capacity and soil basal respiration and promotes lettuce growth and quality (Cavalheiro et al. 2021). The growth of vegetables was enhanced with organic fertilizers, compared to the non-fertilized vegetable. Among organic fertilizers, the growth, ascorbic acid content and Brix were comparable between MOF and other fertilizer treatments. Although the actual yield of lettuce increased with organic fertilizers, MOF treatment still yielded 2.5–3.5 tons per ha and 2.7–3.8 tons per ha more than the other fertilizer treatments in the first and second planting. The results of this study are consistent with earlier reports, using compost or vermicompost-based organic fertilizers (Coria-Cayupán et al. 2009; Masarirambi et al. 2010). Vitamin C is an essential antioxidant because it contributes 24.5% to the overall antioxidant activity in lettuce (Nicolle et al. 2004). This study found the highest ascorbic acid content in plants treated with MOF. The actual yield of mustard spinach grown in the first and second planting under treatment MOF reached 25.9 tons per ha and 26.8 tons per ha, respectively. However, the plants of this treatment yielded lower than those treated with *Moringa* foliar fertilizer (Chanthanousone et al. 2020). In another study, organic amendments such as green manure, poultry, cow, pig and rabbit manure, when being applied at 120 kg per ha, significantly increased the organic matter, Ca and Mg in soil and further enhanced okra yield, protein and mucilage contents (Adekiya et al. 2020). However, the effect of cow manure on the cultivation of leafy vegetables was less profound than that of MOF in this study. Therefore, *Moringa* organic fertilizer derived from non-edible moringa parts is promising for sustainable organic farming.

Conclusion

In conclusion, the moringa non-edible parts, such as stems, branches and leaf petioles, were promising materials to produce organic fertilizers. Optimal moringa organic fertilizer (MOF) was obtained after a seven-week incubation period. Furthermore, applying 25 tons of MOF per ha enhanced the yield and quality of leafy vegetables. MOF is a promising alternative to cow manure and other commercial bio-organic fertilizers to ensure safe and sustainable vegetable farming.

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Declaration of Competing Interest

The authors declare no competing interests.

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