HUE UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

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STUDY ON THE EFFICIENCY OF BIOFERTILIZER FROM MORINGA RESIDUES FOR SOME LEAFY VEGETABLES

SUMMARY PhD DISSERTATION

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The dissertation was completed at the Institute of Biotechnology Hue University.

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CHAPTER 1. INTRODUCTION

1.1. Background

Moringa oleifera Lam. (commonly known as drumstick) is a multipurpose tree species, nutritional rich and is distributed throughout South India, Southeast Asia, South America and Africa (Alavilli et al., 2022). Additionally, *M. oleifera* parts are also rich in minerals, protein, vitamins, phenolic and flavonoid compounds (Hassan et al., 2021). In Vietnam, *M. oleifera* leaf is used for vegetable, tea and veggie powder, and the seed is for propagation. Hence, stem, root and bark of *M. oleifera* are garbage. Thus, the use of residues of *M. oleifera* to produce biofertilizer is necessary, Thus, it is critical to develop cultivars with high tolerance to waterlogged conditions, to expand drumstick cultivation areas to provide materials for Moringa biofertilizer productions. Therefore, it is necessary to conduct "Study on the efficiency of Biofertilizer from Moringa residues for some leafy vegetables".

1.2. Research objectives

1.2.1. Overall objective

Products of biofertilizers from Moringa residues (stem, old petiole, and other unused parts) to serve organic agricultural production and contribute solving environmental pollution and soil structure degradation that improving plant growth and yield, and having safety foods.

1.2.2. Details objective

- Selecting waterlogging and good characteristics of *M. oleifera* lines for biomass production in Thua Thien Hue.
- Evaluating influence of Moringa foliar biofertilizer on growth, yield and quality of leafy vegetables.
- Evaluating influence of Moringa organic fertilizer on the growth performance of lettuce and mustard spinach.
- Evaluating efficiency of Moringa foliar biofertilizer (MFB) on leafy vegetables.
- Evaluating efficiency of Moringa organic fertilizer (MOF) on leafy vegetables.

1.3. New findings

- Selection of three lines (SPLs 7, 18 and 65) for waterlogging tolerance and three lines (SPLs 21, 27, 66 and 73) for high phenolic and flavonoid contents for future Moringa breeding programs in Vietnam as well as in Thua Thien Hue.

- Identification of the right time and ingredients to process the best quality of MFB and MOF fertilizers.

- Determination of the appropriate amount of MFB and MOF fertilizers for some leafy vegetables in Thua Thien Hue province.

CHAPTER 2. LITERATUR REVIEW

2.1. Theoretical basics of the research

2.1.1. Introduction a bout Moringa

2.1.1.1. Biodiversity and botany of Moringa

2.1.1.1.1. Biodiversity of Moringa

The genus Moringa includes 13 species that are found in the sub-Himalayan ranges of India, Sri Lanka, North Eastern and South Western Africa, Madagascar, and Arabia. *Moringa pterygosperma* Gaerthn (syn. *Moringa oleifera* Lam) is the most well-known and widespread species. The followings are white or pink flowered *Moringa peregrina*. Forsk, *Moringa optera* Gaerthn, *Moringa zeylanica* sieb., *Moringa arabica* (Boopathi & Raveendran, 2021).

2.1.1.1.2. Botany of Moringa

Moringa is a softwood tree, native to India that grows wild in the sub-Himalayan regions of Northern India and is now planted all over the world in the tropics and sub-tropics. It is grown throughout India for its sensitive pods, as well as its leaves and flowers. Moringa pods are a common vegetable in South Indian cuisine and are prized for their peculiar flavor. *Moringa oleifera* is found in all tropical countries.

Botanical classification of Moringa:

Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order - Brassicales
Family - Moringaceae
Genus - Moringa
Species – oleifera

2.1.1.2. Genetic diversity assessment of M. oleifera

The genetic variation of plant species is the primary source of distinction in characters, which improves their adaptability and distribution (Carvalho et al., 2019).

2.1.1.2.1. Morphological marker

Conventionally, various quantitative and qualitative morphological characters have been used to identify species, and distinguish cultivars or accessions (Adhikari et al., 2017).

2.1.1.2.2. Phytochemical components

Antioxidants (vitamins A, C, and E, β-carotene), biochemicals (amino acids, glucosinolates, chlorophyll, sugars, seed protein, and total protein), macronutrients micronutrients, anti-nutritional factors and polyphenols have been used to assess genetic variability among *M. oleifera* accessions and advanced breeding lines from India, Thailand, Laos, the Philippines, China, Taiwan, Saudi Arabia, Tanzania, and the United States (Zhu et al., 2020).

2.1.1.3. Molecular markers

Molecular markers are classified based on the method of analysis as hybridization-based (e.g., restriction fragment length polymorphism (RFLP)), polymerase chain reaction (PCR)-based (e.g., random amplified polymorphic DNA (RAPD)), or sequencing-based (e.g., single nucleotide polymorphisms (SNPs)) (Adhikari et al., 2017).

2.1.1.3.1. Random amplified polymorphic DNA (RAPD)

RAPD is a PCR-based technique that uses short (decamer) and random oligonucleotide primers and does not require sequence information or radioactive probes; DNA fragments separated by agarose gel electrophoresis and then visualized by staining with ethidium bromide.

2.1.1.3.2. Sequence-Related Amplified Polymorphism (SRAP)

SRAP marker technique is a simple and efficient method for amplifying open reading frames (ORFs) by using a 17-18-mer oligonucleotide with core sequences at the 5′ end that included 13-14-mer oligonucleotide with different filler sequences containing no specific sequences such as CCGG and AATT in the forward and reverse primers, respectively and three selective nucleotides at the 3′ end (Li & Quiros, 2001).

2.1.2. Introduction about Biofertilizer

2.1.2.1. Biofertilizer

Biofertilizers are substances of biological origin (microorganisms), which are added to the soil and building to enhance the fertility and ability of plant growth, biofertilizers has includes fungi, blue-green algae, and bacteria or their combinations of organisms, biofertilizers are nutrients and are economical, practical, and renewable sources chemical fertilizer for the plant. (Baboo, 2009).

2.1.2.2. Foliar Biofertilizer

The use of foliar fertilization is an efficient approach for improving crop nutritional characteristics (Otalora et al., 2018). It is improved physiological properties of plants, particularly in drought and light stress environment (Ruiz-Navarro et al., 2019).

2.1.3. Leafy vegetable

2.1.3.1. Definition of leafy vegetable

Leafy vegetables are important in human nutrition, particularly as sources of vitamins, minerals, dietary fiber, and phytochemicals (Yahia et al., 2019), as well as for food security (Rani, 2020).

2.1.3.2. Leafy vegetable production

Vegetable production is carried out by smallholder farmers/company using various production strategies. Vegetables are acknowledged as a profitable venture for improving farmers' livelihoods and solving concerns of self-sufficiency, food security, and remote economic development (Chagomoka et al., 2015).

2.1.4. Role of nutrient of leafy vegetables

2.1.4.1. Nitrogen (N)

Nitrogen is a principal element of nutrient that plants need for the growth of leaves, trees are able to get Nitrogen from fertilizer, compost, air, and soils, nitrogen, Gaseous chemical element (Yousaf et al., 2021).

2.1.4.2. Phosphorus (P)

Phosphorus is one of the most abundant macronutrients in plant tissues and is required for several key plant functions such as energy transfer, photosynthesis, sugar and starch transformation, nutrient movement within the plant, and genetic trait transfer from generation to the next ones (Baroowa et al., 2022).

2.1.4.3. Potassium (K)

Potassium (K^+), along with nitrogen (N) and phosphorus (P), is one of the essential plant nutrients required for development and physiology (Perelman et al., 2022).

2.1.4.4. Calcium

Calcium is an essential inorganic nutrient for higher plants; It is necessary for structural roles in the cell wall and membranes as the divalent cation (Ca^{2+}), as a counteraction for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol (Marschner, 1995)

2.1.4.5. Magnesium (Mg)

Magnesium is known to be an essential nutrient for many living organisms, including plant species, animals, and humans. result in decreased productivity and quality in forestry (Mitchell et al., 1999).

2.1.4.6. Sulphur (S)

Sulfur is one of the basic elements required by plants. It is a component of proteinaceous amino acids like methionine and cysteine, vitamins (biotin and thiamine), glutathione, phytochelatins, coenzyme A, chlorophyll, and S-adenosyl-methionine (Nakai & Maruyama-Nakashita, 2020).

2.1.4.7. Biostimulant to supplement synthetic fertilizers from Moringa

Fertilizers are elements used to increase growth and yield of plant (Bulgari et al., 2019). As a result, chemical fertilizers have become an essential aspect of modern agriculture, delivering essential plant nutrients such as nitrogen, phosphorus, and potassium (Savci, 2012).

2.2. Practical basics of the research

2.2.1. Production of Moringa in the world and Vietnam

2.2.1.1. Production of Moringa in the world

Moringa oleifera Lam. (commonly known as drumstick) is a multi-purpose tree species, nutritional rich and is distributed throughout South India, Southeast Asia, South America and Africa (Alavilli et al., 2022). *M. oleifera* parts are also rich in minerals, protein, vitamins, phenolic and flavonoid compounds (Hassan et al., 2021).

2.2.1.2. Production of Moringa in Vietnam

In Vietnam, Moringa grows natively in provinces Ninh Thuan, Binh Thuan, Dong Nai, and Kien Giang. Because of its high nutritional value and medicinal materials, as well as wide adaptability, in recent years, the Moringa cultivation has appeared in many provinces and cities across the country. Demand for Moringa leaves for making vegetables, producing tea bags, nutritional powders is increasing, while there is no supplier in large-scale having stable quantities and quality assurance according to food hygiene and safety standards, and GMP standards of the Ministry of Health (Chau, 2016).

2.2.1.3. Production of Moringa in Thua Thien Hue

Since *M. oleifera* is poorly tolerant to waterlogged conditions. Currently, the requirement for well-drained soil makes it unsuitable for drumstick to be cultivated in areas with frequent rain fall and floods (Dania et al., 2014). In addition, Thua Thien Hue province is located in the center of Vietnam, where is experienced adverse downpours and floods because of low pressure affection. Nguyen et al., (2023) selected a parental line and three self-pollinated lines with high level of water logging resistance in Thua Thien Hue that were used for biomass production in an area of 500 m^2 to provide materials for making biofertilizers. Thus, production area is necessary to be enlarged to produce biomass for fertilizer production in future.

2.2.2. M. oleifera breeding in the world and in Vietnam

2.2.2.1. M. oleifera breeding in the world

M. oleifera is a cross-pollinated species and is also naturalized in many areas; they exhibit variations in morphologies, yields and photochemical contents (Lakshmidevamma et al., 2021). Gandji and co-workers (2019) also observed diversity in morphological traits of *M. oleifera* with changing climate and cultivation practice. (Drisya et al., 2021).

Moringa Oleifera can adapt and grows well in a wide range of altitudes, from 600 to 1200 m in the tropics, with annual rainfall ranging from 250 to 1500 mm, and temperatures ranging from 25 to 35°C. In addition, it can be tolerated to light frost, higher temperature that about 48°C in the shade and well-drained sandy loam to clay loam, but susceptible to waterlogged soil and poor drainage (Alavilli et al. 2022). In China, a Moringa breeding program is focused on identification of association of functionally diverse genes and important agronomical traits (Deng et al. 2016).

2.2.2.2. Moringa Oleifera breeding in Vietnam

Moringa (*Moringa oleifera* Lam.) is grown commercially and used widely in pharmaceutical technology, cosmetics, beverage, nutrition and functional foods in more than 80 countries around the world. Truong and co-workers (2017) found that Moringa accession VI08718, which is originated from Thai Lan, is the most adapted variety for growing in Thua Thien Hue province, whereas, PKM–1, which is originated from Philippines, showed a good adaptation in Quang Tri province (Nguyen et al. 2017).

2.2.3. Production and use of biofertilizer

2.2.3.1. Production and use of biofertilizer in the world

Total of 11.3% of the value of the global fertilizer market in 2021 was attributable to the foliar technique of fertilizer application. Field crops made up 83.65% of the market for fertigation fertilizers in 2021, followed by horticultural crops (11.2%), turfs and decorative crops (7.1%), and field crops (11.2). For foliar fertilizers in field crops, the Asia-Pacific and European regions held market shares of 40.2% and 33.8%,

2.2.3.2. Production and use of biofertilizer in Vietnam

Vietnam's organic market generates $132.15 million annually. The majority of Vietnamese organic products are exported to other countries. The Vietnamese government supports the development of organic fertilizer in Vietnam and encourages its application and manufacturing (Van Toan et al., 2019).

2.2.4. The use of Moringa oleifera as fertilizer

2.2.4.1. The use of Moringa oleifera as fertilizer in the world

Biofertilizers (organic fertilizers) are essential for the production of safe leafy vegetables. Furthermore, the use of biofertilizers helps to protect the environment from soil degradation and groundwater pollution. One of the biofertilizers which are widely investigated for their potential of improving plant yield and growth is moringa leaf extract, produced from *Moringa oleifera* (Zulfiqar et al., 2020; Karthiga et al., 2022).

2.2.4.2. The use of Moringa oleifera as fertilizer in Vietnam

All 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 nonedible parts promotes the growth, yield, ascorbic acid content and Brix of lettuce, Mustard spinach (Chanthanousone et al. 2020; Chanthanousone et al. 2022) and Mustard green (Truong et al. 2023).

CHAPTER 3. MATERIALS AND METHODS

3.1. Research contents

- Selection of promising *M. oleifera* lines for biomass production in Thua Thien Hue.
- Influence of Moringa foliar biofertilizer (MFB) on growth, yield and quality of leafy vegetables.
- Influence of Moringa organic fertilizer (MOF) on the growth performance of leafy vegetables.
- Demonstration of Moringa foliar biofertilizer (MFB) on leafy vegetables
- Demonstration Moringa organic fertilizer (MOF) on leafy vegetables

3.2. Research materials

- A hundred self-pollinated seeds were randomly harvested from a single parental plant of accession VI048718.
- Lettuce (*Lactuca sativa* L.) variety obtained from Phu Nong Seeds company and a mustard spinach (*Brassica juncea*) variety obtained from Ha Noi Xanh company, Ceylon spinach obtained from Trang Nong seed company.
- Total of 200 0UBC (University of British Columbia) RAPD primers (synthesized by Bioneer, Korea).
- Moringa residues (including stems, branches, and leaf petioles).
- Ground moringa residues.
- Seaweed organic foliar fertilizer that originated from Canada, and NPK foliar fertilizer that produced by Southern Fertilizer Joint Stock Company.

Table 1. *Sequence of primers used for characterize polymorphism in 76 M. oleifera self-pollinated lines*

No.	Primer name	Sequence (5’-3’)
1	UBC#350	TGACGCGCTC
2	UBC#368	ACTTGTGCGG
3	UBC#413	GAGGCGGCGA
4	UBC#433	TCACGTGCCT

5	UBC#437	AGTCCGCTGC
6	UBC#448	GTTGTGCCTG
7	UBC#489	CGCACGCACA
8	me_1F	TGAGTCCAAACCCGATA
	em_4R	GACTGCGTACGAATTTGA
9	me_2F	TGAGTCCAAACCGGAGC
	em_1R	GACTGCGTACGAATTAAT
10	me_2F	TGAGTCCAAACCGGAGC
	em_4R	GACTGCGTACGAATTTGA

3.3. Research methods

3.3.1. Selection of promising M. oleifera lines for biomass production in Thua Thien Hue

3.3.1.1. Morphology and waterlogging tolerance

After transplanting for forty days, the waterlogging tolerance of the 76 SPLs was assayed by watering with 10 L of water every day for twenty days. Colours were determined using the Methuen Handbook of Colours.

3.3.1.2. Genetic diversity analysis

- DNA extraction: Genomic DNAs of the parental plant and 76 SPLs were extracted from fresh leaves following the CTAB (cetyl-trimethyl ammonium bromide) procedure of Doyle and Doyle (1986).

- *RAPD-PCR amplification:* A total of 200 UBC RAPD primers (Bioneer, Korea). The polymorphic UBC RAPD primers were used to genotype 76 SPLs. PCR reactions were carried out as described previously (Truong et al. 2013).

- Sequence-related amplified polymorphism (SRAP)-PCR amplification: Sequence-related amplified polymorphism was examined using fifteen primer combinations (three forward and five reverse primers) (Ridwan et al., 2020).

3.3.1.3. Total phenolic content assay

The total phenolic content of *M. oleifera* leaves was determined using the Folin–Ciocalteu assay as previously described (Siddhuraju & Becker, 2003). The absorbance was measured at 758 nm (Hitachi U-2910, Japan).

3.3.1.4. Total flavonoid content assay

- The ethanol extract was prepared as above, and a ten-fold dilution was carried out in 70% ethanol. The total flavonoid content was determined as described by Siddhuraju and Becker (2003).

3.3.2. Influence of Moringa foliar biofertilizer (MFB) on growth, yield and quality of leafy vegetables

3.3.2.1. Moringa foliar biofertilizer (MFB) preparation

Moringa foliar biofertilizer was prepared following the non-aerated process. Briefly, 70 kg of moringa residues (including stems, branches, and leaf petioles) were washed with water to remove dust particles before being chopped into small parts. In a 100-liter container, the chopped moringa residues were spread to form a 20- cm layer. Second, molasses (5 L) and effective microorganism (EM) products (0.2 kg) were subsequently added to the top of the layer. The container was filled with chopped materials and water was added to 2/3 of the container. The container was then tightly covered. The mixture in the container was stirred once every month until the end of the composting period (three to four months).

3.3.2.2. Effect of composting time on the quality of MFB

To evaluate the effect of composting time on the quality of MFB, the residue was incubated for either 3, 3.5, or 4 months. Nutrition properties of the extract including the percentages of nitrogen (N), phosphorus (P), phosphorus pentoxide (P_2O_5), potassium (K), potassium oxide (K_2O), and organic matter (OM) were determined.

3.3.2.3. Primarily screening of Moringa foliar biofertilizer on growth and yield of leafy vegetables

Three-to-four-leaf lettuce, mustard spinach and ceylon spinach grown in 10-m^2 plots were sprayed with either 20, 25, 33.3, 50 or 100 ml of MFB diluted in water (to a total volume of 1 L) (Nwokeji et al. 2022). Seaweed organic foliar fertilizer and NPK foliar fertilizer were used as controls. Foliar fertilizers were sprayed every five days until five days prior to harvest. The experiment was designed in a Randomized Completely Block Design (RCBD) with five fertilizer doses and three replicates per treatment.

3.3.2.4. Effect of different doses of MFB on growth, yield, and quality of lettuce and mustard spinach

Three to four leaf plants in a 10 m^2 plot were sprayed with either 100 ml, 50 ml, 33.3 ml, 25 ml, or 20 ml of MFB diluted in 1 L of water

(Nwokeji et al. 2022). The experiment was designed in a Completely Randomized Design (CRD) with five fertilizer doses and three replicates per treatment.

3.3.2.5. Effect of different foliar fertilizers on growth, yield, and quality of lettuce and mustard spinach

Three-to-four leaf lettuce and mustard spinach plants in a 10 m^2 plot were sprayed with MFB (100 ml per Litre), commercial chitosan fertilizer, seaweed fertilizer, and water (control). Commercial fertilizers were diluted with water at a ratio of 1.25:1 (volume: volume). The experiment was designed in a Completely Randomized Design (CRD) with five fertilizer doses and three replicates per treatment.

3.3.3. Influence of Moringa organic fertilizer (MOF) on the growth performance of leafy vegetables

3.3.3.1. Moringa organic fertilizer (MOF) preparation

MOF was prepared from Moringa non-edible parts, including stems, branches and leaf petioles. 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%. After three weeks, water was supplemented, and the mixture was stirred and incubated for another 5, 7 or 9 weeks.

3.3.3.2. 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.

3.3.3.3. Effect of MOF amounts on the growth, yield and quality of lettuce and mustard spinach

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 was planted with a density of 33 plants per m^2.

3.3.3.4. Effect of various organic fertilizers on growth, yield and quality of lettuce and mustard spinach

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.

3.3.4. Demonstration of Moringa foliar biofertilizer (MFB) on leafy vegetables

Lettuce and mustard spinach were planted with a density of 33 plants per m^2 on 100-m^2 plots. Three-to-four-leaf lettuce and mustard spinach plants were sprayed with MFB (100 ml diluted with water to a total volume of 1 L) (Model 1). For control (Model 2), Nitrate Magness fertilizer was sprayed following manufacturer's recommendation (3.125 g in 1 L of water). Foliar fertilizers were applied every five days until five days prior to harvest. The experiment was designed in a completely randomized design (CRD), and three replicates per treatment.

3.3.5. Demonstration moringa organic fertilizer (MOF) on leafy vegetables

The field experiment was carried to compare the effects of MOF (T1; 2.5 kg/m^2) (Model 1) and chemical fertilizer (T2; 7 g N, 7 g P_2O_5 and 4 g K_2O per m^2) (Model 2) on the growth, yield and quality of lettuce and mustard spinach. Fertilizers were applied as basal dressing before planting. Lettuce and mustard spinach were planted with a density of 33 plants per m^2 on 100-m^2 plots. The experiment was conducted in a completely randomized design (CRD) and three replicates per treatment.

3.4. Data collection and analysis

Clear and undistorted DNA bands were scored as “1”, and absent (or faint) bands were scored as “0”. This logical matrix data was used to determine the genetic diversity using POPGENE version 1.32 (Yeh et al., 1999). The phylogenetic tree was constructed using the UPGMA algorithm in NTSYSpc (version 2.1), in which the distance matrix was established based on simple matching similarity coefficient (Sokal & Michener, 1958).

Growth time (day) from sowing to harvest. Growth parameters: plant height (cm), canopy diameter (cm), the number of leaves, and leaf area index (leaf area/ground area) were determined for five plants in each treatment. The yield components included (i) fresh mass/plant (g/plant) (combined weight of stem, leaves, and roots); (ii) estimated yield (ton/ha) (average fresh mass/plant × plant density); (iii) actual yield (ton/ha). Statistical analysis was performed using one ways analysis of variance (ANOVA) followed by Turkey’s test in IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA). Data represented significant differences as $p < 0.05$.

CHAPTER 4. MATERIAL AND METHODOLOGY

4.1. Selection of promising *M. oleifera* lines for biomass production in Thua Thien Hue

4.1.1. Morphology and waterlogging tolerance

Young shoot colour varied from green, greenish purple, light purple to purple. Leaf number ranged from nine leaves (SPL 65) to 21 leaves (SPL 55). Plant heights varied between 36 cm (SPL 61) and 132 cm (SPL 10). Stem circumferences varied between 3.4 cm (SPL 61) and 8.0 cm (SPL 23). Waterlogging treatment was carried out for 20 days. Overall, leaf gain was observed in only three SPLs following the waterlogging treatment: 7, 18 and 65.

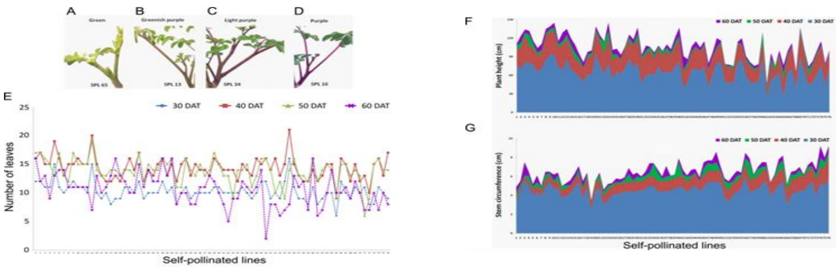
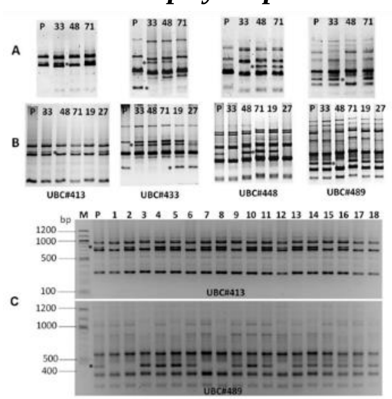


Figure 1. *Waterlogging tolerance of 76 M. oleifera self-pollinated lines (SPLs) at 40 days after transplanting.*

4.1.2. Genetic polymorphism



These primers were then used to genotype the 76 *M. oleifera* self-pollinated lines and the parental plant (Fig. 5C).

Figure 2. Polymorphism within the M. oleifera parental (P) and self-pollinated lines shown by RAPD markers.

4.1.3. PCR result with RAPD and SRAP primers

The polymorphic analyses obtained from PCR reactions using seven RADP primers and three SRAP primer pairs were Across SPLs, the combined number of amplification bands from ten primers/primer pairs ranged from 75 to 83, with SPL 71 yielding the highest number of amplification bands.

4.1.4. Genetic diversity analysis

Genetically, the parental and 76 self-pollinated lines five major groups: group I included SPL 5 and SPL 43, having a similarity coefficient of 0.80. Group II consisted of SPL 3 and SPL 13 whereas group III involved SPL 12 and SPL48. Next, group IV included 14 SPLs (7, 8, 23, 25, 34, 39, 67, 68, 69, 70, 72, 73, 74 and 75) whereas the rest, which included the parental and 56 SPLs, belonged to the largest group - group V. SPL 76 and *P* were genetically close. The lowest similarity was observed between SPL 43 and SPL 48.

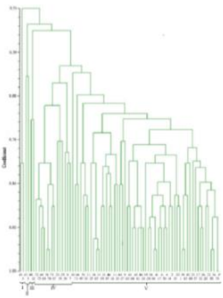
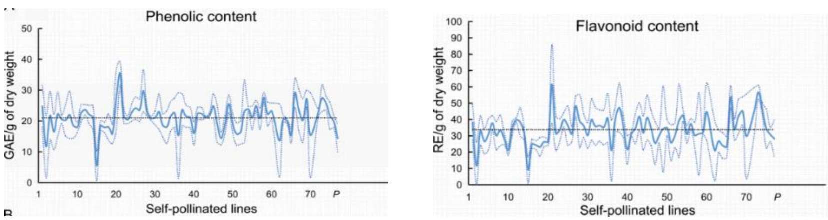


Figure 3. *Dendrogram showing the genetic relationship between the Moringa oleifera parental (P) and 76 self-pollinated lines (SPLs).*

4.1.5. Phenolic and flavonoid contents

The lines with the highest phenolic contents were SPLs 21, 27 and 66 and the lines with the highest flavonoid contents were SPLs 21, 73 and 66. Future work will focus on creating pure breeds from accessions with high waterlogging tolerance (SPLs 7, 18 and 65), and high phenolic and flavonoid contents (SPLs 21, 27, 66 and 73), before outcrossing can be carried out to create elite *M. oleifera* cultivars.



(A) (B)

Figure 4. *Total phenolic and flavonoid contents measured in M. oleifera parental (P) and 76 self-pollinated lines.*

4.2. Influence of Moringa foliar biofertilizer on growth, yield and quality of leafy vegetables

4.2.1. Effect of composting time on the quality of Moringa foliar biofertilizer (MFB)

These parameters peaked after composting for four months (nitrogen content of 11.9% and pH of 5.04).

Table 2. *Effect of composting time on the physicochemical properties of moringa foliar biofertilizer (MFB)*

Composting time	N (%)	P (%)	P_2O_5 (%)	K (%)	K_2O (%)	OM (%)	pH
3 months	4.20^{c}	2.21^{b}	5.06^{b}	7.20^{a}	8.68^{a}	37.73^{a}	3.37^{b}
3.5 months	8.52^{b}	3.04^{a}	6.97^{a}	5.39^{b}	6.49^{b}	29.13^{a}	4.82^{a}
4 months	11.90^{a}	2.63^{ab}	5.89^{ab}	5.07^{b}	6.11^{b}	32.77^{a}	5.04^{a}

4.2.2. Primarily screening of Moringa foliar biofertilizer on growth and yield of leafy vegetables

In summary, the application of MFB at 100 ml and 30 ml per Liter helped to shorten the growth and development time of lettuce and mustard spinach. For lettuce, the actual yield was the highest in treatment 6 using Seaweed organic foliar fertilizer with 2.45kg/m^2. Treatment with 100 ml of MFB per L of water and NPK chemical foliar fertilizer control (treatment 7) produced similar yields (2.38 and 2.35kg/m^2 respectively). For mustard spinach, the highest actual yield (2.82 kg/m^2) was recorded when 100 ml of MFB per L of water was sprayed (treatment 8), followed by the NPK chemical foliar fertilizer control (2.59 kg/m^2, treatment 14). Similar results were obtained with Ceylon spinach (3.14 kg/m^2 - treatment 15 and 2.33 kg/m^2 - treatment 21).

4.2.3. MFB doses influence on growth, yield and quality of leafy vegetables

Lettuce was grown from 35 days to 37 days in the first planting, and from 32 days to 34 days in the second planting. Foliar application of MFB at 100 ml per liter significantly increased the fresh mass and estimated yield compared to the lower doses. The actual yields were comparable between 100 and 50 ml per litre treatments and were significantly higher than those of other treatments. (Table 3).

Mustard spinach also has a similar grown period to lettuce and it was recorded from 33 to 36 days in the first planting, and from 28 to 32 days in the second planting. The highest dose of MFB (100 ml per Litre) correlated with the freshest weight and highest yield of mustard spinach at both times of planting. The ascorbic acid content remained relatively constant across a range of MFB doses. On the other hand, the data for Brix were not reproducible and it decreased from 8.07 (100 ml/L) to 5.26 (20 ml/L) in the first planting but it did not significantly change in the second planting. (Table 4)

Table 3. *Effect of different doses of MFB on the yield and quality of lettuce*

Dose (ml per Litre)	**Fresh weight (g per plant)**	**Estimated yield (ton per ha)**	**Actual yield (ton per ha)**	**Ascorbic acid (%)**	**Brix (%)**
First planting					
100	127.3^{a}±9.02	33.7^{a}±2.40	21.3^{a}±0.60	2.67^{a}±0.12	5.53^{a}±0.25
50	108.6^{b}±6.43	29.0^{b}±1.07	19.7^{ab}±0.95	2.57^{ab}±0.15	5.10^{a}±0.15
33.3	106.0^{bc}±4.01	28.0^{bc}±1.71	18.3^{bc}±1.03	2.34^{bc}±0.21	4.53^{b}±0.11
25	96.0^{c}±6.24	26.7^{bc}±0.53	18.2^{bc}±0.67	2.19^{c}±0.07	4.47^{b}±0.18
20	100.0^{bc}±2.18	25.6^{c}±1.66	17.7^{c}±0.43	2.16^{c}±0.16	4.43^{b}±0.24
$LSD_{0.05}$	10.88	2.95	1.68	0.28	0.43
Second planting					
100	140.2^{a}±8.26	34.4^{a}±1.83	21.7^{a}±1.26	3.45^{a}±0.38	5.45^{a}±0.15
50	117.0^{b}±6.15	28.7^{b}±1.91	20.0^{ab}±0.95	2.94^{a}±0.27	4.94^{a}±0.26
33.3	107.3^{bc}±5.23	27.0^{bc}±1.34	19.0^{bc}±0.78	3.01^{a}±0.41	5.01^{a}±0.68
25	101.6^{c}±2.55	26.3^{bc}±0.95	18.0^{bc}±1.14	3.07^{a}±0.06	5.07^{a}±0.22
20	99.3^{c}±4.79	25.8^{c}±1.06	17.3^{c}±0.87	3.04^{a}±0.09	5.04^{a}±0.17
$LSD_{0.05}$	10.85	2.54	2.36	0.72	0.71

Table 4. *Effect of different doses of moringa foliar fertilizer (MFB) on the yield and quality of mustard spinach*

Dose (ml per Litre)	**Fresh weight (g per plant)**	**Estimated yield (ton per ha)**	**Actual yield (ton per ha)**	**Ascorbic acid (%)**	**Brix (%)**
First planting					
100	133.0^{a}±8.47	35.3^{a}±1.47	28.0^{a}±1.17	5.76^{a}±0.12	8.07^{a}±0.09
50	115.7^{b}±5.32	30.7^{b}±2.21	24.3^{b}±1.35	5.54^{a}±0.07	7.13^{b}±0.11
33.3	113.0^{bc}±2.19	30.3^{bc}±1.05	24.6^{b}±0.98	5.69^{a}±0.05	7.01^{b}±0.10
25	112.0^{bc}±6.20	29.6^{bc}±2.14	23.7^{b}±1.61	5.68^{a}±0.10	6.77^{b}±0.07
20	101.7^{c}±7.56	27.0^{c}±3.02	22.3^{b}±2.21	5.62^{a}±0.09	5.26^{c}±0.13
$LSD_{0.05}$	11.67	3.41	3.14	0.23	0.48
Second planting					
100	137.7^{a}±4.41	37.0^{a}±1.92	29.7^{a}±0.66	5.52^{a}±0.21	4.80^{a}±0.24
50	126.0^{b}±6.92	33.7^{b}±2.04	27.3^{b}±1.05	5.02^{a}±0.34	4.20^{a}±0.19
33.3	119.3^{bc}±4.65	31.6^{bc}±1.99	25.3^{c}±1.24	4.73^{a}±0.08	4.53^{a}±0.20
25	114.7^{c}±8.07	30.7^{c}±2.31	24.0^{c}±0.68	5.28^{a}±0.17	4.43^{a}±0.16
20	102.3^{d}±5.42	27.3^{c}±2.11	21.7^{d}±0.41	5.20^{a}±0.09	4.40^{a}±0.32
$LSD_{0.05}$	9.53	2.50	1.91	0.86	0.62

4.2.4. Effect of various foliar fertilizers on growth, yield, and quality of leafy vegetables

The results suggested that the application of MFB promoted the growth of lettuce. Furthermore, the growth time, the number of leaves, canopy diameter, and leaf area index of lettuce plants applied with MFB was comparable to those sprayed with commercial biofertilizers. The plant height of lettuce slightly changed among foliar treatments in the second planting and peaked at 24.3 cm in plants treated with MFB. The yield of lettuce was enhanced by spraying foliar fertilizers at both plantings. The treatment of MFB increased the fresh weight of lettuce. Estimated yields ranged from 33.8 tons per ha to 37.5 tons per ha and actual yields ranged from 21.3 tons per ha to 23.9 tons per ha across foliar treatments. (Table 5).

Mustard spinach growth was also affected by foliar treatments. In the first planting, plant height and leaf area index did not vary between different treatments. In the second planting, plant height, the number of leaves, and leaf area index were similar among foliar treatments and higher than those of the control. Canopy diameter ranged from 27.2 cm (chitosan fertilizer) to 31.7 cm (seaweed fertilizer), compared to 25.4 cm of the control. The highest fresh weight and estimated yield of mustard spinach grown in the first planting were found in those treated with MFB but these results were not reproducible in the second planting (Table 6).

The ascorbic acid of plants grown in the first planting varied from 3.31% (control) to 5.21% (seaweed fertilizer treated).

Table 5. *Effect of various foliar fertilizers on the yield and quality of lettuce*

Treatment	Fresh weight (g/plant)	Estimated yield (ton/ha)	Actual yield (ton/ha)	Ascorbic acid (%)	Brix (%)
First planting					
MFB	$146.7^{a} \pm 12.12$	$37.5^{a} \pm 3.23$	$23.9^{a} \pm 1.07$	$4.59^{a} \pm 0.37$	$5.13^{a} \pm 0.27$
Chitosan fertilizer	$132.3^{ab} \pm 11.46$	$35.3^{a} \pm 2.39$	$21.9^{ab} \pm 1.92$	$4.77^{a} \pm 0.29$	$5.10^{a} \pm 0.13$
Seaweed fertilizer	$127.3^{b} \pm 4.16$	$33.9^{a} \pm 2.67$	$21.4^{b} \pm 1.06$	$4.87^{a} \pm 0.55$	$4.53^{b} \pm 0.15$
Control	$105.3^{c} \pm 5.04$	$28.0^{b} \pm 1.81$	$17.7^{c} \pm 0.84$	$3.96^{a} \pm 0.77$	$4.27^{b} \pm 0.19$
$LSD_{0.05}$	15.17	3.66	2.10	1.92	0.33
Second planting					
MFB	$137.7^{a} \pm 3.05$	$34.7^{a} \pm 1.55$	$23.5^{a} \pm 1.42$	$4.77^{a} \pm 0.27$	$5.34^{a} \pm 0.34$

Chitosan fertilizer	129.6^{b} ± 4.14	34.6^{a} ± 2.01	21.8^{ab} ± 1.15	4.68^{a} ± 0.13	4.93^{a} ± 0.15
Seaweed fertilizer	123.0^{c} ± 2.39	33.8^{a} ± 1.79	21.3^{b} ± 1.08	4.72^{a} ± 0.56	5.00^{a} ± 0.09
Control	101.7^{d} ± 1.81	27.1^{b} ± 1.43	17.8^{c} ± 1.41	3.63^{b} ± 0.48	4.96^{a} ± 0.47
$LSD_{0.05}$	4.92	2.29	1.87	0.88	0.72

Table 6. *Effect of various foliar fertilizers on the yield and quality of mustard spinach*

Treatment	Fresh weight (g per plant)	Estimated yield (ton per ha)	Actual yield (ton per ha)	Ascorbic acid (%)	Brix (%)
First planting					
MFB	158.0^{a}±5.55	37.1^{a}±1.06	26.7^{a}±1.29	3.92^{b}±0.61	6.47^{a}±0.49
Chitosan fertilizer	140.2^{b}±3.60	32.9^{b}±1.60	24.4^{b}±0.76	4.06^{b}±0.78	6.60^{a}±0.08
Seaweed fertilizer	136.7^{b}±6.01	32.1^{b}±1.42	25.6^{ab}±1.22	5.21^{a}±0.30	6.67^{a}±0.34
Control	116.0^{c}±5.78	27.3^{c}±0.95	19.2^{c}±0.87	3.31^{b}±0.54	6.33^{a}±0.44
$LSD_{0.05}$	7.89	1.85	1.75	0.88	1.73
Second planting					
MFB	157.3^{a}±10.78	37.1^{a}±2.05	25.4^{a}±1.75	5.22^{a}±0.06	6.73^{a}±0.49
Chitosan fertilizer	146.7^{a}±12.24	32.9^{b}±3.32	23.0^{b}±0.99	5.12^{a}±0.14	6.82^{a}±0.35
Seaweed fertilizer	155.6^{a}±13.42	36.6^{a}±2.69	25.2^{ab}±1.42	5.73^{a}±0.45	6.98^{a}±0.10
Control	117.3^{b}±9.97	27.5^{c}±3.02	18.6^{c}±1.86	5.08^{a}±0.58	6.07^{a}±0.38
$LSD_{0.05}$	17.07	3.61	2.33	0.87	1.05

4.3. Influence of Moringa organic fertilizer on the growth performance of leafy vegetables

4.3.1. Nutrient contents of Moringa organic fertilizer at different incubation periods

Moringa organic fertilizer (MOF) prepared with seven-week was quality best.

Table 7. *Effect of incubation periods on the quality of MOF*

Treatment	N (%)	P (%)	P_2O_5 (%)	K_2O (%)	Organic matter (%)	pH
I1	0.82^{c}±0.01	2.02^{a}±0.19	4.62^{a}±2.05	25.58^{a}±4.41	6.58^{a}±1.42	6.27^{a}±0.03
I2	3.57^{a}±0.11	3.50^{a}±0.64	8.00^{a}±1.90	20.63^{a}±5.84	11.49^{a}±4.12	6.13^{a}±0.02
I3	2.29^{b}±0.17	3.76^{a}±1.39	8.61^{a}±2.42	26.24^{a}±4.63	8.12^{a}±0.75	5.88^{b}±0.17
$LSD_{0.05}$	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 5% probability. I1: 5 weeks, I2: 7 weeks, I3: 9 weeks. LSD: Least significant difference.

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

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. At both planting times, the fresh mass, theoretical yield, and actual yield of lettuce grown 25 tons of MOF per ha were significantly higher than those grown at 15 and 20 tons of MOF per ha (Table 8).

The mustard spinach plants 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 4.20). 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 9).

Table 8. *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	$100.3^{b}\pm6.66$	$26.7^{b}\pm0.63$	$19.0^{c}\pm1.67$	$2.767^{a}\pm0.11$	$4.93^{a}\pm0.31$
R2	$101.7^{b}\pm4.23$	$27.0^{b}\pm1.78$	$20.3^{bc}\pm2.01$	$2.730^{a}\pm0.14$	$4.76^{ab}\pm0.46$
R3	$123.3^{a}\pm5.04$	$32.7^{a}\pm0.53$	$23.7^{a}\pm1.30$	$2.741^{a}\pm0.30$	$5.17^{a}\pm0.25$
R4	$125.4^{a}\pm6.50$	$33.0^{a}\pm1.34$	$22.7^{ab}\pm1.71$	$2.693^{a}\pm0.15$	$4.90^{a}\pm0.32$
$LSD_{0.05}$	7.89	3.12	2.56	0.41	0.39
Second planting					
R1	$99.9^{c}\pm2.01$	$25.7^{c}\pm0.54$	$20.8^{c}\pm0.42$	$2.607^{b}\pm0.11$	$4.40^{b}\pm0.26$
R2	$110.0^{bc}\pm5.29$	$29.3^{b}\pm1.42$	$22.9^{bc}\pm1.10$	$2.770^{ab}\pm0.23$	$4.76^{a}\pm0.33$
R3	$122.7^{a}\pm4.73$	$31.7^{a}\pm0.67$	$25.6^{a}\pm0.98$	$2.863^{a}\pm0.05$	$5.10^{a}\pm0.36$
R4	$117.8^{b}\pm9.62$	$30.0^{ab}\pm0.85$	$24.5^{ab}\pm2.00$	$2.874^{a}\pm0.07$	$4.86^{a}\pm0.29$
$LSD_{0.05}$	12.0	2.1	2.5	0.2	0.4

Table 9. *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^{b}±4.17	29.7^{b}±1.21	19.3^{b}±0.54	4.1^{b}±0.66	3.5^{a}±0.32
R2	121.3^{b}±5.42	32.0^{b}±2.12	21.0^{b}±0.67	5.4^{a}±0.35	3.4^{a}±0.17
R3	149.3^{a}±8.15	39.3^{a}±0.69	25.7^{a}±0.47	5.7^{a}±0.44	4.5^{a}±0.51
R4	146.0^{a}±3.67	38.7^{a}±0.47	25.3^{a}±0.36	5.3^{a}±0.51	4.4^{a}±0.46
$LSD_{0.05}$	13.68	2.92	2.49	1.18	1.16
Second planting					
R1	108.7^{b}±2.89	28.7^{d}±0.96	18.7^{c}±0.50	4.5^{b}±0.36	3.9^{b}±0.33
R2	115.3^{b}±9.18	32.1^{c}±0.70	19.6^{c}±1.51	5.4^{a}±0.51	4.3^{b}±0.58
R3	146.1^{a}±4.78	38.0^{a}±0.81	25.7^{a}±0.94	5.7^{a}±0.57	5.4^{a}±0.16
R4	136.7^{a}±2.35	35.3^{b}±1.05	23.0^{b}±0.58	5.4^{ab}±0.39	5.2^{a}±0.29
$LSD_{0.05}$	11.7	1.9	2.2	0.9	0.6

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

Furthermore, applying 25 tons of MOF per hectare enhanced the yield and quality of leafy vegetables

- For lettuce fresh mass, theoretical yield and actual yield 25.6 to 25.5 T/ha were higher in MOF treatment than in other treatments (Table 10).

- For mustard spinach MOF had more fresh mass than other organic fertilizers during both planting times. The MOF treatment also produced 25.9 to 26.8 T/ha (actual yield) more than the cow manure treatment (Table 11).

Table 10. *Effect of various organic fertilizers on the yield and quality of lettuce*

Treatment	Fresh mass (g $plant^{-1}$)	Theoretical yield (ton ha^{-1})	Actual yield (ton ha^{-1})	Ascorbic acid (%)	Brix (%)
First planting					
F1	150.0^{a}±3.05	38.7^{a}±0.81	25.6^{a}±1.22	5.2^{a}±0.22	5.0^{a}±0.43
F2	133.7^{b}±2.57	35.6^{b}±0.39	23.1^{b}±0.76	5.2^{a}±0.31	4.7^{a}±0.49
F3	128.3^{b}±6.02	33.5^{b}±2.11	22.1^{b}±1.18	5.3^{a}±0.16	5.0^{a}±0.47
Control	105.0^{c}±3.78	28.0^{c}±1.18	18.0^{c}±1.34	4.3^{b}±0.56	3.6^{b}±0.26
$LSD_{0.05}$	12.31	2.30	1.40	0.6	0.6

Second planting					
F1	$145.7^{a}\pm3.52$	$37.4^{a}\pm0.53$	$25.5^{a}\pm0.34$	$5.6^{a}\pm0.30$	$5.1^{a}\pm0.10$
F2	$129.6^{b}\pm4.04$	$34.0^{b}\pm0.59$	$22.8^{b}\pm0.73$	$5.7^{a}\pm0.23$	$5.0^{a}\pm0.26$
F3	$123.5^{c}\pm4.92$	$33.5^{b}\pm1.67$	$21.7^{b}\pm1.42$	$5.7^{a}\pm0.29$	$5.1^{a}\pm0.15$
Control	$101.7^{d}\pm5.44$	$26.2^{c}\pm1.26$	$18.1^{c}\pm0.95$	$4.7^{b}\pm0.27$	$3.9^{b}\pm0.49$
LSD$_{0.05}$	5.99	2.12	1.55	0.3	0.2

Table 11. *Effect of various organic fertilizers on the yield and quality of mustard spinach*

Treatment	**Fresh mass (g plant^{-1})**	**Theoretical yield (ton ha^{-1})**	**Actual yield (ton ha^{-1})**	**Ascorbic acid (%)**	**Brix (%)**
First planting					
F1	$158.0^{a}\pm8.93$	$38.7^{a}\pm0.38$	$25.9^{a}\pm0.51$	$5.7^{a}\pm0.38$	$4.5^{a}\pm1.01$
F2	$140.3^{b}\pm9.14$	$37.3^{a}\pm1.55$	$23.3^{b}\pm11.35$	$5.6^{a}\pm0.56$	$4.4^{a}\pm0.76$
F3	$136.7^{b}\pm7.70$	$37.0^{a}\pm1.97$	$24.3^{ab}\pm1.42$	$5.7^{a}\pm0.63$	$4.5^{a}\pm0.95$
Control	$111.3^{c}\pm7.26$	$28.2^{b}\pm1.70$	$18.4^{c}\pm0.98$	$4.2^{b}\pm0.74$	$3.6^{a}\pm2.14$
LSD$_{0.05}$	14.4	2.4	1.8	1.2	1.2
Second planting					
F1	$155.0^{a}\pm6.39$	$37.4^{a}\pm0.66$	$26.8^{a}\pm0.66$	$5.5^{a}\pm0.19$	$5.9^{a}\pm0.28$
F2	$138.1^{b}\pm4.55$	$35.3^{b}\pm1.87$	$23.9^{b}\pm1.24$	$5.2^{a}\pm0.84$	$4.7^{b}\pm0.74$
F3	$130.3^{b}\pm8.95$	$34.8^{b}\pm1.16$	$24.1^{b}\pm1.28$	$5.3^{a}\pm0.58$	$5.5^{a}\pm0.32$
Control	$110.4^{c}\pm8.04$	$27.3^{c}\pm1.81$	$19.9^{c}\pm0.93$	$4.4^{b}\pm0.60$	$4.0^{b}\pm1.01$
LSD$_{0.05}$	9.4	1.9	1.1	0.7	0.7

4.4. Demonstration of biofertilizer from Moringa residue on lettuce and mustard

4.4.1. Demonstration of Moringa foliar fertilizer on lettuce

The yield in Model 1 using moringa foliar biofertilizer reached 21.32 tons ha^{-1}, which is significantly higher than the farmer's practice (19.45 tons ha^{-1}). This means that Moringa foliar fertilizer has a great influence on the growth characteristics, yield, and quality of lettuce in large-scale production.

4.4.2. Demonstration of Moringa foliar fertilizer on mustard spinach

These explained why the actual yield of mustard spinach was significantly higher in the demonstration model using moringa foliar biofertilizer. We can conclude that MFB applied at a ratio 1: 10 could improve the growth characteristics of lettuce and mustard spinach.

4.5. Demonstration of Moringa organic fertilizer (MOF) on leafy vegetables

4.5.1. Demonstration of Moringa organic fertilizer on lettuce

The yield in model 1 using MOF reached 23.62 tons ha^{-1}, significantly higher than in model 2 (21.22 tons ha^{-1}). Besides higher yield, the quality of lettuce tended to be higher in model 1 than in model 2. The Brix content were 6.77% and 5.50% the in the two models. Additionally, the vitamin C value model 1 was higher than those in Model 2.

4.5.2. Demonstration of Moringa organic fertilizer on mustard spinach

The yield and quality of mustard spinach in Table 4.33 showed the brix and vitamin C contents in the model using MOF were 6.60% and 8.70%, respectively. These values were significantly higher than those in the farmer practice demonstration. The actual yield of mustard spinach was found 22.54 tons ha^{-1} and 19.12 tons ha^{-1} in Model 1 and Model 2, respectively. The differences in actual yield might be due to the differences in fresh weight.

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The waterlogged tolerant lines were found to be SPLs 7, 18 and 65. The lines with the highest phenolic contents were SPL 21 (35.6 mg of GAE/g of dry weight), SPL 27 (29.7 mg of GAE/g of dry weight), and SPL 66 (29.2 mg of GAE/g of dry weight), and the lines with the lowest phenolic contents were SPL 15 (5.5 mg of GAE/g of dry weight). The lines with the highest flavonoid contents were SPL 21 (61.6 mg of RE/g of dry weight), SPL 73 (56.7 mg of RE/g of dry weight), and SPL 66 (53.9 mg of RE/g of dry weight), and the lines with the lowest flavonoid contents were SPL 15 (9.1 mg/RE/g of dry weight).

- Moringa residues were fermented using EM product and molasses to produce Moringa foliar biofertilizer (MFB) in four months of composting time.
- Optimal Moringa organic fertilizer (MOF) was obtained after a seven-week incubation period.
- The application of MFB with 100 mL per liter of MFB spray improved the yield of leafy vegetables, which peaked at 23.5-23.9 tons/ha for lettuce and 25.4-26.7 tons/ha for mustard spinach, and produced similar effects compared to the chitosan and seaweed fertilizers. However, MFB promoted the growth and yield of mustard spinach more than the other fertilizer at both plantings.

- Applying 25 tons of MOF per hectare enhanced the yield and quality of leafy vegetables, which peaked at 25.5-25.6 tons/ha for lettuce and 25.9–26.8 tons/ha for mustard spinach. MOF is a promising alternative to cow manure and other commercial bio-organic fertilizers for safe and sustainable vegetable farming.
- Both Moringa foliar biofertilizer (MFB) and Moringa organic fertilizer (MOF) improved yields of leafy vegetables more than chemical fertilizers.

5.2. Recommendations

- Future Moringa breeding should be focused on creating pure breeds from accessions with high waterlogging tolerance (SPLs 7, 18 and 65), and high phenolic and flavonoid contents (SPLs 21, 27, 66 and 73).
- Moringa non-edible parts can make organic fertilizer and foliar biofertilizer to enhance growth, yield, and quality of leafy vegetables.
- Both Moringa foliar Biofertilizer (MFB) and Moringa organic fertilizer (MOF) can be used in organic production of leafy vegetables.
- Large-scale Moringa plantation for biomass production should be considered to provide materials for MFB and MOF production in Thua Thien Hue.

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2. Chanthanousone, H., Phan, T. T., Nguyen, C. Q., Nguyen, T. D. T., Dang, L. T., Hoang Ho, N. T., Le Nguyen, B. Q., & Truong, H. T. H. (2022). Influence of foliar application with Moringa oleifera residue fertilizer on growth, and yield quality of leafy vegetables. *Journal of Experimental Biology and Agricultural Sciences*, 10(6): 1453-1461. https://doi.org/10.18006/2022.10(6).1453.1461.

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