HUE UNIVERSITY

INSTITUTE OF BIOTECHNOLOGY

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

PhD DISSERTATION

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PREFACE

I hereby declare that this is my own research work. The data in the dissertation has a clear origin. Data for the research process are collected from conducting experiments, and analyzing are honest and have never been published before.

Hue city, November 2023

Author:

HATSADONG CHANTHANOUSONE

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Hue city, November 2023

Author:

HATSADONG CHANTHANOUSONE

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GLOSSARY OF SYMBOLS, MEASURE UNITS, ABBREVIATIONS AND TERMS

AVRDC	Asian Vegetable Research and Development Center
ANOVA	Analysis of Variance
Cal	Calories
^{o}C	Degrees Celsius
cm	Centimeter
CRD	Completely Randomized Design
CTAB	Cetyl-Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
DUS	Discriptors of Distinct Ness Uniformity and Stability
EM	Effective Microorganic
g	Gram
GAE	Gallic Acid Equivalent
GAE/g	Gallic Acid Equivalent Per Gram
GMP	Good Manufacturing Practice (Gmp)
ha	hectare
HPLC	High-Performance Liquid Chromatography
Kg	Kilogram
L	Liter
LSD	Least Significant Difference
M	Meter
m^2	Square Meter
mg	Milligram
mg/kg	Milligram Per Kilogram
Kg/ha	Kilogram Per Hectar
mL	milliliters
MLE	Moringa Leaf Extract
MO	*Moringa oleifera* Lam.
MFB	Moringa Foliar Biofertilizer
MOF	Moringa Organic Fertilizer
NISF	National Institute for Soil and Fertilizers

NUE	Nitrgen Utilization Efficiency
N	Nitrogen
P	Phosphorus
K	Potassium
KCl	Potassium Chloride
OM	Organic Matter
ORFs	Open Reading Frame
PCR	Polymerase Chain Reaction
PGPR	Plant Growth Promoting Rhizobacteria
IPGRI	International Plant Genetic Resources Institute
PKM1	Periyakulum 1
pH	Potential of Hydrogen
POPGENE	Population Genetic Analysis
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RE	Rutin Equivalent
RE/g	Rutin Equivalents Per Gram
RFLP	Restriction Fragment Length Polymorphism
Rpm	Revolutions Per Minute
SMs	Secondary Metabolites
SNPs	Single Nucleotide Polymorphism
SPL	Self – Pollinated Line
SPSS	Statistical Package Social Sciences
SRAP	Sequence -Relate Amplified Polymorphism
T	Tone
Ton/ha	Tone Per Hectare
µg/l	Microgram/Liter
UBC	University of British Columbia
UPGMA	Unweighted Pair Group Method Arithmetic Mean
USA	United States of American
USD	United States Dollar
VAM	Vesicular-Arbuscular Mycorrhizal Fungi

CHAPTER 1. INTRODUCTION

1.1. Background

Moringa oleifera Lam. (commonly known as drumstick) is a multipurpose tree species, nutritionally rich and is distributed throughout South India, Southeast Asia, South America and Africa (Dhakad et al., 2019; Singh et al., 2020; George et al., 2021; Alavilli et al., 2022). Drumstick leaves and pods are used as a vegetable for human consumption and serve as ingredients for animal feeds (Moyo et al., 2011). Additionally, *M. oleifera* parts are also rich in minerals, protein, vitamins, phenolic and flavonoid compounds (Singh et al., 2020; Hassan et al., 2021). Furthermore, hydrogels prepared with *M. oleifera* seed extract help to promote wound healing (Ali et al., 2022). Moringa dried leaves contain protein (30.3%), iron (490 mg/kg), selenium (363 mg/kg), manganese (86.8 mg/kg), and zinc (13.03 mg/kg), α-Linolenic acid (44.57%), heneicosanoic (14.41%), copper (8.25%), calcium (3.65%), potassium (1.5%), magnesium (0.5%), phosphorus (0.3%), g-linolenic (0.20%) palmitic (0.17%), sodium (0.164%), capric acid (0.07%), sulfur (0.63%), Vitamin E (77 mg/100 g), beta-carotene (18.5 mg/100 g) ((Farooq et al., 2012)). The values of amino acids, fatty acids, minerals, and vitamin profiles reflect a desirable nutritional balance (Oparinde et al., 2014). Verma and Nigam (2014) investigated the nutritive values of all parts of *M. oleifera* and reported that they all have nutritionally important minerals and can be devoid of toxic heavy metals. Whereas, stem, root, and bark showed lower amounts of fiber, carbohydrate, and protein, but higher amounts of Zn, Fe, Ca, K, and Mg compared to leaf, fruit, and seed (Verma and Nigam, 2014) (Verma and Nigam, 2014). Therefore, using liquid Moringa organic fertilizer increases plants' N, P, K, and Fe contents and increases dry weight (Rachmawatie et al., 2022). In Vietnam, *M. oleifera* leaf is used for vegetables, tea, veggie powder, and the seed is for propogation. Hence, stem, root and bark of *M. oleifera* are garbaged. Thus, the use of residues of *M. oleifera* to produce biofertilizer is necessary. Even though *M. oleifera* is a fast-growing plant and able to adapt to nutrition soil conditions, drought, or inconvenient climates (Gopalakrishnan et al., 2016; Olson, 2010; (Aslam et al., 2005), it is poorly tolerant to waterlogged conditions, leading limitation of biomass production in central of Vietnam as well as in Thua Thien Hue. Thus, it is critical to develop cultivars with high tolerance to waterlogged conditions and to expand drumstick cultivation areas to provide materials for Moringa biofertilizer productions. Therefore, a “Study on the efficiency of Biofertilizer from Moringa residues for some leafy vegetables” is necessary.

1.2. Research objectives

1.2.1. Overall objective

Product 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. Detailed objectives

- Selecting waterlogging tolerance and good characteristics of *M. oleifera* lines for biomass production in Thua Thien Hue and breeding programs.

- Evaluating the influence of Moringa foliar biofertilizer on growth, yield and quality of leafy vegetables.

- Evaluating the influence of Moringa organic fertilizer on the growth performance of lettuce and mustard spinach.

- Evaluating the efficiency of Moringa foliar biofertilizer (MFB) on leafy vegetables

- Evaluating the efficiency of Moringa organic fertilizer (MOF) on leafy vegetables

1.3. New achievements

- Selection of three lines (SPLs 7, 18 and 65) for waterlogging tolerance and three lines (SPLs 21, 27, and 66 for high phenolic and three lines (SPLs 21, 73, and 66) the 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 about 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).

Moringa sternopetala tree grows wild in Ethiopia around 1000-1800 meters above sea level and is also native to Kenya's Northern Province. Its leaves are consumed throughout the dry season and have local medical purposes. *Moringa longihiba* Engl. is a tiny shrub type found in Kenya's Wajir, Moyale, Garissa, Teita regions. *Morniga concanensis* Nimmo was found in the Yercaud area of the Salem district of Tamil Nadu, South India. *Moringa drouhardii* sumelle is native from Madagascar with a massive trunk, is exceptionally drought resistance and can flourish in saline soils where the seeds exhibit long dormancy but the seedling grows quickly (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

Moringa is a member of the Moringaceae family. The family includes the single genus Moringa, and the tree's botanical name is *Moringa oleifera* Lam. The family is identified by parietal placentation, three-valved fruit, elongated, non-dehiscent berry, and winged seeds (Boopathi & Raveendran, 2021). Pax (1936) and Puri (1942) had identified ten species belonging to the Old-World Tropics, while Philips (1951) listed four species. Bessey (1915) classified the family as Rheoadales. According to Datta and Mitra (1942), it is more closely linked to the Violaceae of the Violales. *M. oleifera* and *M. concanensis* are the two most prevalent species. *M. oleifera* has medium-sized leaves that are generally tripinnate, 12-18 mm long leaflets, and yellow or white petioles with no red streaks. *M. concanensis* is a big tree identified by leaflets with 15-30 mm long, bipinnate leaves petals with red streaks or reddish at the base.

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 (Adhikari et al., 2017; Carvalho et al., 2019). *M. oleifera* is cross-pollinated; therefore, it is expected to have a vast genetic diversity (Makin and Solowey, 2017). The level of genetic variation among individuals of a species can be assessed based on phytochemical, morphological, and molecular markers (Adhikari et al., 2017; Nadeem et al., 2018).

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). A list of descriptors for the selected morphological traits, such as bark color, receptacle leaf shape, leaflets shape, stem color, flower color, flower symmetry, petals, sepals, anthers, seed, seed cover, pod length and habit, was suggested for distinguishing among *M. oleifera* accessions and creating a character state matrix (Mgendi et al., 2011). The International Plant Genetic Resources Institute (IPGRI) established universal standards for crop coding, data recording, and scoring in 2007. As a result, a more thorough list of morphological traits (14 qualitative and 11 quantitative) and 48 other descriptors were developed based on IPGRI recommendations for the characterization and evaluation of *M. oleifera* accessions (Santhoshkumar et al., 2013). Descriptors of distinctness, uniformity, and stability (DUS) have also been employed to assess the diversity of *M. oleifera* genotypes (Meena et al., 2021).

Using various morphological and horticultural traits, the genetic diversity and population structure of several cultivated and non-cultivated accessions collected from various geographical regions around the world (Ethiopia, India, Laos, Indonesia, Philippines, Taiwan, Saudi Arabia, Tanzania, Thailand, and the United States) were

assessed (Resmi et al., 2005; Varalakshmi et al., 2007; Mgendi et al., 2011; Santhoshkumar et al., 2013; Ganesan et al., 2014; Mulugeta et al., 2014; Natarajan et al., 2015; Palada et al., 2015; Palada et al., 2017; Hassanein et al., 2018; Singh et al., 2019; Meena et al., 2021; Paul et al., 2021; Ravi et al., 2021; Ravi et al., 2021; Ridwan et al., 2021). These investigations demonstrated the effectiveness of morphological attributes in determining genetic variation among accessions, facilitating the selection of those with desired characteristics for the future *M. oleifera* improvement effort. However, the number of morphological markers is limited, and they are frequently modified by plant growth and development stages as well as numerous environmental conditions (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 (magnesium [Mg], calcium [Ca], Nitrogen [N], potassium [K], and phosphorus [P]), micronutrients (iron [Fe], copper [Cu], zinc [Zn]), and manganese [Mn], and nutritional and anti-nutritional factors (lead [Pb], oxalate, and oligosaccharides), and polyphenols (caffeic acid, baicalin, cinnamic acid, chlorogenic acid, ferulic acid, coumaric acid, gallic acid, gallogen, kaempferide, isoquercetin, quercetin, rutin, quercitrin, and vanillin) have been used to assess genetic variability among *M. oleifera* accessions and/or advanced breeding lines from India, Thailand, Laos, the Philippines, China, Taiwan, Saudi Arabia, Tanzania, and the United States (Palada et al., 2015; Kleden et al., 2017; Kumar et al., 2017; Tak et al., 2017; Hassanein, 2018; Panwar & Mathur, 2020; Zhu et al., 2020). Zhu and co-workers (2020) found that significant differences in the polyphenol content of *Moringa oleifera* from different regions suggest that *Moringa oleifera*'s genetic diversity was relatively rich. It could be possibly due to differences in cultivation conditions, climate, or soil environment, which resulted in the accumulation of different polyphenols. According to HPLC examination, the concentration of active substances varied greatly among 57 accessions of *M. oleifera* from Banasthali region, India. The data revealed that the polyphenolic component concentration ranged from 0.06 mg/kg (sample KVKB) to 210.5 mg/kg (sample BG). The findings indicate a strong relationship between phytochemical variables and DNA polymorphism. (Panwar & Mathur, 2020). Hassanein (2018) reported that a strong association was detected between nutritional and molecular genotype classifications among *M. oleifera* and *M. peregrina* grown in Saudi Arabia. The effective classification based on four chemical traits may be helpful in Moringa evaluation. However, like morphological markers, phytochemical markers also have several limitations, i.e., their low efficacy in detecting polymorphism and

being affected by the growth and developmental stages of the plant and various biotic and abiotic stresses.

2.1.1.2.3. Molecular markers

Molecular markers are an appealing paradigm because they are automatic, have genomic coverage, are highly reproducible, and are not affected by environmental variations (Adhikari et al., 2017; Gudeta, 2018). 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). Molecular markers can detect the allelic variations of a gene in a heterozygous condition (codominant) or cannot detect (dominant). Although there are numerous molecular markers available, each has unique advantages and disadvantages. As a result, many molecular markers were tested for their usefulness in assessing genetic diversity in *M. oleifera* accessions gathered from various agroclimatic zones throughout the world. Therefore, using molecular markers to assess the genetic diversity of a germplasm is essential for conservation, selection and breeding programs. In this study, we focus on polymerase chain reaction (PCR)-based marker such random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP).

2.1.1.2.3.1. 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. This technique allows detection of several loci (0.5 kb to 5 kb) in the genome revealing DNA polymorphism between individuals (Welsh and McClelland, 1990). Because of its simplicity, cost-effectiveness, and efficacy, RAPD has become a popular dominant marker. Previous works have employed Random Amplified Polymorphic DNA (RAPD) markers to explore the genetic diversity of cultivated or wild accessions of *M. oleifera* (Mgendi et al., 2010; Yusuf et al., 2011; Silva et al., 2012; Saini et al., 2013; Rufai et al., 2013; Popoola et al., 2014; Kleden et al., 2017; Shahzad, et al., 2018; Swati et al., 2020; Drisya et al., 2022). Furthermore, Truong et al. (2018) observed genetic diversity not only among accessions collected from different countries (Thailand, USA, Philippines, Taiwan and Vietnam), but also among individuals derived from the same accession, suggesting that the varieties have been mixed in the process of breeding through cross pollination. As a result, it is not surprising that the adaptable RAPD technique was used in 50% of the investigations involving the use of molecular markers for the detection of genetic

diversity in *M. oleifera* accessions (48-96%) obtained from the differently geographical locations of Thailand, Indonesia, Brazil, India, Malaysia, Pakistan, Nigeria, Taiwan, Tanzania, and the USA (Mgendi et al., 2010; Yusuf et al., 2011; Silva et al., 2012; Saini et al., 2013; Rufai et al., 2013; Popoola et al., 2014; Kleden et al., 2017; Shahzad, et al., 2018; Truong et al., 2018; Swati et al., 2020; Drisya et al., 2022). However, the existence of false-positive results, non-reproducibility, sensitivity to experimental circumstances, and the need for a high concentration of agarose gel for higher resolution are all inherent difficulties with the RAPD approach (Adhikari et al., 2017).

2.1.1.2.3.2. 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). For the first five cycles of amplification, the annealing temperature is set at 35 °C, followed by 35 cycles at 50 °C. The amplified DNA fragments are separated by denaturing acrylamide gels and identified by autoradiography (Li & Quiros, 2001). Twenty percent of the SRAP markers were co-dominant in *Brassica oleracea* L. recombinant inbred and doubled-haploid lines. It is easily amplified in other crop species (Li & Quiros, 2001).

Dominant SRAP markers are useful for understanding genetic diversity across taxa, building linkage maps, and identifying quantitative trait loci (QTL). SRAP markers were used to determine the genetic diversity and population structure of 97 *M. oleifera* accessions that collected from Indian states such Andhra Pradesh, Odisha, and Tamil Nadu (Rajalakshmi et al., 2019) and 10 accessions from different islands in the Indonesian archipelago (Ridwan et al., 2021). SRAP markers demonstrated 70-81% polymorphism among Indian and Indonesian accessions (Rajalakshmi et al., 2019; Ridwan et al., 2021).

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 include 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. Particularly in the present context of the skyrocketing cost of agriculture inputs, the role of biofertilizers in agriculture production shows particular importance; able to prepare material from agricultural

residues for making biofertilizers and good Agri-economic and building sub-stable ecosystems, biofertilizers are highly advantageous over chemical fertilizers, biofertilizers can be mixed together with seeds, sowing, setts, seedlings, and soil, and they improve crop productivity and soil health (Baboo, 2009).

Biofertilizers can yield good yields for crop farmers, increase soil fertility, and supply more nutrients to plants through natural system processes, creating growth-promoting chemicals and Nitrogen fixation, phosphorus solubilization. It greatly rehabilitates the soil's natural nutrient cycle to increase soil organic matter. Good plants were developed by applying biofertilizers while improving the soil's sustainability and health. Biofertilizers will need a decrease in synthetic fertilizers and pesticides, and they will not be able to completely agent all of them. Biofertilizer is a substance that contains microbes that help promote the growth of plants and trees by increasing the quality of essential nutrients in the crop (Karki, 2020).

The optimum fertilization methods for the two dill genotypes' plant height (cm), leaf count per plant, pigment content, antioxidant percentage, total carbohydrate percentage, and N and P percentages were 100% organic fertilization with biofertilizer and 100% chemical fertilizer. On the other hand, 50% organic fertilization with biofertilizers and K percentage with two dill cultivars were found to be the best treatments for nitrate accumulation. Dill (*Anethum graveolens*) growth, nutrient content, and fresh production are all impacted by biofertilizer and organic fertilization. Dill plants were grown in the Experimental Farm Station of the Agriculture Faculty at Cairo University as two cultivars, Balady and Dukat (Elsayed et al., 2020).

2.1.2.2. Foliar biofertilizer

Foliar fertilization is an efficient approach for improving crop nutritional characteristics (Otalora et al., 2018). It improves the physiological properties of plants, particularly in drought and stressful environments (Ruiz-Navarro et al., 2019). Tejada et al. (2018) discovered an increase in corn yield when it was fertilized with foliar biostimulants, which are defined as products formed by organic compounds, microorganisms, or mixtures of such substances that improve nutrition efficiency, abiotic stress tolerance, and crop quality traits regardless of nutrient content (Rouphael et al., 2018). According to Sigurnjak et al. (2017), using liquid biofertilizers has also shown encouraging results in several crops' diverse management and environmental situations, making agricultural systems more efficient, affordable, and sustainable. The activity of biostimulant/biofertilizer is adjusted by interacting parameters such as plant genotypes, growth circumstances, rates, and application period (Rouphael et al., 2018).

2.1.3. Leafy vegetable

2.1.3.1. Definition of leafy vegetables

Leafy vegetables are essential 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). Vegetables are necessary to the diet due to their nutritious properties. However, their consumption is far below the optimum level in many countries, necessitating the implementation of public initiatives to reverse this trend (Moura & Vialta, 2022). Most rural and urban households rely on vegetables to meet their daily dietary and nutritional needs, particularly vitamin A and iron (Chadha, 2006).

Among popular leafy vegetables, lettuce (*Lactuca sativa* L.) and mustard spinach (*Brassicca juncea*) are commonly used because they are essential sources of nutrients, fiber, 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).

2.1.3.2. Leafy vegetables production

Vegetable production is carried out by smallholder farmers/company using various production strategies. Usually, smallholder farmers plant vegetables in various ago-ecological zones ranging from low to mid altitudes, where they intercrop with other food crops. Different vegetables are generally grown in subsistence systems for local consumption, with the excess harvested sold on local or regional markets to generate revenue. These veggies are prepared in many ways depending on the people's traditions, using various recipes. 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). Recently, some market-oriented veggies have recently been rigorously maintained in the world.

2.1.4. Role of nutrient of leafy vegetables

2.1.4.1. Nitrogen

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, chemical symbol (N), atomic number 7. A colorless, odorless, tasteless gas, it makes up 78% of Earth’s atmosphere and is a constituent of all living matter. For most vegetables, approximately 2lb of available Nitrogen per 1,000 sq ft is adequate for early plant growth (Yousaf et al., 2021). Nitrogen (N) fertilizer is required for vegetable cultivation to provide enough yields and high quality

(Tilman et al., 2002; Zhang et al., 2015). N is regarded as one of the most important mineral elements for all living tissues of the plant, from metabolism to resource allocation, growth, and development (Crawford, 1995; Stitt & Krapp, 1999). Nitrogen administration promotes overall radish growth, yield, and quality (Khatri et al., 2019). However, high N fertilizer application rates not only reduced crop yields but also harmed crop quality (Chen et al., 2004). This common and ineffective practice undoubtedly contributes to environmental problems due to primarily nitrate (NO_3) loss in the ecosystem (Ji et al., 2006).

Some popular practices, such as intensive irrigation combined with high N fertilizer application rates, are thought to promote surface and ground water contamination via soil erosion and nitrate (NO_3) leaching (Gastal & Lemaire, 2002). Leafy vegetables have the ability to absorb a large amount of Nitrogenous fertilizer resulting in higher yield and quality. However, increasing the use of nitrate Nitrogen in alkaline soils effectively increases nitrate leaching. NO_3 is thought to play a significant role in gastric cancer, occurrence of methaemoglobinemia, and other disorders (Ishiwata et al., 2002; Ikemoto et al., 2002). As a result, nitrate accumulation in plants is a major worry and a widespread problem in most crops (Cárdenas-Navarro et al., 1999). The efficient use of N contributes lower input of N-fertilizer costs and lower nitrate level of contamination.

2.1.4.2. Phosphorus

Phosphorus (P) 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). P is restricting crop productivity estimating more than 30% of the world's arable land. Vance et al. (2003) forecasted that world supply of cheap phosphorus may be gone by 2050. Despite the presence of both inorganic and organic phosphorus forms in soils, the majority of phosphorus in soils is fixed, while plant accessible phosphorus is rare (Kumar et al., 2018). Furthermore, phosphorus has been linked to increased root growth, increased stalk and stem strength, improved flower formation and seed production, more uniform and earlier crop maturity, increased N-fixing capacity of legumes, crop quality improvements, and increased resistance to plant diseases (Kumar et al., 2018).

2.1.4.3. Potassium

Potassium (K+), along with Nitrogen (N) and phosphorus (P), is one of the essential plant nutrients required for development and physiology. K+ is a cation found in plants that accumulated ranging from 50 to 150 mM in liquid portions, cytoplasm, and vacuole. The concentration of K+ in the cytoplasm is typically constant

at around 50 mM, whereas the quantity in the vacuole can vary significantly. It is a structural component of plants, but it also serves as a regulator in numerous metabolic processes such as protein synthesis, glucose metabolism, and enzyme activation. K+ is required for many physiological activities, including stomatal control and photosynthesis (Perelman et al., 2022). Plants accumulate K+ before stress events such as water deprivation, lodging, cold stress, and salinity stress as a survival strategy. K+ is required in high concentrations within plants beginning with the vegetative growth phase. Extreme shock environmental events like as cold, frost, late season rains, salinity stress, and heat waves can be mitigated by high internal K+ concentrations. Protein structure and activity require high K+ concentrations in the cytosol for optimal plant function. Accumulation of K+ has been proven to prevent plant damage caused by osmotic stress and exceptional physical burden (Perelman et al., 2022). Therefore, the suitable usage of K+ in conjunction with other nutrients obtains stable productivity and quality of the plants, and ensuring nutritional food security for humans and animals.

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 countercation for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol (Marschner, 1995). Although calcium deficiency is uncommon in nature, high Ca limits plant populations on calcareous soils. The roots absorb calcium from the soil solution and transport it to the shoot via the xylem. It can go through the root via the cytoplasm of cells connected by plasmodesmata (the symplast) or the gaps between cells (the apoplast). The transfer of Ca across these pathways, however, must be delicately equaled in order for root cells to signal using cytosolic Ca^{2+} concentration ($[Ca^{2+}]cyt$), control the rate of Ca delivery to the xylem, and avoid harmful cation accumulating in the shoot (White & Broadley, 2003). In addition to metabolic and structural activities, it is crucial in salt detoxification in salty environments (Jin et al., 2007).

2.1.4.5. Magnesium

Magnesium (Mg) is known to be an essential nutrient for many living organisms, including plant species, animals, and humans. As a result, its deficiency may result in decreased productivity and quality in forestry (Mitchell et al., 1999) and agriculture (Hermans et al., 2004). According to Nguyen et al. (2016), magnesium is an essential component of chlorophyll, photosynthesis, enzyme activators, the creation of nucleic acids, carbohydrate metabolism, and increases phosphorus uptake and transport. In an experiment conducted by Tewari et al. (2006) showed that magnesium deficit effects

harmfully on mulberry plants. Maintaining adequate levels of magnesium in agricultural products is therefore a crucial task.

Magnesium is required in sufficient proportions, particularly at key periods of crop growth and development (Alva et al., 2006), as it is regarded as an essential nutrient for growth, productivity, and fruit quality (Nguyen et al., 2016). High agricultural productivity has been facilitated by reliance on the use of mineral fertilizer (Rathke et al., 2006). One of the enormous difficulties of the twenty-first century is managing agricultural nutrients to ensure a secure food supply and a healthy environment (Yousaf et al., 2021).

2.1.4.6. Sulphur

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). In the regulation of proteins and enzymes, sulfur also plays a role in the creation of disulfide bonds, particularly in redox control. Through glutathione and its derivatives, sulfur provides protection against oxidative damage (Aarabi et al., 2020). In addition, it is a component of a number of secondary metabolites (SMs) that are essential for the growth, development, and physiological processes of plants. The types of plantspecies and developmental stages have an impact on the sulfur requirements (Gohain et al., 2019). Brassicaceae crops require more S to obtain growth and yield optimally than other (Anjum et al., 2012).

The storage proteins cruciferin and napin, along with GSLs, are ultimately responsible for storing the majority of S in the mature seeds of oleiferous brassicas (Schatzki et al., 2014). The health of human, plants, and animals is impacted by the wide range of distinctive features of glucosinolates. The higher GSL concentrations, the higher pungency mustard stimulated (Borpatragohain et al., 2019). S, a primary form of sulfate that transported and stored in *Arabidopsis thaliana* (Brassicaceae), is primarily aborted in shoots rather than roots (Hawkesford & De Kok, 2006). Therefore, optimal agronomic approach in terms of S fertilizer use may alter ultimate brassicas production.

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). Chemical fertilizer overuse has been connected to soil degradation and environmental pollution (Phiri, 2010; Abdalla, 2014; Ali et al., 2018). The use of excessive amounts of chemical fertilizers has also been linked to nutritional

imbalances that promote insect and crop disease infestations and the growth of troublesome weeds (Sharma & Singhvi, 2017). Furthermore, due to the high cost of such fertilizers, subsistence and smallholder farmers in several developing nations may be unable to afford (Savci, 2012). Recently, there has been increased interest in using alternate, reliable, and safe natural sources for plant nutrients in order to achieve sustainable agricultural practices and enhance crop yield (Ali et al., 2018; Sharma & Singhvi, 2017; Abdel-Rahman et al., 2008; Jhilik et al., 2017). Therefore, attempts have been recorded to reduce the use of chemical fertilizer and boost nutrient usage efficiency, as well as to alleviate various biotic or abiotic stresses on plants through the use of plant biostimulants (Abdalla, 2014; Bulgari et al., 2019).

Plant biostimulants are a class of bioactive chemicals that can promote physiological processes, enhancing plant growth and economic yield (Jardin, 2015). Nonetheless, the continued use of commercially available synthetic biostimulants is typically costly (making them unaffordable to smallholder farmers) and less environmentally friendly (Pizzale et al., 2002). To achieve optimal crop development, a combination of natural-product-based biostimulants and lower amounts of chemical fertilizers has been proposed (Bulgari et al., 2015). Several novel natural biostimulants have recently been employed to boost crop growth and productivity.

Moringa oleifera, a species from the family Moringaceae, grows well in tropical and subtropical regions. Ogbe and Affiku (2011) investigated the nutrition of *M. oleifera* leaves and discovered a high carbohydrate (63.11% ±0.09) and crude protein (17.01% ±0.1) content. The leaves also contained considerable levels of ash (7.93% ± 0.12), crude fat (2.11% ±0.11), crude fibre (7.09% ±0.11), and fatty acid (1.69% ±0.09). In addition, mineral elements also detected such as Ca (1.91% ± 0.08), Fe (107.48 ± 8.2), K (0.97% ± 0.01), Na (192.95 ± 4.4), Zn (60.06 ± 0.3), Mn (81.65 ± 2.31), and P (30.15 ± 0.5), Magnesium (0.38% ± 0.01) and copper (6.10 ± 0.19). Verma and Nigam (2014) reported that stem of *M. oleifera* contains 45% fiber, 17 % protein, 15% carbohydrate, 11% fat, 9% moisture, 3% Ash, while stem contains 42% fiber, 19 % protein, 20% carbohydrate, 12% fat, 5% moisture, 12% Ash, and bark contains 29% fiber, 27 % protein, 14% carbohydrate, 19% fat, 9% moisture, 2% Ash. Beside the high nutritious value in the leaf, fruit and seed of *M. oleifera*, Zn, Fe, Ca, K, Na, Mg also detected in the bark (11.16, 22.53, 264.12, 259.83, 34.79, and 10.94, respectively), stem (13.22, 2.84, 125.49, 829.79, 19.61, and 12.95, respectively), and root (47.84; 5.04; 286.07; 860.59; 17.17, and 43.79, respectively) (all values are in ppm) (Verma and Nigam, 2014).

Moringa leaf extract (MLE), gained from *M. oleifera* Lam. is one such alternative crop being researched for its effect on crop growth and productivity under standard and stressful settings (Phiri & Mbewe, 2010) due to it generates natural

biostimulants (Jhilik et al., 2017; Khan et al., 2017). Environmentalists, academics, and scientists all over the world are interested in cultivating MLE as a biostimulant for agricultural purposes (Rady & Mohamed, 2015) because of its abundance of growth-promoting elements (Yasmeen et al., 2013; Sakr et al., 2018). MLE comes in a variety of forms, including aqueous extracts, pressured hot water extraction, and solvents. The potential of Moringa as an MLE contributing to the development of nutritious and safe meals through environmentally friendly and sustainable agricultural practices is critical in determining the nutritional advantages and market value of the leaf powder (Abdel-Rahman & Abdel-kader, 2020). Furthermore, increasing the development and productivity of food crops with safe natural biostimulants like MLE is critical in the present period (Elzaawely et al., 2017). Thus, instead of using chemical fertilizers, farmers can use natural biostimulants like MLE as a viable supplement or substitute for inorganic fertilizers to boost crop growth, yield, and quality.

MLE was used to improve seed germination, strong plant growth, and deeper root development, as well as delaying fruit senescence and increasing yield and product quality (Nasir et al., 2016). These benefits are attributed to moringa leaves' high concentration of phytochemicals. Other research using MLE as a growth stimulator reported increased yield and nutrient uptake in a variety of agronomic and horticultural crops (Mvumi et al., 2013; Pervez et al., 2017). It is crucial to note, however, that different plant species react differently to different MLE concentrations (McMahon et al., 2005). However, MLE application reacts variously to different crops. For example: Improving fresh and dry root mass, plant height, above-ground biomass, 1000-grain weight, and straw and grain yield in *Triticum aestivum* L. (Jhilik et al., 2017; Khan et al; 2017; Rehman et al., 2017; Khan et al. 2020); Increasing fresh and dry shoot mass, plant height, number of grains, grain mass/plant, and 100-grain mass in *Zea mays* L. (Mvumi et al., 2013; Biswas et al., 2016); Improving plant height, germination, biomass and grain yield in *Sorghum bicolor* L. (Phiri & Mbewe, 2010; Bashir et al., 2017); Increasing fruit size and sugar concentration in Glycine max L. (Foidl et al., 2001); Increasing vegetative growth, photosynthetic pigments, phytohormonal concentrations of leaves, dry shoot mass, and pod yield in *Phaseolus vulgaris* L. (Elzaawely et al., 2017; Mvumi et al., 2013); Improving germination and seedling survival in *Vigna unguiculata* (L) Walp. and *Arachis hypogaea* L. (Phiri, 2010); Increasing plant height, number of branches, leaf number, leaf lamina thickness, dry shoot biomass, dry root mass, stomatal size, stomatal density, fruit quality, and fruit yield in *Solanum lycopersicum* L. (Ngcobo & Bertling, 2021; Mvumi et al., 2018; Hoque et al., 2022); Reducing fruit drop, increasing fruit set, fruit size, and fruit yield in *Citrus nobilis* Lour × *C. deliciosa* Tenora (Nasir et al., 2016; Nasir et al., 2020); Increasing plant height and leaf number in *Manihot utilissima* Pohl.

(Ndubuaku et al., 2015); Increasing germination percentage, seedling emergence and survival, plant height, dry shoot mass, dry root mass, and yield in *Helianthus annuus* L. (Iqbal et al., 2020); Increasing plant growth and yield in *Basella alba* cv. Red Malabar (Hoque et al., 2022) and in *Ocimum basilicum* L. (Alkuwayti et al., 2020); Producing better growth and seed yield in *Cucurbita pepo* L. (Hegazi et al., 2015); Increased seed germination %, germination index, germination velocity, plant growth, fruit yield and fruit nutrient concentration in *Capsicum annuum* L. (Abou El-Nour & Ewais, 2017); Improving vegetative growth and yield characteristics in *Foeniculum vulgare* Mill. (Abdel-Rahman & Abdel-Kader, 2020); Increasing plant height, branch number, leaf area, overall plant biomass, volatile oil content and geraniol and citronellol in *Pelargonium graveolens* L. (Ali et al., 2018).

Thus, the production of nutritious and safe food through environmentally friendly and sustainable agricultural practices, such as MLE application, is critical in determining their nutritional and market value (Abdel-Rahman et al., 2020). Therefore, efforts should be undertaken to introduce MLE to small-scale (and even commercial) farmers.

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

The plant moringa originated in India, and it is currently grown in tropical and subtropical regions all over the world. India is the largest producer of moringa, with an annual production of 1.2 million tonnes of fruit from an area of 380 km^2 (Radovich, 2011). Estimation of global Moringa production in 2017 was 46 thousand units and up from around 32 thousand units the previous year (Statista[a]). The market size of moringa products was projected to reach 15 billion US dollars in 2028 (Fortune Business Insights, 2022), calling for the expansion of cultivation areas.

In South Africa, Moringa is planted in Limpopo, Gauteng, Pumalanga, KwaZulu-Natal, Free State and North West, but it is mainly grown in Limpopo Province by farmers at household level (Mashamaite et al., 2021). However, Moringa is produced on an area of about 0.25 ha that obtained seed yields of 50–100 kg/ha. Additionally, the estimation of annual enterprise income is about 13.000 USD and gross margin through selling moringa leaves is about 6.000 USD (Mabapa et al., 2017). Tshabalala and co-workers (2020) forecasted that 17% of South Africa's land area (200.837 km^2) having optimum conditions for Moringa plantation. Since moringa production in South Africa is still in its infancy and developing stage, it is challenging to estimate the area under production and the number of hectares for cultivation. Moringa is a perennial and multipurpose crop in India. It is mostly grown in the southern Indian states of

Tamil Nadu, Karnataka, Kerala, and Andhra Pradesh. Though perennial types have been known for a long time, their cultivation is beset with many production constraints (Ramachandran et al., 1980). Accounting of 80 percent of the Moringa exported worldwide was from India in 2015 (Statista[b]). In Ghana, high density planting (300,000-1 million plants ha-1) with either seeds or hardwood stem cuttings (30 cm to 1 m long) has been advised for maximum leaf output. Moringa is also being promoted in some communities for use in agroforestry systems (alley cropping). The crop's beneficial uses have been expanded to include grass-cutter feed and mineral fertilizer substitution in small-holder farms. According to anecdotal evidence and informal information, over 10,000 farmers utilize improved agronomic practices (Adu-Dapaah et al. 2017).

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, including Truong Sa Island district. However, the cultivation applied in Moringa production is primarily spontaneous, as opposed to a scientifically cultivated procedure. Therefore, exploitation of economic, nutritional and medicinal values of Moringa from these farming models has not been very effective and widespread. 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. Recently, in Dong Nai, Ho Chi Minh City had a number of households importing seeds from Thailand to grow as vegetables, nutritional powder, and tea bags. The cultivation is also spontaneous, there is no standard protocol is applied (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. It has a lot of rain (rainy season) falls in the months: May, June, July, August, September, October and November. Therefore, Moringa cannot grow perennially in here due to plants will be died after heavy rain and flooding. Farmers are not interested to grow Moringa due to they don’t have water logging variety and good quality of the seeds. Nguyen and co-

workers (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. Moringa oleifera breeding in the world and in Vietnam

2.2.2.1. Moringa oleifera breeding in the world

Moringa 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; Leone et al., 2015). Morphological diversity was also observed among drumstick landraces in Myanmar (Chan et al., 2018) and Ghana (Amoatey et al., 2012). Similarly, differences in leaf size, stem colours, tree shapes and heights were observed among the drumstick accessions from the South-Southeast of Mexico (Hernández et al., 2021) and India (Kurian et al., 2021). Gandji and co-workers (2019) also observed diversity in morphological traits of *M. oleifera* with changing climate and cultivation practice. Thus, these traits are influenced not only by genetic factors but also by environmental factors (Drisya et al., 2021; Ruiz-Hernández et al., 2022).

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 tolerate 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). Thus, it is critical to develop cultivars with high tolerance to waterlogged conditions, to expand drumstick cultivation areas. This has not been successfully addressed in the *M. oleifera* field of research. A potential approach to solve this problem is to obtain self-pollinated offspring from waterlogged tolerant drumstick plants and to keep selecting for waterlogging tolerant trait. Pure breeds can be obtained, which can then be outcrossed to create elite lines of *M. oleifera* that are tolerant to waterlogged conditions. However, breeding of Moringa for waterloging as well as high quality and high biomass yield is rarely reported. Lalas and Tasaknis (2002) had characterized “Periyakulum 1” (PKM 1) from India, a promising high-yielding line selected through pure line selection, for seed oil that contains high levels of β -sitosterol, stigmasterol, campesterol, α -, γ - and δ –tocopherols. In China, a Moringa breeding program is focused on identification of association of functionally diverse genes and important agronomical traits (Deng et al. 2016). Kumar and co-worker (2017) had improved the genetic resources for development of superior cultivars by assaying the genetic diversity among the advanced breeding lines.

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. However, in Vietnam, it grows naturally in the provinces Ninh Thuan, Binh Thuan, Dong Nai, and Kien Giang. Some regions have grown Moringa for commercial exploitation spontaneously, but not for breeding or scientific farming techniques. Low productivity, cultivation techniques, lack of the good quality seed and the output market are the main reasons for limitation of local drumstick production. Therefore, the economic and nutritional value of Moringa from these farmings is not very effective. Chau (2016) reported that low genetic diversity was observed in drumstick varieties that originated from Ninh Thuan, Binh Thuan, Dong Nai and Ba Ria Vung Tau provinces, whereas, high genetic diversity was detected among drumstick varieties that originated from Thailand. Dong Nai is the one province having high potential for cultivating Moringa as organic-oriented leafy vegetable using Ninh Thuan local varieties with density from 100 to 200 trees/m^2, which can be obtained high leaf productivity (29.3 - 30.8 tons/ha) and flavonoid and nutrient contents. 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%). More than 90% of agricultural land in the world is utilized to grow field crops. For foliar fertilizers in field crops, the Asia-Pacific and European regions held market shares of 40.2% and 33.8%, respectively. In 2021, South America had a share of 22.0%. Due to their simple delivery via foliar spraying methods, which also have superior nutrient uptake efficiency, the usage of foliar fertilizers is growing. The Asia-Pacific and South American regions dominated the usage of foliar fertilizers in horticulture crops in 2021, with market shares of 28.9% and 23.64%, respectively. The largest fertilizer consumption rates are found in the Asia-Pacific region, which includes countries like China and India and has a sizable area set aside for agricultural development. The results show that 73.0% of the world's land area was used for horticulture crop

farming. With 16.0% and 2.0% of the market share, respectively, Europe and North America were in second and third place (ResearchAndMarkets, 2023).

Biofertilizers made of free-living bacteria encourage plant development, increase productivity by fortifying roots, and minimize the need of synthetic fertilizer on crops. The usage of 95 genera and seven phyla of microorganisms as biofertilizers, or Plant Growth-Promoting Rhizobacteria (PGPR), is summarized along with its advantages, drawbacks, and prospects for the future. Through numerous trials conducted in a greenhouse and on a field, it was shown that the experimental biofertilizer produced was efficient. It increased the size of the roots, the number of crockets, the percentage of dry matter, and the yield of the crops. In comparison to conventional farming methods, the evaluations conducted on farmers' fields revealed a 30% increase in yield and a 21% drop in the cost of production per kilogram as a result of the application of biofertilizer plus 50% of the advised chemical fertilization. Through the deployment of this technology, farmers can decrease the usage of synthetic fertilizers while sustainably increasing agricultural productivity (Zambrano-Mendoza et al., 2021).

The usage of biofertilizers or microbial inoculants has significantly expanded over the past 20 years (Yadav et al., 2017). In order to raise crop output, improve and restore soil fertility, promote plant growth, lower production costs, and lessen the environmental effect associated with chemical fertilization; biofertilizers are viewed as an attractive and realistic biotechnological option (Vassilev et al., 2015; Ronga et al., 2019). Numerous microorganisms, such as Nitrogen-fixing soil bacteria (such as *Azotobacter* and *Rhizobium*), Nitrogen-fixing cyanobacteria (such as *Anabaena*), solubilizing phosphate bacteria (such as *Pseudomonas*), and arbuscular mycorrhic fungus, are frequently utilized as biofertilizers. Similar to this, cellulite-causing microbes and bacteria that produce phytohormones (such auxins) are utilized as biofertilizers (Umesha et al., 2018; Thomas & Singh, 2019). Additionally, the utilization of microorganisms can help plant growth under both typical and abiotic stress environments (Singh et al., 2018).

Biofertilizers as well as PGPR have been assessed in different crops such: rice, wheat, sugarcane, tobacco, tea, coffee, cotton, oats, corn, flax, beet, coconut, potato, fan cypress, grass sudan, carrots, cucumber, eggplant, pepper, tomato, lettuce, black pepper, alfalfa, alder, sorghum, pine, strawberries, green soybeans, peanut, beans, neem, and sunflower (García-Fraile, 2015). Of these, the soybean is the most significant example of the application and significance of biofertilizers in crop cultivation. *Bradyrhizobium* spp., which includes *Bradyrhizobium elkanii*, *Bradyrhizobium japonicum*, and *Bradyrhizobium diazoefficiens*, is mostly used to inoculate seeds for soybean production. There are over 70 businesses that make and

commercialize biofertilizers for this crop in Argentina, one of the major producers of soybeans in the world (Lodeiro, 2015).

The advantages of rice-Azolla relationship for rice cultivation in Cuba were assessed by Castro and co-workers (2002). The outcomes demonstrated that the use of Azolla had a favorable impact, allowing for an increase in the number of grains per panicle and panicle per m2, as well as a correspondingly large increase in yields. Additionally, it was noted that the association controlled the pH and temperature of the water. Grageda-Cabrera et al. (2018) assessed the impact of the inoculation of bacterial and fungal isolates on Nitrogen utilization efficiency (NUE) in wheat. In comparison to the non-inoculated treatment, the inoculation of wheat with arbuscular fungus considerably enhanced grain yield up to 1.291 kg ha^{-1}. To ascertain the impact of inoculation on growth and crop yield, the solubilizing phosphate bacteria *Pseudomonas putida*, *Pantoea agglomerans*, and *Microbacterium laevaniformans* were examined in potato. The combination of *P. putida* with P. *agglomerans* or *M. laevaniformans* significantly boosted biomass and enhanced tuber growth. The increased supply of phosphorus (P) from the bacteria to the developing plants may have contributed to the output. *P. agglomerans* considerably increased potato growth and yield by about 20–25% among the microorganisms (Malboobi et al., 2009). The use of growth promoters has an impact on tomato, just like it does on a number of horticulture crops. The inoculation of seedlings with *Burkholderia tropica* resulted in an efficient colonization of the roots that extended to aerial tissues, as demonstrated by Bernabeu et al. (2015). In two growing seasons, this efficient colonization increased tomato production. Mirik et al. (2008) also used Bacillus strains to test on pepper and found the yield inceased up to 23.5%.

Using the bacteria *Azospirillum brasilense* and *Rhizobium etli* as well as the fungus *Glomus intraradices* in cereals, legumes, and citrus, Garza et al. (2003) found that the response of annual and perennial crops to the application of biofertilizers in the central region of Mexico. In comparison to the non-inoculated treatment, production gains of up to 111% in oranges, 85% in wheat, 74% in barley, 60% in maize, 36% in beans, and 25% in oats (biomass) were observed.

2.2.3.2. Production and use of biofertilizer in Vietnam

A major part of Vietnam's agriculture, crop production has made significant strides in the past 30 years and is based on intensive farming practised with rising pesticide and fertilizer use. The negative effects on the environment, human health, and food safety are considered consequences. Globally, organic farming has gained popularity and is expanding quickly. Since 2012, the area of certified organic farming in Vietnam has increased. According to estimates, Vietnam's organic market generates

$132.15 million annually. The majority of Vietnamese organic products are exported to other countries. The government of Vietnam has made using organic fertilizer for agriculture one of its top goals. Although Vietnam has manufactured organic fertilizer in the past using a variety of materials and manufacturing techniques, its production capacity is insufficient to match the demand for organic farming. The Vietnamese government supports the development of organic fertilizer in Vietnam and encourages its application and manufacturing (Van Toan et al., 2019).

Biofertilizers, which are given to seeds or plants to encourage growth, are created in a lab using living or dormant cells of organisms, such as Nitrogen fixers, phosphate solubilizers, cellulites bacteria, and growth promoters. Biofertilizers, as opposed to synthetic fertilizers, contain microorganisms that don't produce nutrients on their own but instead assist plants to access nutrients that are present in the rhizosphere (Umesha et al., 2018).

In Vietnam, there are five main categories of microbial fertilizers: (1) microbial fertilizers for Nitrogen fixation; (2) microbial fertilizers for soluble phosphate; (3) mixed microbial fertilizers for soluble phosphate and Nitrogen fixation; (4) microbial fertilizers for organic matter decomposition; and (5) vesicular-arbuscular mycorrhizal fungi (VAM). Farmers utilize these biofertilizers in their fields because they effectively increase crop yields and quality. They are essential to the growth of Vietnamese agriculture. From 1980 to date, the government has invested in a National Biotechnological Programmed for Research and Development on Biofertilizers. This program involves more than ten research institutes and universities with about 100 researchers, including the NISF (National Institute for Soils and Fertilizers). At the moment technological production of microbial fertilizer in Vietnam has been researched and perfected for every crop and for some crop groups, Microbial preparations can be used as a type of general fertilizer or mixed with organic matter to create microbial organic fertilizer.

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.

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).

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). Besides, Moringa is mainly cultivated for its leaves which are consumed as a vegetable. Recently, aqueous extracts of different parts of moringa (leaves, seeds, and roots) have been used to produce agricultural products. Its aqueous extract reduces the reproduction and galling of root knot nematodes, and helps to improve plant growth and yield parameters of pea plants (Youssef & El-Nagdi, 2021). Previous studies demonstrated that moringa leaf extracts increase the growth and yield of various plants such as pepper (Matthew, 2016), tomatoes (Culver et al., 2012), and maize (Biswas et al., 2016).

Moringa leaf and seed extracts are also effective in extending the shelf-life of cut rose flowers (Hassan et al., 2020). The extract products derived from Moringa leaves help promote crops' growth and yield (Culver et al., 2012; Matthew 2016). Moringa leaf extract is extensively studied (Foidl et al., 2001; McMahon et al., 2005; Phiri & Mbewe, 2010; Mvumi et al., 2013; Ndubuaku et al., 2015; Hegazi et al., 2015; Biswas et al., 2016; Nasir et al., 2016; Elzaawely et al., 2017; Pervez et al., 2017; Bashir et al., 2017; Khan et al; 2017; Jhilik et al., 2017; Rehman et al., 2017; Mvumi et al., 2018; (Kanchani, 2019), Iqbal et al., 2020; Khan et al. 2020; Hoque et al., 2022; Alkuwayti et al., 2020; Abou El-Nour & Ewais, 2017; Ali et al. 2018; Abdel-Rahman and Abdel-Kader, 2020; Nasir et al., 2020; Ngcobo & Bertling, 2021; Hoque et al., 2022;), but the production of moringa biofertilizer using residues parts and its impact on vegetable growth still remained under-explored. 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).

2.2.4.2. The use of Moringa oleifera as fertilizer in Vietnam

All of the Moringa parts contain a high concentration of nutritionally important minerals and are free of toxic heavy metals, making them suitable for human and animal consumption (Verma & Nigam, 2014). 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 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. RESEARCH CONTENTS, MATERIALS AND METHODS

3.1. Research contents

- Selection of promising *Moringa 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 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, kindly provided by AVRDC - The World Vegetable Center (Truong et al., 2017). The parental plant was planted in 2015 and survived a historical flood in 2020 while all other accessions cultivated in the same area did not. The seeds were a result of self-pollination in 2020 and were matured in 2021. The seeds were sowed in pots containing a 1:1:1 mixture of sand, garden soil and commercial organic fertilizer. Drumstick seedlings were generated as described in AVRDC International Cooperators' Guide: Suggested Cultural Practices for Moringa (Palada & Chang, 2003). The germination rate was 82% and the survival rate was 93%. The seedlings (76 self-pollinated lines, SPLs) were placed in a net house for eight weeks before being transplanted to plastic pots (36 x 29 x 29 cm) containing 25 kg of alluvial soil, 20 g of N:P:K (30:30:30) and 150 g of Super Organic 3-2-2. Soil properties (Table 3.1) were measured as described in Ruíz-Valdiviezo and co-workers (2010). These materials were used for selection of waterlogging tolerance as well as biomass production for making foliar and Moringa organic fertilizers.

Table 3.1. *Characteristics of the soil used growing 76 M. oleifera self-pollinated lines*

Soil property	Values
Soil density (g/cm^3)	1.07
Absolute density (g/cm^3)	2.50
Porosity (%)	52.04
pH_{KCl}	5.70
Total N (%)	0.18
Available N (mg/100 g)	3.13
Total P (%)	0.42
Available P_2O_5 (mg/100 g)	45.40
Total K (%)	0.81
Available K_2O (mg/100 g)	35.24
OC (%)	3.20
Cu (mg/kg)	25.14
Pb (mg/kg)	0.22
Zn (mg/kg)	112.0

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, were used for primarily screening, evaluation and demonstration of Moringa foliar biofertilizer (MFB) and Moringa organic fertilizer (MOF) on leafy vegetables. The seeds were sowed in a 72-hole-plastic tray (hole size: W4.0 × L4.0 × H5.0 cm) that containing mixture consisted of sand, soil, rice husks and compost in the ratio of 1:3:1:1. The seedlings with three to four fully expanded true leaves were used for transplanting in experiments related to Moringa foliar biofertilizer (MFB) and Moringa organic fertilizer (MOF) evaluations.

Total of 200 UBC (University of British Columbia) RAPD primers (synthesized by Bioneer, Korea) were used to access genetic diversity of 76 *M. oleifera* self-pollinated lines.

Moringa residues (including stems, branches, and leaf petioles), 5 L of molasses and 0,2 kg of effective microorganism (EM) products were used for Moringa foliar biofertilizer (MFB) preparation.

Ground moringa residues, 50 kg of manure, 0.2 kg of Tricho–compost (Trichoderma–based product) and 2.0 kg of superphosphate (Lam Thao Fertilizers and Chemicals JSC) were used for Moringa organic fertilizer (MOF) preparation.

Seaweed organic foliar fertilizer that originated from Canada, and NPK foliar fertilizer that produced by Southern Fertilizer Joint Stock Company were used as control checks and sprayed as recommendation in primarily screening of Moringa foliar biofertilizer experiment.

Nitrate Magness foliar fertilizer, chemical fertilizers such Nitrogen (N), phosphorus pentoxide (P_2O_5), and potassium oxide (K_2O) were used as control checks and applied as factor's recommendation in the demonstration experiments.

The experiments were conducted from January 2019 to April 2023 at Institute of Biotechnology, Hue University (Hue, Vietnam).

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 as described by Abud-Archila and co-workers (2018). Each pot was watered with 10 L of water every day for twenty days. Growth parameters including leaf number, plant height (cm), stem circumference (cm), biomass yield (g), stem fresh yield (g), leaf fresh yield (g), leaf dry yield (g) and leaf dry matter (%) were measured. Colours were determined using the Methuen Handbook of Colours (Kornerup & Wanscher, 1978).

3.3.1.2. Genetic diversity analysis

3.3.1.2.1. 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). In particular, 0.5 g of leaves was washed and ground with a mortar and pestle in 500 µL of CTAB extraction buffer (100 mM Tris.HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% CTAB). The mixture was transferred to 1.5 mL tubes and incubated at 65°C for 30 minutes. Afterwards, an equal volume of chloroform:isoamyl alcohol (24:1) mixture was added and the mixture was shaken at 500 rpm for 30 minutes. The tubes were centrifuged at 17,000 *x g* for 10 minutes at 4°C and the aqueous phases (upper phases) were transferred to clean 1.5 mL tubes. Isopropanol (2/3 volume) was added, inverted to mix and the mixture was incubated in -20°C for

30 minutes to precipitate DNA. Genomic DNA was harvested by centrifugation (17,000 *x g* for 10 minutes at 4°C) and the pellets were washed with 500 µL of 70% ethanol (17,000 *x g* for 10 minutes at 4°C). DNA pellets were air-dried for 10 minutes on the bench to remove ethanol residues before being dissolved in 100 µL TE buffer (pH 7.5). DNA quality was examined by gel electrophoresis (1% agarose in 0.5 x TBE buffer). Genomic DNAs were either used directly or subjected to further purification using spin columns (DNeasy Plant mini kit, QIAGEN, Germany).

3.3.1.2.2. RAPD-PCR amplification

A total of 200 UBC RAPD primers (Bioneer, Korea) were used to pre-screen the parental plant (*P*) and three SPLs (33, 48 and 71). The primer pairs yielding polymorphism were then confirmed using five SPLs (33, 48, 71, 19 and 27) and *P*. The polymorphic UBC RAPD primers were used to genotype 76 SPLs. PCR reactions were carried out as described previously (Truong et al., 2013). Briefly, 15-µL PCR reactions contained 1x MyTaq DNA polymerase mix (Bioline-Meridian, UK), 0.67 µM of primers and 100 ng of genomic DNA. The thermocycling program included 94°C for 3 min, 40 cycles of amplification (94°C for 1 min, 37°C for 1 min and 72°C for 2 min), followed by a final extension at 72°C for 7 min. The PCR products were resolved by gel electrophoresis (2% agarose gel in 0.5 × TBE buffer) for 4 hours at 120 V, stained with SYBR Green I (Invitrogen, USA) and visualized under UV illumination.

3.3.1.2.3. 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). The PCR reactions were performed as above, in which thermocycling program included an initial denaturation at 94ºC for 5 minutes, 40 cycles of amplification (94°C for 1 minute, 50ºC for 45 sec and 72ºC for 2 minutes) and a final extension at 72ºC for 5 minutes. The PCR products were resolved by gel electrophoresis (2% agarose gel in 0.5 x TBE buffer), stained with SYBR Green I and visualized under UV illumination.

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) with modifications. Briefly, leaves were dried in an oven at 50ºC for 48 hours and then were ground with a mortar and pestle. Next, 50 mg of ground powder were extracted with 1 mL of 70% aqueous ethanol in 2 mL tubes and shaken (500 rpm) at 30ºC for 24 hours. Then, the tubes were centrifuged at 13,000 rpm for 5 minutes. The ethanol extract was diluted in 70% ethanol (20 µL of extract in 980 µL of 70% ethanol) and 0.2 mL of the diluted extract was added to 1.2 mL of MilliQ water in 2 mL tubes.

Folin–Ciocalteu's phenol reagent (0.1 mL) was added to the mixture, mixed and incubated for 5 minutes. Next, 0.3 mL of 20% Na_2CO_3 solution was added, followed by 0.2 mL of MilliQ water. After a 45-min incubation at room temperature, the absorbance was measured at 758 nm (Hitachi U-2910, Japan). Standards of gallic acid were prepared in 70% ethanol (20, 40, 60, 80, 100 and 120 mg/L). Total phenolic content of *M. oleifera* leaves was expressed as mg of gallic acid equivalents (GAE) per gram of dry weight. Results represent averages of three technical repeats.

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). In 2-mL tubes, 0.12 mL of diluted ethanol extract, 1.36 mL of 30% methanol, 0.06 mL of $NaNO_2$ (0.5 M) and 0.06 mL of $AlCl_3.6H_2O$ (0.3 M) were mixed. After 5 minutes, 40 µL of NaOH (1 M) was added to the mixture. The absorbance was measured at 506 nm (Hitachi U-2910, Japan). The standard curve was constructed using rutin standard solutions (100, 200, 300, 400 and 500 mg/L). The total flavonoid contents were expressed as milligrams of rutin equivalents per gram of dry weight. Results represent averages of three technical repeats.

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 dried stems, branches, and dried leaf petioles processed from grinder machine) 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). The extract was collected and filtered. The obtained fertilizer was kept in an airtight container.

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. Nutritional analysis of MFB: nitrogen was determined by the Kjeldahl method, phosphorus by the ammonium molybdate spectrometric method, potassium by the tetraphenylborate method, and pH by paper chromatography. The Walkley-Black method was applied to determine OC, and then OM was calculated as follows: OM (%) = OC (%) × 2.2.

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 (Table 3.2). MFB was sprayed every five days until five days before harvest with 100 mL of diluted MFB in 1 m^2. The experiment was designed in a Randomized Completely Block Design (RCBD) with five fertilizer doses and three replicates per treatment.

Table 3.2. *The experimental treatments*

Leafy vegetables	Treatment	Fertilizer doses*
Lettuce	1	MFB - 100 mL
	2	MFB - 50 mL
	3	MFB - 33.3 mL
	4	MFB - 25 mL
	5	MFB - 20 mL
	6	Seaweed organic fertilizer (0.5 g)
	7	NPK foliar fertilizer (1.25 g)
Mustard spinach	8	MFB - 100 mL
	9	MFB - 50 mL
	10	MFB - 33.3 mL
	11	MFB - 25 mL
	12	MFB - 20 mL
	13	Seaweed organic fertilizer (0.5 g)
	14	NPK foliar fertilizer (1.25 g)
Ceylon spinach	15	MFB - 100 mL
	16	MFB - 50 mL
	17	MFB - 33.3 mL
	18	MFB - 25 mL

19	MFB - 20 mL
20	Seaweed organic fertilizer (0.5 g)
21	NPK foliar fertilizer (1.25 g)

**diluted of water to a total volume of 1 liter*

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, 50, 33.3, 25, or 20 mL of MFB is diluted of water to a total volume of 1 liter (Nwokeji et al. 2022). MFB has sprayed every five days intervals until five days before harvest. 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 is diluted of water to a total volume of 1 liter), commercial chitosan fertilizer, seaweed fertilizer, and water (control). MFB has sprayed every five days intervals until five days before harvest. 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 kg of ground moringa residues, 50 kg of manure, 0.2 kg of Tricho–compost (Trichoderma–based product) and 2.0 kg 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^oC), 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 is diluted of water to a total volume of 1 liter) (Model 1). For control (Model 2), Nitrate Magness fertilizer was sprayed following manufacturer's recommendation (3.125 g in 1 Liter of water). Foliar fertilizerswere 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". The size of each band was estimated based on the molecular weight markers. 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) was the time taken from sowing to harvest. Growth parameters including 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 plant height (cm) was measured from the ground to the highest point of the leaves. The leaf area index is the multiplication of the number of plants/ground area (m^2) and the leaf area (m^2)/plant. 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 $p < 0.05$.

Vitamin C content assay: The vitamin C content assay was performed as previously described by Kareem et al. (2020) with modifications. Briefly, 5 g of lettuce and mustard leaves are finely ground in a porcelain mortar with 50 mL of sterile distilled water. Ground leaves were then transferred to a 50-mL Falcon tube and centrifuged at 13,000 rpm for 10 minutes. The supernatant (10 mL) was then transferred to a 250-mL conical flask containing 150 mL of distilled water and 1 mL of starch indicator solution (0.5%). Samples were titrated with a 5 mM iodine solution. The endpoint of the titration was identified as the first distinct trace of a dark blue-black color due to the starch-iodine complex. Results represent averages of three technical replicates.

Total nitrogen by the Kjeldahl method: Materials and method: Samples (Biofertilizer) 10–20 g/bottle, put in CuSO45H2O about 1g, added H2 SO4 about 50 mL, then put in the hot oven (for stylization by hitting 2 hours), prepared Machine Gerhard (Run analysis), added H3PO2 about 50 ml, put in bottle size 100 mL, added Methyl Red about 1–3 drops changes

color (light purple), then started at Gerhard titration. After that, look at the color change (if purple). That is no nitrogen and stop analysis. If there is a color difference, we can continue to the next step.

Phosphorus by the ammonium molybdate spectrometric method: Use sample Organic fertilizer, about 10 g, added sulfuric acid H2SO4 (about 50%). 5 mL/10 g, and cover with paper aluminum. added the water, still about 5 ml, then put it in the auto cave for about 121^{o}C and 30 minutes. Preparing the bottle by water still: bring water still 50 mL (put in bottle control and organic 5 mL/bottle), add organic 5 mL, and use (NH4)6HO6 about 5 mL (put in bottle sample and control). Preparing vitamin C: about 0.3g + 5 mL of water (use fitter paper). Preparing 1 mL of KH2PO4 + water is still 100 mL. After, put water in the bottle sample and control (20 ml/sample bottle), and after, put Vitamin C about 0.3g + 5 mL of water still (use fitter paper) about 2.5 mL–5 mL/bottle sample and control (look for the color blue and put water still). Test the Abs 710 computer with a spectrophotometer, U-2910, HITACHI, and record data.

Potassium by the tetraphenylborate method: The first prepared a sample of Organic fertilizer, about 1 g, added a solution of formalin, about 1 mL, and put in the water still 5 mL in the bottle. Adstran B is about 1 mL (use after mixing in Centerful for 10 minutes). Used fitter paper, put in 2-3 Phytanoyl, and added NaOH (looks purple). The solution of Natri tetraphenol borane was 35%. Preparing a new bottle for fitter paper Acid provides.

CHAPTER 4. RESULTS AND DISCUSSION

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

4.1.1. Morphology and waterlogging tolerance

At 40 days post transplantation, morphological variations were observed amongst 76 SPLs. As an example, young shoot colour varied from green, greenish purple, light purple to purple (Fig. 1A-D). Leaf number ranged from nine leaves (SPL 65) to 21 leaves (SPL 55) (Fig. 1E, red line). Plant heights varied between 36 cm (SPL 61) and 132 cm (SPL 10) (Fig. 1F, red upper edge). Stem circumferences varied between 3.4 cm (SPL 61) and 8.0 cm (SPL 23) (Fig. 1G, red upper edge). Furthermore, the number of leaves, plant height and stem circumference of self-pollinated line population were distributed normally (Fig. 2), thus these traits were likely to be regulated by multiple genes.

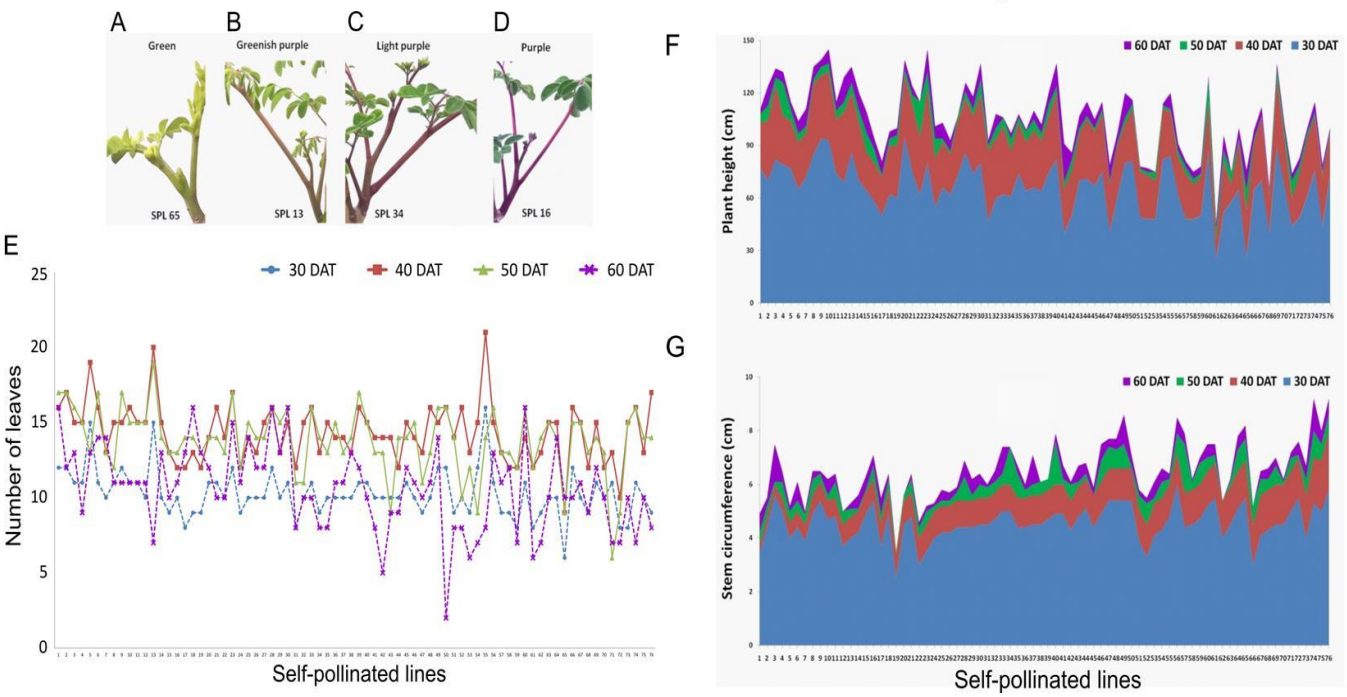

Figure 1. *Waterlogging tolerance of 76 M. oleifera self-pollinated lines (SPLs) at 40 days after transplanting. (A-D) Colour variation observed in young shoots of M. oleifera self-pollinated lines. (E-G) Growth parameters observed in M. oleifera SPLs following waterlogging treatment. (E) Number of leaves, (F) plant height and (G) stem circumference prior to waterlogging treatments (30 DAT and 40 DAT), 10 days (50 DAT) or 20 days (60 DAT) into the waterlogging treatment (60 DAT). DAT: days after transplanting.*

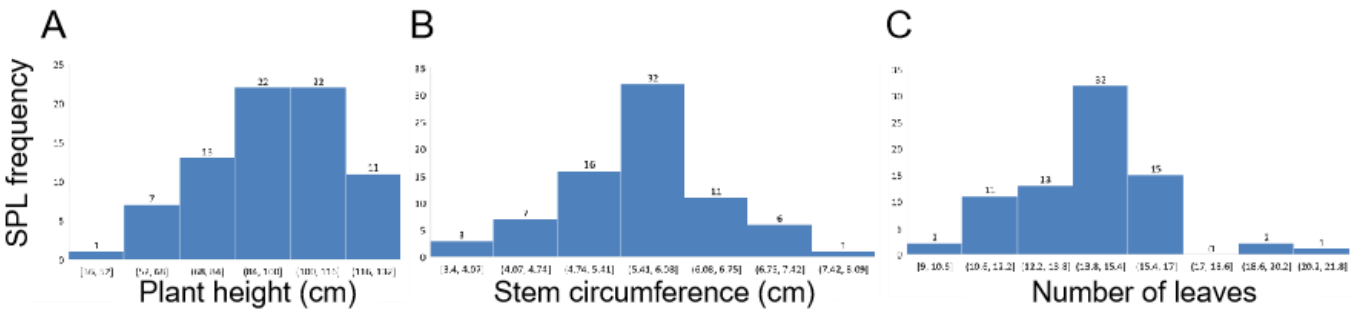

Figure 2. *Distribution of (A) plant height, (B) stem circumference and (C) number of leaves in 76 M. oleifera self-pollinated lines 40 days after transplantation.*

Waterlogging treatment was carried out for 20 days, during which the number of leaves, plant heights and stem circumferences were monitored. Ten days into the waterlogging treatment, *M. oleifera* leaves from most SPLs turned yellow (Fig. 3). Leaf dropping was observed in most SPLs at the end of the 20-day waterlogging treatment (Fig. 1E and Fig. 3C). Overall, leaf gain was observed in only three SPLs following the waterlogging treatment: 7, 18 and 65. Furthermore, the rates of plant height and stem circumference increase reduced during the waterlogging treatment (Fig. 1F-G). Taken together, these observations demonstrated poor tolerance of SPLs towards waterlogged conditions.

Figure 3. *Waterlogging treatment on Moringa oleifera self-pollinated lines. (A) Before, (B) 10 days into the waterlogging treatment or (C) at the end of the 20-day waterlogging treatment. (D) Differences in waterlogging tolerance ability amongst M. oleifera self-pollinated lines 10 days into the waterlogging treatment (at 50 days after transplanting).*

Following the 20-day waterlogging treatment, the drumstick biomasses were harvested by cutting at position of 55 cm from the soil surface. Variations in biomass yield, stem fresh yield, leaf fresh yield and leaf dry yield were observed among 76 SPLs (Fig. 4). The highest biomass yield and stem fresh yield were obtained in SPL 23 (220.3 g and 213.4 g, respectively), followed by SPL 1 (168.1 g and 138.3 g, respectively). The highest leaf fresh yield and leaf dry yield were found in SPL 24 (42.3 g and 11.1 g, respectively), followed by SPL 12 (41.5 g and 9.8 g, respectively). SPL 61 had the lowest biomass yield, stem fresh yield, leaf fresh yield and leaf dry yield (0.9 g, 0.8 g, 0.1 g and 0.02 g, respectively). Although the highest biomass yield and stem fresh yield were recorded in SPL 23, its leaf fresh yield was low (6.95 g), thus, the ratio of leaf fresh yield and biomass yield was only 3.15%. The highest leaf fresh yield and leaf dry yield were recorded in SPL 24, and the highest ratio of leaf fresh yield/biomass (34%).

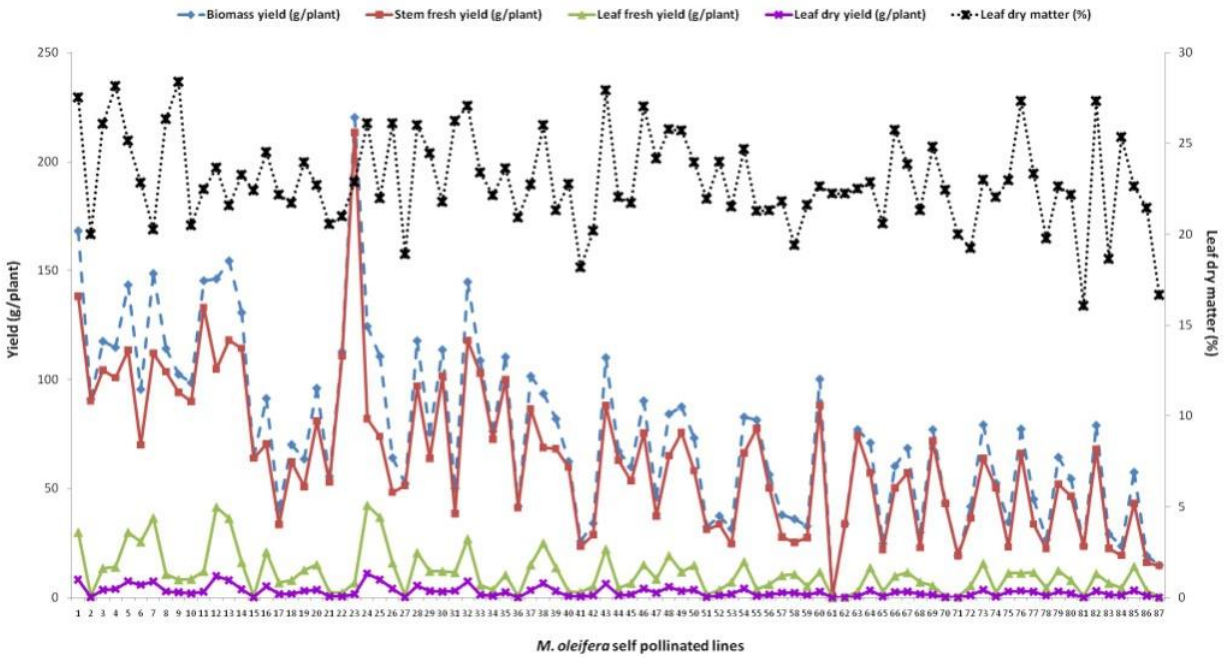

Figure 4. *Biomass yield, stem fresh yield, leaf fresh yield, leaf dry yield and leaf dry matter of Moringa oleifera self-pollinated lines following the waterlogging treatment.*

4.1.2. Genetic polymorphism

Polymorphism was screened on the parental plant and three randomly selected SPLs 33, 48 and 71, using a total of 200 UBC RAPD primers and 15 SRAP primer pairs. Of these, 17 UBC RAPD primers and eight SRAP primer pairs were found to yield polymorphism (Fig. 5A). When the screen was expanded to include SPLs 19 and 27, only seven UBC RAPD primers and three SRAP primer pairs yielded clear and stable polymorphic fragments (Fig. 5B, Table 4.1). These primers were then used to genotype the 76 *M. oleifera* self-pollinated lines and the parental plant (Fig. 5C).

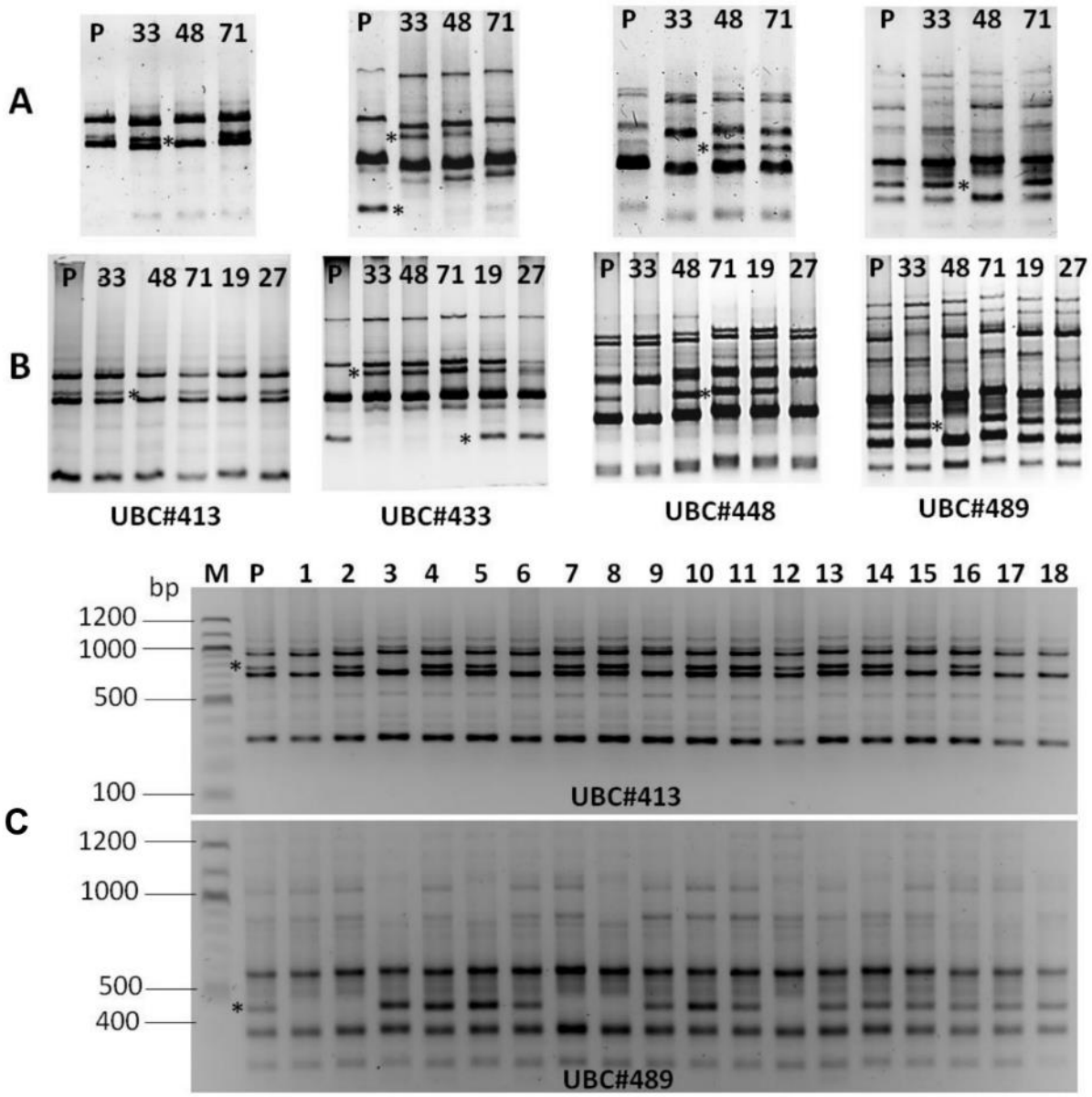

Figure 5. *Polymorphism within the M. oleifera parental (P) and self-pollinated lines shown by RAPD markers. (A) Three SPLs (33, 48 and 71) were randomly selected to screen for suitable primers in a collection of 200 UBC RAPD primers and 15 SRAP primer pairs. (B) The screen was expanded to include SPLs 19 and 27 to identify seven UBC RAPD primers and three SRAP primer pairs for polymorphic analyses. (C) PCR products obtained with RAPD UBC#413 and UBC#489 primers and DNA from the M. oleifera parental plant (P) and 18 self-pollinated lines. Products were resolved on 2% agarose gel. M, 100-bp molecular weight markers; asterisk denotes polymorphic bands.*

Table 4.1. *Sequence of primers used for characterising polymorphism in 76 M. oleiferaself-pollinated lines*

No.	Primername	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

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 displayed in Tables 4.2 and 4.3. A total of 92 bands were observed, with 25 bands being polymorphic (27%). The band sizes ranged from 300 to 1800 base pairs. Most primer pairs yielded low polymorphic band ratios, except UBC#350 and the pair me_1F/em_4R, both of which gave rise to a polymorphic rate of 50%. The pair me_2F/em_4R yielded the most polymorphic bands (6 bands, Table 4.3). In contrast, primer UBC#433 yielded the lowest rate of polymorphic band (10%). One characteristic band (450 bp), which appeared only in the PCR products of SPL 48 and not in others, was observed when primer UBC#368 was used. 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 (Table 4.2).

Table 4.2. *Number of PCR bands observed when genomic DNA of M. oleifera parental and self-pollinated lines were amplified using ten different primers/primer pairs*

No.	Line	UBC#							SRAP primer pair			Total
		350	368	413	433	437	448	489	me_1F/ em_4R	me_2F/ em_1R	me_2F/ em_4R	
1	*P*	4	7	9	10	9	7	11	4	7	10	78
2	SPL 1	4	7	8	10	9	7	10	4	7	10	76
3	SPL 2	5	7	9	9	9	6	10	4	7	10	76
4	SPL 3	5	7	8	10	9	6	9	4	7	11	76
5	SPL 4	5	7	9	10	9	7	11	4	7	10	79
6	SPL 5	8	7	9	10	9	7	9	4	7	11	81
7	SPL 6	4	7	8	9	9	7	11	4	7	10	76
8	SPL 7	6	7	9	10	9	7	10	4	7	11	80
9	SPL 8	6	7	9	10	9	6	8	4	7	11	77
10	SPL 9	4	7	8	10	9	6	11	4	6	11	76
11	SPL 10	5	7	9	10	9	7	11	4	7	11	80
12	SPL 11	5	7	9	10	9	7	11	4	7	11	80
13	SPL 12	4	7	9	10	9	6	9	4	7	11	76
14	SPL 13	6	7	9	9	9	7	11	4	7	10	79
15	SPL 14	4	7	9	9	9	6	10	4	7	10	75

No.	Line	UBC#							SRAP primer pair			Total
		350	368	413	433	437	448	489	me_1F/ em_4R	me_2F/ em_1R	me_2F/ em_4R	
16	SPL 15	4	7	8	10	9	7	11	4	7	11	78
17	SPL 16	4	7	9	9	9	7	10	4	7	10	76
18	SPL 17	4	7	8	10	9	7	10	4	7	10	76
19	SPL 18	4	7	8	10	9	6	11	4	7	10	76
20	SPL 19	4	7	8	10	9	7	11	4	7	10	77
21	SPL 20	6	7	8	10	9	7	9	4	7	10	77
22	SPL 21	4	7	9	10	9	6	9	4	7	10	75
23	SPL 22	4	7	8	10	9	6	10	4	7	10	75
24	SPL 23	4	7	9	9	9	7	10	4	7	11	77
25	SPL 24	4	7	8	9	9	7	11	4	7	10	76
26	SPL 25	5	7	9	10	9	6	11	4	7	11	79
27	SPL 26	4	7	9	9	9	7	11	4	7	11	78
28	SPL 27	4	7	9	10	9	6	10	4	7	11	77
29	SPL 28	4	7	9	10	9	7	10	4	7	11	78
30	SPL 29	4	7	8	10	9	6	11	4	7	10	76
31	SPL 30	4	7	9	10	9	7	10	4	7	10	77
32	SPL 31	4	7	9	10	9	7	10	4	7	11	78

No.	Line	UBC#							SRAP primer pair			Total
		350	368	413	433	437	448	489	me_1F/ em_4R	me_2F/ em_1R	me_2F/ em_4R	
33	SPL 32	4	7	9	9	9	7	9	4	7	11	76
34	SPL 33	4	7	9	9	9	6	10	4	7	11	76
35	SPL 34	7	7	8	10	9	7	11	4	7	11	81
36	SPL 35	4	7	9	10	9	7	9	4	7	10	76
37	SPL 36	4	7	9	10	9	7	10	4	7	10	77
38	SPL 37	4	7	9	10	9	7	10	4	7	11	78
39	SPL 38	4	7	9	10	9	7	11	4	7	10	78
40	SPL 39	6	7	9	10	9	7	9	4	7	11	79
41	SPL 40	6	7	9	10	9	7	11	4	7	11	81
42	SPL 41	4	7	9	9	9	7	10	4	7	11	77
43	SPL 42	4	7	9	10	10	7	11	4	7	12	81
44	SPL 43	8	7	9	9	9	6	10	4	7	11	80
45	SPL 44	4	7	8	10	9	6	11	4	7	10	76
46	SPL 45	4	7	9	10	9	7	11	4	7	11	79
47	SPL 46	4	7	8	9	9	6	10	4	7	11	75
48	SPL 47	4	7	8	9	9	7	11	4	7	11	77
49	SPL 48	4	8	8	10	9	7	9	4	7	11	77

No.	Line	UBC#							SRAP primer pair			Total
		350	368	413	433	437	448	489	me_1F/ em_4R	me_2F/ em_1R	me_2F/ em_4R	
50	SPL 49	4	7	9	10	9	7	10	4	7	12	79
51	SPL 50	4	7	9	10	9	7	10	4	7	11	78
52	SPL 51	4	7	9	10	9	7	10	4	7	10	77
53	SPL 52	4	7	8	10	9	7	10	4	7	11	77
54	SPL 53	4	7	8	10	10	7	11	4	7	11	79
55	SPL 54	4	7	9	9	9	7	9	4	7	11	76
56	SPL 55	4	7	9	9	9	6	11	4	7	11	77
57	SPL 56	4	7	9	9	9	7	11	4	7	11	78
58	SPL 57	4	7	9	10	10	7	11	4	7	11	80
59	SPL 58	4	7	9	10	9	7	9	4	7	11	77
60	SPL 59	4	7	9	10	9	7	11	4	7	12	80
61	SPL 60	4	7	9	10	9	7	9	4	7	10	76
62	SPL 61	4	7	8	10	9	7	11	4	7	11	78
63	SPL 62	4	7	9	10	9	7	10	4	7	11	78
64	SPL 63	5	7	9	10	9	6	11	4	7	10	78
65	SPL 64	4	7	9	9	9	7	11	4	7	11	78
66	SPL 65	5	7	9	10	9	6	10	4	7	10	77

No.	Line	UBC#							SRAP primer pair			Total
		350	368	413	433	437	448	489	me_1F/ em_4R	me_2F/ em_1R	me_2F/ em_4R	
67	SPL 66	5	7	9	10	9	7	9	4	7	12	79
68	SPL 67	4	7	8	10	9	6	10	4	7	12	77
69	SPL 68	5	7	9	10	9	7	11	4	7	10	79
70	SPL 69	5	7	9	10	10	7	10	4	7	10	79
71	SPL 70	4	7	9	10	9	6	9	4	7	11	76
72	SPL 71	7	7	9	10	9	7	11	4	7	12	83
73	SPL 72	5	7	9	10	9	7	10	4	7	11	79
74	SPL 73	4	7	9	10	10	7	9	4	7	10	77
75	SPL 74	4	7	9	10	9	6	8	4	7	11	75
76	SPL 75	4	7	9	10	9	6	11	4	7	11	78
77	SPL 76	4	7	9	10	9	7	11	4	7	10	78
Total		346	540	672	752	698	516	784	308	538	824	5978

Table 4.3. *Polymorphic analysis of the M. oleifera self-pollinated lines based on PCR products obtained withten primers/primer pairs*

Number of bands	Number of polymorphic bands	Primer/ Primer pair	Percentage of polymorphic bands (%)	Size (bp)
8	4	UBC#350	50.0	570 - 1517
8	1	UBC#368	12.5	450 - 1550
9	1	UBC#413	11.1	300 - 1150
10	1	UBC#433	10.0	300 - 1517
10	2	UBC#437	20.0	450 - 1300
7	1	UBC#448	14.3	400 - 1250
11	3	UBC#489	27.3	300 - 1500
6	3	me_1F and em_4R	50.0	320 - 1800
9	3	me_2F and em_1R	33.3	450 - 1800
14	6	me_2F and em_4R	42.9	300 - 1700
92	25	Total	27.2	300 - 1800

4.1.4. Genetic diversity analysis

POPGENE (version 1.32) was employed to determine the genetic diversity indices. The number of expected alleles, the number of effective alleles, Nei's gene diversity (*h*) and Shannon's information index (*I*) were found to be 1.2609, 1.1358, 0.0791 and 0.1200 respectively (Table 4.4). These figures indicated that the self-pollinated lines were quite diverse genetically. Genetically, the parental and 76 self-pollinated lines were separated into five major groups: group I included SPL 5 and SPL 43, having a similarity coefficient of 0.80 (Fig. 6). 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 (Table 4.5).

Table 4.4. *Genetic diversity indices of Moringa oleifera self-pollinated lines*

Indices	Number of expected alleles	Number of effective alleles	Nei's gene diversity (*h*)	Shannon's information index (*I*)
	1.2609	1.1358	0.0791	0.1200
Standard error	0.4415	0.2951	0.1590	0.2301

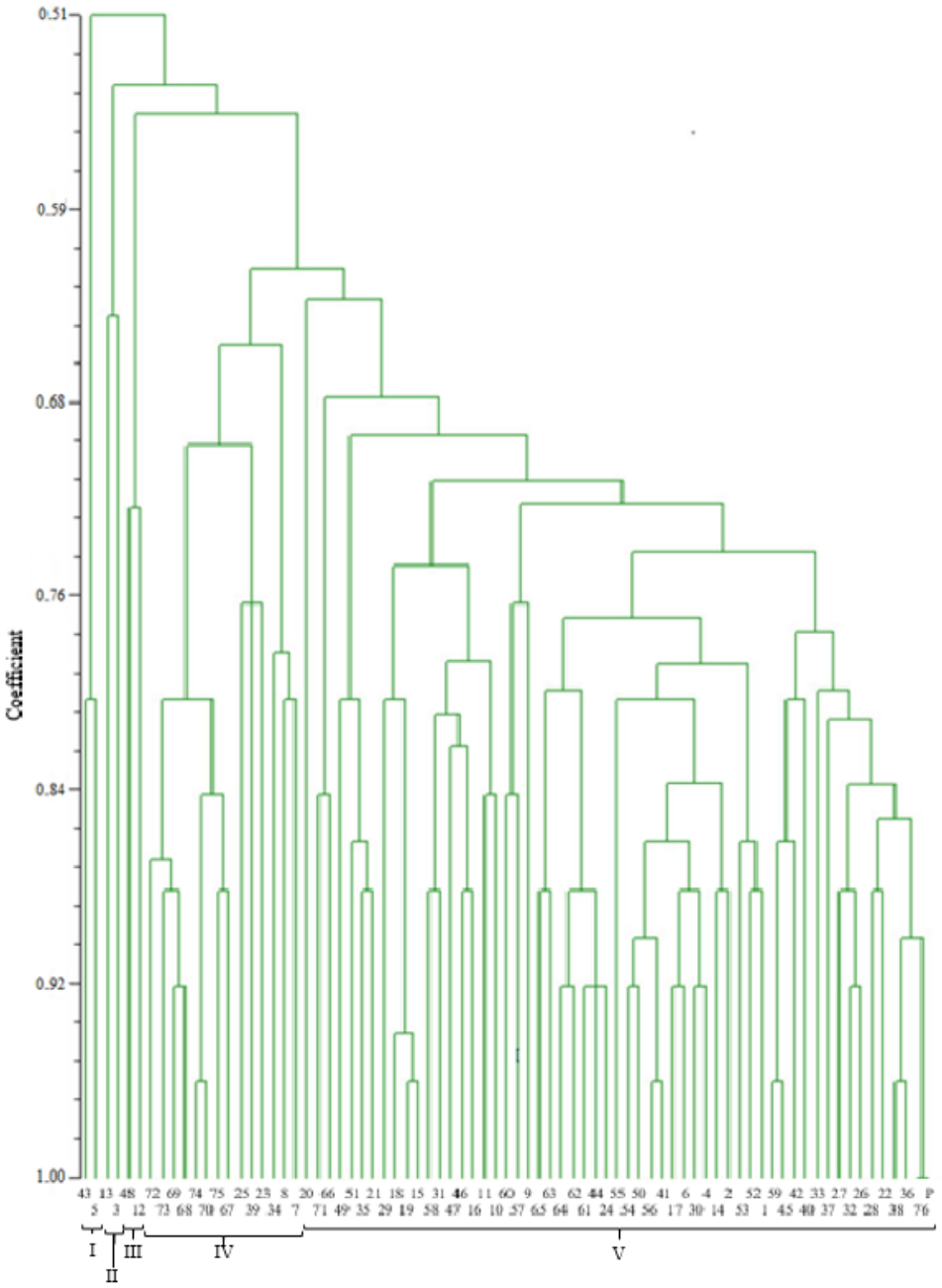

Figure 6. *Dendrogram showing the genetic relationship between the Moringa oleifera parental (P) and 76 self-pollinated lines (SPLs).*

Table 4.5. *Genetic distance between 77 individuals of M. oleifera (parent and 76 SPLs)*

pop ID	P	SPL 1	SPL 2	SPL 3	SPL 4	SPL 5	SPL 6	SPL 7	SPL 8	SPL 9	SPL 10	SPL 11	SPL 12	SPL 13	SPL 14	SPL 15	SPL 16	SPL 17	SPL 18	SPL 19	SPL 20	SPL 21	SPL 22	SPL 23	SPL 24	SPL 25	SPL 26	SPL 27	SPL 28	SPL 29	SPL 30	SPL 31	SPL 32	SPL 33	SPL 34	SPL 35	SPL 36	SPL 37	SPL 38	SPL 39	SPL 40	SPL 41	SPL 42	SPL 43	SPL 44	SPL 45	SPL 46	SPL 47	SPL 48	SPL 49	SPL 50	SPL 51	SPL 52	SPL 53	SPL 54	SPL 55	SPL 56	SPL 57	SPL 58	SPL 59	SPL 60	**SPL 61**SPL 62 SPL 63 SPL 64 SPL 65 SPL 66 SPL 67 SPL 68 SPL 69 SPL 70 SPL 71 SPL 72 SPL 73 SPL 74 SPL 75 SPL 76
P	1,00																																																													
SPL 1	0,68	1,00																																																											
SPL 2	0,68	0,76	1,00																																																									
SPL 3	0,48	0,48	0,40	1,00																																																							
SPL 4	0,80	0,80	0,88	0,44	1,00																																																					
SPL 5	0,56	0,40	0,48	0,44	0,60	1,00																																																			
SPL 6	0,76	0,84	0,84	0,40	0,88	0,48	1,00																																																	
SPL 7	0,60	0,68	0,52	0,48	0,56	0,56	0,52	1,00																																															
SPL 8	0,56	0,64	0,56	0,52	0,52	0,52	0,48	0,80	1,00																																													
SPL 9	0,72	0,64	0,64	0,68	0,68	0,36	0,72	0,40	0,44	1,00																																												
SPL 10	0,76	0,68	0,68	0,32	0,80	0,56	0,76	0,68	0,64	0,64	1,00																																											
SPL 11	0,76	0,68	0,76	0,48	0,88	0,48	0,76	0,52	0,48	0,72	0,84	1,00																																										
SPL 12	0,60	0,52	0,52	0,56	0,48	0,48	0,44	0,52	0,48	0,56	0,52	0,60	1,00																																									
SPL 13	0,68	0,60	0,60	0,64	0,64	0,40	0,60	0,60	0,56	0,64	0,60	0,60	0,36	1,00																																								
SPL 14	0,72	0,72	0,88	0,44	0,84	0,52	0,88	0,48	0,60	0,68	0,72	0,72	0,56	0,56	1,00																																							
SPL 15	0,68	0,76	0,60	0,48	0,72	0,32	0,76	0,52	0,48	0,72	0,76	0,84	0,68	0,52	0,64	1,00																																						
SPL 16	0,76	0,68	0,76	0,40	0,80	0,48	0,84	0,52	0,56	0,64	0,84	0,84	0,52	0,60	0,80	0,76	1,00																																					
SPL 17	0,76	0,84	0,76	0,48	0,88	0,56	0,92	0,52	0,56	0,72	0,76	0,76	0,44	0,52	0,80	0,76	0,84	1,00																																				
SPL 18	0,68	0,76	0,68	0,48	0,72	0,32	0,76	0,52	0,56	0,72	0,76	0,76	0,68	0,52	0,72	0,92	0,76	0,76	1,00																																			
SPL 19	0,72	0,80	0,64	0,44	0,76	0,36	0,80	0,56	0,52	0,68	0,80	0,80	0,64	0,56	0,68	0,96	0,80	0,80	0,96	1,00																																		
SPL 20	0,72	0,56	0,48	0,44	0,60	0,68	0,64	0,48	0,60	0,60	0,72	0,64	0,56	0,48	0,60	0,64	0,72	0,72	0,64	0,68	1,00																																	
SPL 21	0,64	0,56	0,64	0,60	0,68	0,60	0,64	0,56	0,60	0,60	0,72	0,72	0,72	0,40	0,76	0,64	0,80	0,72	0,72	0,68	0,68	1,00																																
SPL 22	0,80	0,72	0,72	0,52	0,76	0,60	0,80	0,40	0,52	0,76	0,64	0,64	0,64	0,48	0,84	0,64	0,64	0,80	0,72	0,68	0,76	0,68	1,00																															
SPL 23	0,72	0,72	0,64	0,44	0,68	0,60	0,72	0,56	0,60	0,52	0,64	0,56	0,48	0,64	0,68	0,56	0,72	0,72	0,56	0,60	0,60	0,52	0,68	1,00																														
SPL 24	0,84	0,76	0,76	0,48	0,80	0,40	0,92	0,60	0,56	0,72	0,76	0,76	0,44	0,68	0,80	0,76	0,84	0,84	0,76	0,80	0,64	0,64	0,72	0,64	1,00																													
SPL 25	0,64	0,80	0,72	0,44	0,76	0,52	0,72	0,72	0,76	0,60	0,80	0,64	0,48	0,64	0,76	0,64	0,64	0,72	0,72	0,68	0,52	0,60	0,68	0,76	0,64	1,00																												
SPL 26	0,84	0,68	0,76	0,40	0,80	0,56	0,84	0,44	0,40	0,72	0,68	0,76	0,60	0,60	0,80	0,68	0,76	0,76	0,60	0,64	0,64	0,56	0,80	0,80	0,76	0,64	1,00																											
SPL 27	0,80	0,64	0,72	0,52	0,76	0,60	0,72	0,40	0,52	0,76	0,64	0,72	0,64	0,48	0,76	0,64	0,72	0,80	0,64	0,60	0,68	0,68	0,84	0,76	0,64	0,68	0,88	1,00																										
SPL 28	0,84	0,68	0,68	0,40	0,80	0,72	0,76	0,52	0,56	0,64	0,76	0,68	0,60	0,52	0,80	0,60	0,68	0,76	0,60	0,64	0,72	0,64	0,88	0,80	0,68	0,72	0,84	0,80	1,00																									
SPL 29	0,76	0,76	0,60	0,56	0,64	0,40	0,68	0,52	0,56	0,72	0,68	0,68	0,68	0,60	0,64	0,76	0,68	0,68	0,84	0,80	0,72	0,64	0,80	0,72	0,68	0,72	0,68	0,72	0,68	1,00																								
SPL 30	0,80	0,80	0,80	0,44	0,92	0,60	0,88	0,56	0,60	0,68	0,80	0,80	0,56	0,56	0,92	0,72	0,80	0,88	0,72	0,76	0,68	0,76	0,84	0,68	0,80	0,76	0,80	0,76	0,88	0,64	1,00																							
SPL 31	0,84	0,60	0,60	0,48	0,72	0,56	0,68	0,44	0,48	0,72	0,76	0,84	0,68	0,52	0,64	0,76	0,84	0,76	0,68	0,72	0,80	0,72	0,72	0,72	0,68	0,56	0,84	0,88	0,76	0,76	0,72	1,00																						
SPL 32	0,76	0,60	0,68	0,48	0,72	0,64	0,76	0,36	0,48	0,64	0,60	0,68	0,60	0,52	0,80	0,60	0,76	0,76	0,52	0,56	0,72	0,64	0,80	0,80	0,68	0,56	0,92	0,88	0,84	0,60	0,80	0,84	1,00																					
SPL 33	0,84	0,52	0,68	0,56	0,64	0,48	0,68	0,44	0,56	0,72	0,60	0,68	0,68	0,60	0,80	0,60	0,68	0,60	0,60	0,56	0,64	0,64	0,80	0,64	0,76	0,56	0,84	0,80	0,76	0,68	0,72	0,76	0,84	1,00																				
SPL 34	0,64	0,72	0,48	0,44	0,60	0,52	0,64	0,80	0,76	0,52	0,72	0,56	0,32	0,64	0,52	0,64	0,56	0,64	0,64	0,68	0,60	0,44	0,52	0,60	0,72	0,76	0,48	0,44	0,56	0,64	0,60	0,48	0,40	0,48	1,00																			
SPL 35	0,68	0,68	0,68	0,56	0,80	0,72	0,76	0,60	0,56	0,56	0,68	0,68	0,60	0,44	0,80	0,60	0,76	0,84	0,60	0,64	0,64	0,88	0,72	0,64	0,68	0,64	0,68	0,72	0,76	0,52	0,88	0,68	0,76	0,60	0,48	1,00																		
SPL 36	0,88	0,72	0,72	0,44	0,84	0,68	0,80	0,48	0,52	0,68	0,72	0,72	0,56	0,56	0,76	0,64	0,80	0,88	0,64	0,68	0,76	0,68	0,84	0,84	0,72	0,68	0,88	0,92	0,88	0,72	0,84	0,88	0,88	0,72	0,52	0,80	1,00																	
SPL 37	0,76	0,60	0,60	0,56	0,72	0,72	0,68	0,52	0,40	0,64	0,60	0,68	0,76	0,44	0,72	0,60	0,60	0,68	0,52	0,56	0,64	0,72	0,80	0,64	0,60	0,56	0,84	0,80	0,84	0,60	0,80	0,76	0,84	0,76	0,40	0,84	0,80	1,00																
SPL 38	0,92	0,76	0,76	0,40	0,88	0,64	0,84	0,52	0,48	0,72	0,76	0,76	0,60	0,60	0,80	0,68	0,76	0,84	0,68	0,72	0,72	0,64	0,88	0,80	0,76	0,72	0,92	0,88	0,92	0,76	0,88	0,84	0,84	0,76	0,56	0,76	0,96	0,84	1,00															
SPL 39	0,64	0,72	0,56	0,36	0,60	0,68	0,56	0,72	0,76	0,44	0,64	0,48	0,56	0,56	0,60	0,48	0,48	0,56	0,48	0,52	0,68	0,44	0,68	0,76	0,48	0,76	0,64	0,60	0,80	0,64	0,68	0,56	0,64	0,56	0,68	0,56	0,68	0,64	0,72	1,00														
SPL 40	0,80	0,64	0,72	0,36	0,84	0,76	0,72	0,56	0,52	0,60	0,80	0,72	0,48	0,64	0,68	0,56	0,64	0,72	0,56	0,60	0,68	0,52	0,76	0,76	0,64	0,76	0,80	0,76	0,88	0,64	0,76	0,72	0,72	0,64	0,60	0,64	0,84	0,72	0,88	0,76	1,00													
SPL 41	0,72	0,80	0,88	0,36	0,84	0,44	0,88	0,56	0,52	0,68	0,72	0,80	0,56	0,56	0,84	0,72	0,80	0,80	0,64	0,68	0,52	0,60	0,68	0,68	0,80	0,68	0,88	0,76	0,72	0,56	0,84	0,72	0,80	0,72	0,52	0,72	0,76	0,72	0,80	0,60	0,68	1,00												
SPL 42	0,72	0,72	0,72	0,44	0,84	0,60	0,80	0,64	0,52	0,68	0,80	0,80	0,56	0,48	0,76	0,72	0,72	0,80	0,64	0,68	0,52	0,68	0,68	0,68	0,72	0,76	0,80	0,76	0,80	0,56	0,84	0,72	0,72	0,64	0,60	0,80	0,76	0,80	0,80	0,60	0,76	0,84	1,00											
SPL 43	0,44	0,44	0,68	0,40	0,64	0,80	0,60	0,60	0,56	0,40	0,60	0,52	0,44	0,44	0,72	0,36	0,52	0,52	0,44	0,40	0,48	0,64	0,56	0,48	0,52	0,64	0,52	0,48	0,60	0,36	0,64	0,36	0,52	0,52	0,56	0,68	0,48	0,60	0,52	0,56	0,64	0,56	0,64	1,00										
SPL 44	0,84	0,76	0,76	0,56	0,80	0,40	0,84	0,60	0,64	0,80	0,76	0,76	0,52	0,60	0,80	0,76	0,76	0,84	0,84	0,80	0,64	0,72	0,80	0,56	0,92	0,72	0,68	0,72	0,68	0,76	0,80	0,68	0,60	0,76	0,72	0,68	0,72	0,60	0,76	0,48	0,64	0,72	0,72	0,52	1,00									

pop ID	P	SPL 1	SPL 2	SPL 3	SPL 4	SPL 5	SPL 6	SPL 7	SPL 8	SPL 9	SPL 10	SPL 11	SPL 12	SPL 13	SPL 14	SPL 15	SPL 16	SPL 17	SPL 18	SPL 19	SPL 20	SPL 21	SPL 22	SPL 23	SPL 24	SPL 25	SPL 26	SPL 27	SPL 28	SPL 29	SPL 30	SPL 31	SPL 32	SPL 33	SPL 34	SPL 35	SPL 36	SPL 37	SPL 38	SPL 39	SPL 40	SPL 41	SPL 42	SPL 43	SPL 44	SPL 45	SPL 46	SPL 47	SPL 48	SPL 49	SPL 50	SPL 51	SPL 52	SPL 53	SPL 54	SPL 55	SPL 56	SPL 57	SPL 58	SPL 59	SPL 60	**SPL 61**SPL 62 SPL 63 SPL 64 SPL 65 SPL 66 SPL 67 SPL 68 SPL 69 SPL 70 SPL 71 SPL 72 SPL 73 SPL 74 SPL 75 SPL 76
SPL 45	0,80	0,72	0,72	0,44	0,84	0,60	0,80	0,56	0,52	0,76	0,80	0,72	0,48	0,64	0,76	0,64	0,72	0,80	0,64	0,68	0,60	0,60	0,76	0,76	0,72	0,76	0,80	0,76	0,88	0,64	0,84	0,72	0,72	0,64	0,60	0,72	0,84	0,72	0,88	0,68	0,84	0,76	0,84	0,56	0,72	1,00																
SPL 46	0,64	0,64	0,72	0,52	0,68	0,36	0,80	0,40	0,52	0,76	0,72	0,80	0,56	0,48	0,76	0,80	0,88	0,80	0,80	0,76	0,68	0,76	0,68	0,60	0,80	0,60	0,72	0,76	0,56	0,72	0,68	0,80	0,72	0,72	0,52	0,64	0,68	0,56	0,64	0,36	0,52	0,76	0,68	0,48	0,80	0,60	1,00															
SPL 47	0,80	0,64	0,64	0,44	0,68	0,44	0,80	0,40	0,36	0,76	0,72	0,80	0,64	0,56	0,68	0,80	0,80	0,72	0,72	0,76	0,76	0,60	0,76	0,68	0,80	0,52	0,88	0,76	0,72	0,80	0,68	0,88	0,80	0,80	0,52	0,56	0,76	0,72	0,80	0,52	0,68	0,76	0,68	0,40	0,72	0,68	0,84	1,00														
SPL 48	0,64	0,56	0,40	0,60	0,44	0,44	0,48	0,56	0,44	0,52	0,40	0,48	0,72	0,40	0,36	0,64	0,48	0,56	0,56	0,60	0,52	0,52	0,52	0,52	0,56	0,36	0,56	0,60	0,48	0,56	0,44	0,64	0,56	0,56	0,44	0,56	0,60	0,64	0,56	0,44	0,44	0,52	0,52	0,24	0,56	0,44	0,52	0,60	1,00													
SPL 49	0,72	0,56	0,56	0,60	0,68	0,60	0,64	0,72	0,60	0,60	0,72	0,72	0,64	0,48	0,68	0,64	0,64	0,64	0,56	0,60	0,52	0,76	0,60	0,52	0,72	0,60	0,64	0,60	0,72	0,48	0,76	0,64	0,64	0,72	0,60	0,80	0,60	0,80	0,64	0,52	0,60	0,68	0,84	0,64	0,72	0,68	0,60	0,60	0,60	1,00												
SPL 50	0,76	0,76	0,76	0,48	0,88	0,56	0,84	0,52	0,56	0,72	0,76	0,84	0,52	0,52	0,80	0,76	0,84	0,92	0,68	0,72	0,64	0,72	0,72	0,72	0,76	0,72	0,84	0,88	0,76	0,60	0,88	0,84	0,84	0,68	0,56	0,84	0,88	0,76	0,84	0,56	0,72	0,88	0,88	0,52	0,76	0,80	0,80	0,72	0,56	0,72	1,00											
SPL 51	0,80	0,64	0,64	0,60	0,76	0,60	0,72	0,72	0,60	0,60	0,72	0,72	0,56	0,56	0,68	0,64	0,80	0,80	0,64	0,68	0,60	0,84	0,60	0,60	0,80	0,60	0,64	0,68	0,64	0,56	0,76	0,72	0,64	0,64	0,60	0,88	0,76	0,72	0,72	0,44	0,60	0,68	0,76	0,56	0,80	0,68	0,68	0,60	0,68	0,84	0,80	1,00										
SPL 52	0,72	0,88	0,80	0,44	0,84	0,44	0,88	0,56	0,52	0,76	0,72	0,80	0,56	0,48	0,76	0,80	0,72	0,88	0,72	0,76	0,60	0,60	0,76	0,60	0,80	0,68	0,80	0,76	0,72	0,64	0,84	0,72	0,72	0,64	0,60	0,72	0,76	0,72	0,80	0,60	0,68	0,92	0,84	0,48	0,80	0,76	0,76	0,76	0,60	0,68	0,88	0,68	1,00									
SPL 53	0,64	0,88	0,64	0,60	0,76	0,44	0,80	0,64	0,52	0,68	0,64	0,72	0,56	0,56	0,68	0,80	0,64	0,80	0,72	0,76	0,52	0,60	0,68	0,68	0,72	0,76	0,72	0,68	0,64	0,72	0,76	0,64	0,64	0,56	0,68	0,72	0,68	0,72	0,72	0,60	0,60	0,76	0,84	0,48	0,72	0,68	0,68	0,68	0,60	0,68	0,80	0,68	0,84	1,00								
SPL 54	0,68	0,68	0,76	0,48	0,80	0,56	0,84	0,44	0,56	0,64	0,68	0,76	0,52	0,52	0,88	0,68	0,84	0,84	0,60	0,64	0,64	0,72	0,72	0,72	0,76	0,64	0,84	0,80	0,76	0,52	0,88	0,76	0,92	0,76	0,48	0,84	0,80	0,76	0,76	0,56	0,64	0,88	0,80	0,60	0,68	0,72	0,80	0,72	0,48	0,72	0,92	0,72	0,80	0,72	1,00							
SPL 55	0,64	0,64	0,80	0,52	0,76	0,52	0,80	0,56	0,44	0,68	0,64	0,72	0,64	0,48	0,84	0,64	0,72	0,72	0,64	0,60	0,44	0,76	0,68	0,60	0,72	0,68	0,80	0,76	0,64	0,56	0,76	0,64	0,72	0,72	0,44	0,80	0,68	0,80	0,72	0,44	0,60	0,84	0,84	0,72	0,72	0,68	0,76	0,68	0,52	0,76	0,80	0,76	0,76	0,76	0,80	1,00						
SPL 56	0,76	0,76	0,84	0,40	0,88	0,48	0,92	0,52	0,48	0,72	0,76	0,84	0,52	0,60	0,88	0,76	0,84	0,84	0,68	0,72	0,56	0,64	0,72	0,72	0,84	0,72	0,92	0,80	0,76	0,60	0,88	0,76	0,84	0,76	0,56	0,76	0,80	0,76	0,84	0,56	0,72	0,96	0,88	0,60	0,76	0,80	0,80	0,80	0,48	0,72	0,92	0,72	0,88	0,80	0,92	0,88	1,00					
SPL 57	0,84	0,60	0,60	0,64	0,72	0,48	0,68	0,60	0,48	0,80	0,68	0,76	0,60	0,68	0,64	0,68	0,68	0,68	0,60	0,64	0,56	0,64	0,64	0,56	0,76	0,56	0,76	0,72	0,68	0,60	0,72	0,76	0,68	0,76	0,56	0,68	0,72	0,76	0,76	0,48	0,64	0,72	0,80	0,44	0,76	0,80	0,64	0,72	0,64	0,80	0,76	0,80	0,72	0,72	0,68	0,72	0,76	1,00				
SPL 58	0,72	0,64	0,64	0,52	0,68	0,44	0,64	0,48	0,52	0,76	0,72	0,80	0,64	0,56	0,60	0,72	0,80	0,72	0,64	0,68	0,68	0,68	0,60	0,60	0,64	0,52	0,72	0,76	0,64	0,64	0,68	0,88	0,72	0,64	0,44	0,64	0,76	0,64	0,72	0,52	0,60	0,76	0,68	0,32	0,64	0,76	0,76	0,76	0,60	0,60	0,80	0,68	0,76	0,60	0,72	0,60	0,72	0,80	1,00			
SPL 59	0,76	0,68	0,68	0,48	0,80	0,56	0,76	0,52	0,48	0,80	0,76	0,76	0,52	0,60	0,72	0,68	0,68	0,76	0,60	0,64	0,56	0,56	0,72	0,72	0,68	0,72	0,84	0,80	0,84	0,60	0,80	0,76	0,76	0,68	0,56	0,68	0,80	0,76	0,84	0,64	0,80	0,80	0,88	0,52	0,68	0,96	0,64	0,72	0,48	0,72	0,84	0,64	0,80	0,72	0,76	0,72	0,84	0,84	0,80	1,00		
SPL 60	0,84	0,60	0,60	0,64	0,72	0,56	0,68	0,52	0,64	0,72	0,68	0,68	0,52	0,68	0,72	0,60	0,76	0,76	0,60	0,64	0,72	0,72	0,72	0,64	0,76	0,56	0,68	0,72	0,76	0,60	0,80	0,76	0,76	0,76	0,56	0,76	0,80	0,68	0,76	0,56	0,64	0,64	0,64	0,44	0,76	0,80	0,64	0,64	0,56	0,72	0,76	0,80	0,64	0,56	0,76	0,56	0,68	0,84	0,80	0,76	1,00	
SPL 61	0,84	0,76	0,68	0,56	0,80	0,40	0,84	0,60	0,56	0,80	0,76	0,84	0,52	0,60	0,72	0,84	0,76	0,84	0,76	0,80	0,64	0,64	0,72	0,56	0,92	0,64	0,76	0,72	0,68	0,68	0,80	0,76	0,68	0,76	0,72	0,68	0,72	0,68	0,76	0,48	0,64	0,80	0,80	0,44	0,92	0,72	0,80	0,80	0,64	0,80	0,84	0,80	0,88	0,80	0,76	0,72	0,84	0,84	0,72	0,76	0,76	1,00
SPL 62	0,84	0,68	0,68	0,56	0,80	0,48	0,76	0,60	0,64	0,72	0,76	0,84	0,60	0,60	0,80	0,76	0,76	0,76	0,68	0,72	0,64	0,72	0,72	0,56	0,84	0,64	0,76	0,72	0,76	0,60	0,88	0,76	0,76	0,84	0,64	0,76	0,72	0,76	0,76	0,56	0,64	0,80	0,80	0,52	0,84	0,72	0,72	0,72	0,56	0,88	0,84	0,80	0,80	0,72	0,84	0,72	0,84	0,84	0,72	0,76	0,84	0,921,00
SPL 63	0,84	0,68	0,84	0,56	0,88	0,48	0,76	0,60	0,64	0,72	0,76	0,84	0,52	0,68	0,80	0,68	0,76	0,76	0,76	0,72	0,56	0,72	0,72	0,56	0,84	0,72	0,68	0,72	0,68	0,68	0,80	0,68	0,60	0,76	0,64	0,68	0,72	0,60	0,76	0,48	0,72	0,72	0,72	0,60	0,92	0,72	0,72	0,64	0,48	0,72	0,76	0,80	0,72	0,64	0,68	0,72	0,76	0,76	0,64	0,68	0,76	0,840,84 1,00
SPL 64	0,84	0,68	0,76	0,48	0,80	0,40	0,84	0,60	0,56	0,72	0,76	0,84	0,52	0,68	0,80	0,76	0,84	0,76	0,68	0,72	0,56	0,64	0,64	0,64	0,92	0,64	0,84	0,72	0,68	0,60	0,80	0,76	0,76	0,84	0,64	0,68	0,72	0,68	0,76	0,48	0,64	0,88	0,80	0,52	0,84	0,72	0,80	0,80	0,56	0,80	0,84	0,80	0,80	0,72	0,84	0,80	0,92	0,84	0,72	0,76	0,76	0,920,92 0,84 1,00
SPL 65	0,72	0,80	0,80	0,60	0,76	0,36	0,64	0,72	0,76	0,60	0,64	0,72	0,56	0,72	0,68	0,64	0,64	0,64	0,72	0,68	0,44	0,60	0,60	0,60	0,72	0,76	0,56	0,60	0,56	0,72	0,68	0,56	0,48	0,64	0,68	0,56	0,60	0,48	0,64	0,60	0,60	0,68	0,60	0,48	0,80	0,60	0,60	0,52	0,52	0,60	0,64	0,68	0,68	0,68	0,56	0,60	0,64	0,64	0,60	0,56	0,64	0,720,72 0,88 0,72 1,00
SPL 66	0,72	0,64	0,72	0,52	0,76	0,52	0,64	0,64	0,68	0,60	0,72	0,80	0,48	0,56	0,60	0,64	0,72	0,72	0,56	0,60	0,52	0,60	0,52	0,60	0,72	0,60	0,64	0,68	0,64	0,48	0,68	0,72	0,64	0,64	0,60	0,64	0,68	0,56	0,64	0,52	0,68	0,76	0,76	0,48	0,72	0,68	0,68	0,60	0,60	0,76	0,80	0,76	0,76	0,60	0,72	0,60	0,72	0,72	0,76	0,72	0,72	0,800,80 0,80 0,80 0,76 1,00
SPL 67	0,72	0,64	0,48	0,68	0,52	0,44	0,56	0,56	0,68	0,68	0,56	0,56	0,56	0,56	0,52	0,64	0,56	0,64	0,64	0,60	0,60	0,52	0,68	0,76	0,64	0,68	0,64	0,76	0,64	0,80	0,52	0,72	0,64	0,72	0,68	0,48	0,68	0,56	0,64	0,60	0,60	0,52	0,60	0,32	0,72	0,60	0,68	0,68	0,68	0,60	0,64	0,60	0,60	0,68	0,56	0,52	0,56	0,64	0,60	0,64	0,64	0,720,64 0,64 0,64 0,68 0,68 1,00
SPL 68	0,88	0,72	0,64	0,60	0,76	0,52	0,64	0,64	0,60	0,60	0,64	0,72	0,56	0,80	0,60	0,64	0,64	0,64	0,64	0,68	0,60	0,52	0,68	0,76	0,72	0,68	0,72	0,68	0,72	0,80	0,68	0,72	0,64	0,72	0,68	0,56	0,76	0,64	0,80	0,68	0,76	0,60	0,60	0,40	0,72	0,68	0,52	0,68	0,60	0,60	0,64	0,68	0,60	0,68	0,56	0,52	0,64	0,72	0,60	0,64	0,72	0,720,72 0,80 0,72 0,84 0,68 0,76 1,00
SPL 69	0,80	0,64	0,56	0,68	0,68	0,60	0,56	0,64	0,60	0,52	0,56	0,64	0,64	0,72	0,60	0,56	0,56	0,56	0,56	0,60	0,60	0,60	0,68	0,68	0,64	0,60	0,64	0,60	0,72	0,72	0,68	0,64	0,64	0,72	0,60	0,64	0,68	0,72	0,72	0,68	0,68	0,52	0,60	0,48	0,64	0,60	0,44	0,60	0,60	0,68	0,56	0,68	0,52	0,68	0,56	0,52	0,56	0,72	0,52	0,56	0,72	0,640,72 0,72 0,64 0,76 0,60 0,68 0,92 1,00
SPL 70	0,76	0,60	0,52	0,64	0,56	0,56	0,52	0,60	0,80	0,56	0,60	0,52	0,60	0,60	0,64	0,52	0,60	0,60	0,60	0,56	0,64	0,64	0,72	0,80	0,60	0,72	0,60	0,72	0,76	0,76	0,64	0,68	0,68	0,76	0,64	0,60	0,72	0,60	0,68	0,72	0,64	0,48	0,56	0,44	0,68	0,64	0,56	0,56	0,56	0,64	0,60	0,64	0,48	0,56	0,60	0,48	0,52	0,60	0,56	0,60	0,76	0,600,68 0,68 0,60 0,72 0,64 0,88 0,80 0,80 1,00
SPL 71	0,72	0,56	0,64	0,44	0,76	0,60	0,64	0,64	0,60	0,60	0,80	0,80	0,40	0,64	0,60	0,64	0,64	0,64	0,56	0,60	0,52	0,52	0,52	0,52	0,72	0,68	0,64	0,60	0,64	0,48	0,68	0,64	0,56	0,64	0,76	0,56	0,60	0,56	0,64	0,52	0,76	0,68	0,76	0,64	0,72	0,68	0,60	0,60	0,44	0,76	0,72	0,68	0,68	0,60	0,64	0,60	0,72	0,72	0,60	0,72	0,64	0,800,80 0,80 0,80 0,68 0,84 0,60 0,68 0,60 0,56 1,00
SPL 72	0,80	0,64	0,56	0,68	0,68	0,52	0,56	0,56	0,60	0,60	0,56	0,72	0,56	0,72	0,52	0,64	0,64	0,64	0,56	0,60	0,60	0,52	0,60	0,76	0,64	0,60	0,72	0,76	0,64	0,72	0,60	0,80	0,72	0,72	0,60	0,56	0,76	0,64	0,72	0,60	0,68	0,60	0,60	0,32	0,64	0,60	0,60	0,68	0,68	0,60	0,72	0,68	0,60	0,68	0,64	0,52	0,64	0,72	0,68	0,64	0,72	0,720,72 0,72 0,72 0,76 0,76 0,84 0,92 0,84 0,80 0,68 1,00
SPL 73	0,80	0,64	0,48	0,68	0,60	0,60	0,56	0,64	0,68	0,52	0,56	0,56	0,64	0,64	0,60	0,56	0,64	0,64	0,56	0,60	0,68	0,68	0,68	0,76	0,64	0,60	0,64	0,68	0,72	0,72	0,68	0,72	0,72	0,72	0,60	0,72	0,76	0,72	0,72	0,68	0,60	0,52	0,60	0,40	0,64	0,60	0,52	0,60	0,68	0,68	0,64	0,76	0,52	0,68	0,64	0,52	0,56	0,72	0,60	0,56	0,80	0,640,72 0,64 0,64 0,68 0,60 0,76 0,84 0,92 0,88 0,52 0,84 1,00
SPL 74	0,72	0,64	0,56	0,60	0,52	0,52	0,48	0,64	0,84	0,52	0,56	0,48	0,64	0,56	0,60	0,48	0,56	0,56	0,56	0,52	0,60	0,60	0,68	0,76	0,56	0,68	0,56	0,68	0,72	0,72	0,60	0,64	0,64	0,72	0,60	0,56	0,68	0,56	0,64	0,76	0,60	0,52	0,52	0,40	0,64	0,60	0,52	0,52	0,60	0,60	0,56	0,60	0,52	0,52	0,56	0,44	0,48	0,56	0,60	0,56	0,72	0,560,64 0,64 0,56 0,76 0,68 0,84 0,76 0,76 0,96 0,52 0,76 0,84 1,00
SPL 75	0,84	0,68	0,60	0,64	0,64	0,40	0,60	0,60	0,64	0,72	0,60	0,68	0,68	0,68	0,64	0,68	0,60	0,60	0,68	0,64	0,56	0,56	0,72	0,72	0,68	0,72	0,76	0,80	0,68	0,84	0,64	0,76	0,68	0,84	0,64	0,52	0,72	0,68	0,76	0,64	0,64	0,64	0,64	0,36	0,76	0,64	0,64	0,72	0,64	0,64	0,68	0,64	0,64	0,72	0,60	0,64	0,68	0,76	0,64	0,68	0,68	0,760,76 0,76 0,76 0,80 0,64 0,88 0,88 0,80 0,84 0,64 0,88 0,80 0,80 1,00
SPL 76	1,00	0,68	0,68	0,48	0,80	0,56	0,76	0,60	0,56	0,72	0,76	0,76	0,60	0,68	0,72	0,68	0,76	0,76	0,68	0,72	0,72	0,64	0,80	0,72	0,84	0,64	0,84	0,80	0,84	0,76	0,80	0,84	0,76	0,84	0,64	0,68	0,88	0,76	0,92	0,64	0,80	0,72	0,72	0,44	0,84	0,80	0,64	0,80	0,64	0,72	0,76	0,80	0,72	0,64	0,68	0,64	0,76	0,84	0,72	0,76	0,84	0,840,84 0,84 0,84 0,72 0,72 0,72 0,88 0,80 0,76 0,72 0,80 0,80 0,72 0,84 1,00

4.1.5. Phenolic and flavonoid contents

The total phenolic and flavonoid contents were measured in the *Moringa oleifera* parental and self-pollinated lines (Fig. 7). The variations in phenolic contents mirrored those of flavonoid contents (compared Fig. 7A and 7B), which is consistent with the fact that flavonoids are a group of chemicals in the phenolic family. Across the self-pollinated lines, SPL 21 had the highest phenolic and flavonoid contents (35.6 mg of GAE/g of dry weight and 61.6 mg of RE/g of dry weight respectively). The SPLs with the second and third highest phenolic contents were SPL 27 and SPL 66 (29.7 and 29.2 mg of GAE/g of dry weight respectively) (Fig. 7A). On the other hand, SPL 15, SPL 2 and SPL 20 had the lowest, second and third lowest phenolic contents (5.5 mg, 11.7 mg and 12.0 mg of GAE/g of dry weight respectively). The phenolic content of the parent was 14.4 mg of GAE/g of dry weight, below the averaged value of 75 SPLs (20.8 mg of GAE/g of dry weight). The SPL with the highest phenolic content (SPL 21) had more than six-fold higher phenolic content than that of the lowest (SPL 15).

The SPLs with the second and third highest flavonoid contents were SPL 73 and SPL 66 (56.7 and 53.9 mg of RE/g of dry weight respectively) (Fig. 7B). On the other hand, SPL 15, SPL 2 and SPL 62 had the lowest, second and third lowest flavonoid contents (9.1mg, 11.6 mg and 20.9 mg of RE/g of dry weight respectively). The flavonoid content of the parent was 28.2 mg of RE/g of dry weight, below the averaged value of 75 SPLs (33.8 mg of RE/g of dry weight). The SPL with the highest flavonoid content (SPL 21) had almost seven-fold higher flavonoid content than that in SPL 15, which contained the lowest amount of flavonoids.

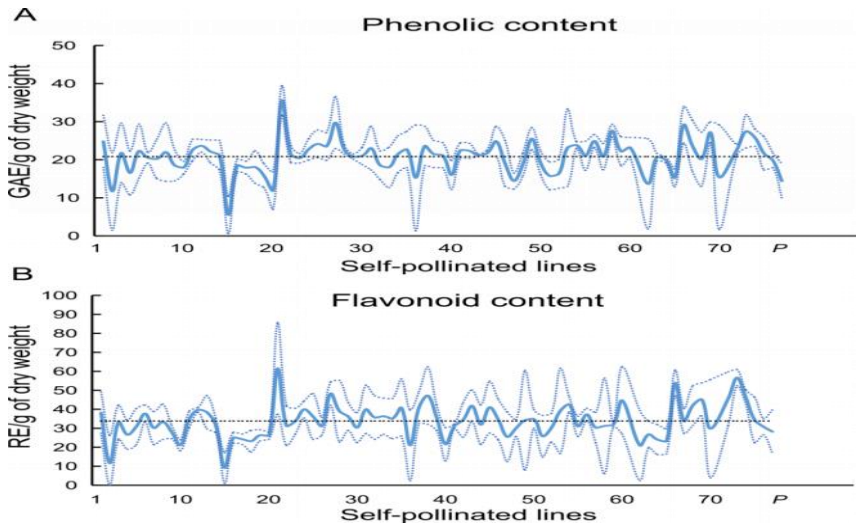

Figure 7. *Total phenolic and flavonoid contents measured in M. oleifera parental (P) and 76 self-pollinated lines. (A) Total phenolic content was determined as mg of gallic acid equivalents per gram of dry weight (GAE/g of dry weight). (B) Total flavonoid content was determined as mg of rutin equivalents per gram of dry weight (RE/g of dry weight). Solid lines and dashed blue lines represent the mean and standard deviations (three repeats) respectively. Dashed black lines represent averaged values across the parental and 76 self-pollinated lines.*

Moringa oleifera leaves are consumed as a vegetable in parts of Asia, although its nutritional and health benefits have not been fully realised. Due to their poor tolerance for waterlogging, it is useful to develop cultivars that are more tolerant to waterlogged conditions to expand cultivation areas. In this study, 76 self-pollinated lines derived from a waterlogging tolerant *M. oleifera* tree were characterised. They exhibited a range of morphologies, yields and tolerance to waterlogged conditions. Following a 20-day waterlogging treatment, leaf gain was only observed in three SPLs: 7, 18 and 65, indicating high levels of tolerance to waterlogged conditions by these lines. However, the phenolic and flavonoid contents in these SPLs were only around the averaged values.

On the other hand, SPL 21 had the highest phenolic and flavonoid contents among the 76 SPLs and doubled those from the parental tree. The averaged phenolic content reported in this work is similar to those obtained in *M. oleifera* from Madagascar (24 mg GAE/g of dry weight; Rodríguez-Pérez et al., 2015), South Africa (15-32 mg GAE/g of dry weight; Chitiyo et al., 2021) and Indonesia (25-30 mg GAE/g of dry weight; Sulastri et al., 2018) but somewhat lower than those reported by Siddhuraju and Becker (2003) (89-123 mg GAE/g of dry weight). Similarly, the averaged flavonoid content reported here is also similar to those measured by Chitiyo and co-workers (2021) but lower than values obtained by Siddhuraju and Becker (2003) (58-140 mg RE/g of dry weight). The variations in phenolic and flavonoid contents among SPLs were large, with the richest line (SPL 21) containing six- and seven-fold higher phenolic and flavonoid contents respectively than the poorest line (SPL 15). The variation is consistent with the differences in morphologies, waterlogging tolerance and genetic diversity; all pointed towards allelic segregation in the self-pollinated lines.

The genetic diversity within *M. oleifera* germplasms is well-known; previous studies using SRAP markers and RADP markers have shown polymorphism percentages to be in the range of 48% to 90% (Ridwan et al., 2021; Saini et al., 2013; Drisya et al., 2022). In terms of the number of expected alleles, the number of effective alleles, Nei's gene diversity and Shannon's diversity index, figures observed in this study (Table 5) are somewhat lower than those reported by Drisya and co-workers (2022), but comparable to those reported by Rufai and co-workers (2013). However, previously reported germplasms were collected from various geographical locations, and therefore the polymorphic ratios were higher than that observed in this study (27%), reflecting a higher genetic diversity.

Thus, most investigations on *M. oleifera* cultivation have been focussed on traits such as yields (Zheng et al., 2016), seed oil content and resistance to pests (Leone et al., 2016). This work presents a new direction where *M. oleifera* was selected for

waterlogging tolerance and high phenolic and flavonoid contents. The waterlogged tolerant lines were found to be SPLs 7, 18 and 65. However, these lines only contained averaged amounts of phenolic and flavonoid compounds. 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.

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)

Results of the study revealed that the chemical properties of MFB depended on the composting time (Table 4.6). Results presented in Table 4.6 showed that the Nitrogen content and pH increased with composting time. These parameters peaked after composting for four months (Nitrogen content of 11.9% and pH of 5.04). On the other hand, the contents of P and P_2O_5 were similar between 3.5 and 4 months, which were higher than those of 3 months. However, the contents of K and K_2O at 3 months were higher than those of 3.5 and 4 months. OM varied between 29% and 38% after 4 months of composting.

Table 4.6. *Effect of composting time on the physicochemical properties of Moringa foliar biofertilizer (MFB)*

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

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

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

Growth rate is an important factor to determine crop season, and apply appropriate techniques. Table 4.7 shows the growthand development rate (in days) of the three leafy vegetables. The time from transplanting to harvesting ranged from 31 - 38 days. For lettuce, the treatment using 100 mL and 33.3 mL of MFB that diluted with water to a total volume of 1 litter resulted in the earliest harvesting time (31 days), similar to

mustard spinach treated with 100 mL of MFB diluted. Lettuce and mustard spinach had the same harvesting time when using seaweed organic foliar fertilizer and NPK chemical foliar fertilizer with 33 days (treatment 6 and treatment 13) and 32 days (treatment 7 and treatment 14), respectively. All treatments of Ceylon spinach had the same harvesting time with 38 days. In summary, the application of MFB at 100 mL and 30 mL (diluted with water to a total volume of 1 liter) gained shorter time of the growth and development of lettuce and mustard spinach.

Table 4.7. *Influence of MFB on the growth rates of leafy vegetables*

Leafy vegetables	Treatment	Fertilizer doses*	Number of days from transplantation		
			Spread of leaves	Intersecting canopy	Harvesting
Lettuce	1	MFB - 100 mL	14	20	31
	2	MFB - 50 mL	13	22	32
	3	MFB - 33.3 mL	15	21	31
	4	MFB - 25 mL	13	22	33
	5	MFB - 20 mL	14	22	33
	6	Seaweed organic fertilizer (0.5 g)	15	22	33
	7	NPK foliar fertilizer (1.25 g)	13	20	32
Mustard spinach	8	MFB - 100 mL	13	19	31
	9	MFB - 50 mL	12	19	32
	10	MFB - 33.3 mL	14	20	34
	11	MFB - 25 mL	13	19	34
	12	MFB - 20 mL	14	21	35
	13	Seaweed organic fertilizer (0.5 g)	17	23	33
	14	NPK foliar fertilizer (1.25 g)	13	20	32
Ceylon spinach	15	MFB - 100 mL	9	14	38
	16	MFB - 50 mL	9	14	38
	17	MFB - 33.3 mL	10	15	38
	18	MFB - 25 mL	10	15	38
	19	MFB - 20 mL	10	16	38
	20	Seaweed organic fertilizer (0.5 g)	11	16	38
	21	NPK foliar fertilizer	9	14	38

(1.25 g)

**diluted with water to a total volume of 1 liter*

Growth ability showed that the number of leaves/stems, leaf length and leaf width of the vegetables increased with the growth time (Table 4.8). At 28 DAT, the controlsapplied with seaweed organic foliar fertilizer (treatment 6, treatment 13 and treatment 20) produced the lowest number of leaves/stems. Result of lettuce with 30 mL of MFB (diluted with water to a total volume of 1 liter) (treatment 3) yielded the highest number of leaves/stem (48.67). For mustard spinach and Ceylon spinach, the highest numbers of leaves/stem, 17.53 and 18.13 respectively, were obtained when MFB was sprayed at 100 mL of MFB (diluted with water to a total volume of 1 liter). The differences between treatment 3 and treatment 5, treatment 8 were significant.

The other treatments using MFB yielded longer leaves than the control check using NPK chemical foliar fertilizer, ranging from 11.02 cm (treatment 7) to 11.55 cm (treatment 3). For mustard spinach, at 28 DAT, the leaf length of the treatments was above 25.00 cm. When sprayed with seaweed organic foliar fertilizer (treatment 13) and NPK chemical foliar fertilizer (treatment 14), leaf lengths were different compared to MFB treatments. The longest leaves were recorded in treatment 8 with 30.84 cm, and the shortest in treatment 12 with 27.52 cm. Similar results were also observed in Ceylon spinach with leaf length ranging from 16.59 cm (treatment 19) to 21.41 cm (treatment 15). The difference in leaf lengths were statistically significant (Table 4.8). On the other hand, lettuce leaf widthswere over 12.00 cm in all treatments. The widest leavesin mustard spinach and Ceylon spinach were observed in treatment 8 and treatment 15 with 17.28 cm and 17.09 cm, respectively.

Table 4.8. *Influence of MFB on the growth ability of leafy vegetables*

Leafy vegetables	Treatment	Number of leaves/ stem (leaves)			Leaf length (cm)			Leaf width (cm)		
		Day after transplanting								
		14	21	28	14	21	28	14	21	28
Lettuce	1	12.67^{a}	23.33^{a}	45.00^{ab}	9.21^{b}	9.89^{b}	11.31^{ab}	9.39^{a}	11.07^{a}	12.26^{a}
	2	13.07^{a}	23.87^{a}	42.00^{ab}	9.73^{ab}	10.18^{b}	11.13^{b}	9.45^{a}	10.84^{a}	12.82^{a}
	3	13.27^{a}	25.60^{a}	48.67^{a}	10.15^{ab}	10.70^{ab}	11.55^{ab}	9.57^{a}	10.83^{a}	12.47^{a}
	4	11.27^{a}	23.53^{a}	38.73^{ab}	9.25^{b}	10.06^{b}	10.93^{b}	9.73^{a}	10.68^{a}	12.05^{a}
	5	12.67^{a}	22.53^{a}	39.33^{ab}	9.43^{ab}	10.43^{b}	11.06^{b}	10.28^{a}	11.49^{a}	12.55$_{a}$
	6	13.53^{a}	24.47^{a}	37.87^{b}	9.00^{a}	11.50^{a}	12.00^{a}	9.85^{a}	11.41^{a}	12.61^{a}
	7	13.07^{a}	23.60^{a}	44.93^{ab}	9.71^{ab}	10.21^{b}	11.02^{b}	9.72^{a}	11.10^{a}	12.56^{a}
Mustard spinach	8	10.33^{a}	12.53^{ab}	17.53^{a}	15.90^{a}	25,03^{a}	30,84^{a}	7,20^{a}	13.61^{a}	17.28^{a}
	9	8.47^{a}	10.80^{b}	15.13^{ab}	15.58^{a}	25,25^{a}	27,90^{a}	6.57^{a}	11.45^{b}	15.27^{b}
	10	9.73^{a}	13.87^{a}	16.47^{ab}	14.32^{a}	24,71^{a}	28,02^{a}	6.29^{a}	11.49^{b}	15.05^{b}
	11	8.87^{a}	11.73^{b}	16.80^{ab}	14.79^{a}	24.31^{a}	28.99^{a}	6.56^{a}	11.61^{b}	14.90^{b}
	12	9.67^{a}	11.87^{b}	16.27^{ab}	15.14^{a}	23.79^{a}	27.52^{a}	7.20^{a}	12.83^{b}	15.06^{b}
	13	8.73^{a}	11.73b	14.13^{b}	14.47^{a}	25.01^{a}	28.83^{a}	6.52^{a}	12.40^{b}	13.94^{b}
	14	7.93^{a}	10.67^{b}	15.80^{ab}	16.70^{a}	21.15^{a}	29.56^{a}	6.30^{a}	11.40^{b}	14.57^{b}
Ceylon spinach	15	7.60^{a}	15.80^{a}	18.13^{a}	12.61^{a}	18.32^{a}	21.41^{a}	9.27^{a}	14.67^{a}	17.09^{a}
	16	6.33^{a}	14.07^{a}	15.13^{ab}	11.77^{a}	16.56^{abc}	18.95^{b}	8.53^{a}	12.32^{b}	14.76^{b}
	17	6.73^{a}	14.07^{a}	14.87^{ab}	12.66^{a}	16.63^{abc}	18.90^{b}	8.92^{a}	12.27^{b}	14.88^{b}
	18	7.00^{a}	13.93^{a}	14.40^{b}	11.37^{a}	15.99^{abc}	18.66^{bc}	8.45^{a}	12.37^{b}	14.66^{b}
	19	7.20^{a}	13.07^{a}	14.47^{b}	12.44^{a}	14.63^{c}	16.59^{c}	8.52^{a}	11.35^{b}	13.65^{b}
	20	7.00^{a}	13.00^{a}	13.47^{b}	11.93^{a}	15.85^{bc}	18.03^{bc}	8.77^{a}	11.82^{b}	13.82^{b}
	21	7.40^{a}	14.40^{a}	15.20^{ab}	10.52^{a}	17.53^{ab}	19.65^{ab}	7.63^{a}	12.65^{b}	15.00^{b}

Means with different letters in each column indicate significant difference at α = 0.05.

The aim of this study was to determine the most suitable fertilizer treatment for each leafy vegetable. Fresh weight represents growth ability in terms of biomass. For lettuce, the average weight/plant was high when applied 30 mL of MFB diluted of water to a total volume of 1 liter was sprayed (156.33 g), followed by the NPK chemical foliar fertilizer control (treatment 7, 145.33 g) and the Seaweed organic foliar fertilizer control (treatment 6, 139.60 g). For mustard spinach and Ceylon spinach, the treatment used Moringa organic foliar fertilizer (100 mL of MFB diluted of water to a total volume of 1 liter) showed positive results in terms of the average weight/plant with 164.67 g (treatment 8) and 192.33 g (treatment 15), respectively. The other treatments produced lower average weight/plant than those using NPK chemical foliar fertilizer (Table 4.9).

The edible weight/plant in treatment 3 and treatment 6 of lettuce was not much different but higher than the other treatments, being 85.67 g and 85.73 g respectively. For mustard spinach and Ceylon spinach, treatment 8 and treatment 15 yielded the highest edible weight/plant compared to the other vegetables with 123.33 g and 152.67 g respectively. The NPK chemical foliar fertilizer control showed positive results in this parameter. The edible percentage in lettuce ranged from 52.11% (treatment 4) to 61.40% (treatment 6). For mustard spinach and Ceylon spinach, the highest edible percentage (74.52% in treatment 8 and 77.14% in treatment 15) was obtained when 100 mL of MFB diluted of water to a total volume of 1 liter was sprayed (Table 4.9).

Table 4.9. *Yield and yield components of leafy vegetables*

Leafy vegetables	Treatment	Average weight/ plant (g)	Edible weight/ plant (g)	Edible percentage (%)	Theoretical yield (kg/m^2)	Actual yield (kg/m^2)
Lettuce	1	138.67^{a}	83.33^{a}	60.44^{a}	3.170^{a}	2.381^{a}
	2	127.67^{a}	73.00^{a}	57.54^{ab}	2.918^{a}	2.086^{a}
	3	156.33^{a}	85.67^{a}	56.38^{b}	3.573^{a}	2.448^{a}
	4	141.33^{a}	73.33^{a}	52.11^{c}	3.231^{a}	2.095^{a}
	5	119.00^{a}	66.00^{a}	55.71^{bc}	2.720^{a}	1.886^{a}
	6	139.60^{a}	85.73^{a}	61.40^{a}	3.191^{a}	2.449^{a}
	7	145.33^{a}	82.20^{a}	56.20^{b}	3.322^{a}	2.348^{a}
Mustard spinach	8	164.67^{a}	123.33^{a}	74.52^{a}	3.764^{a}	2.819^{a}
	9	142.00^{a}	112.00^{ab}	73.77^{a}	3.46^{ab}	2.560^{a}
	10	138.67^{a}	104.67^{ab}	73.49^{a}	3.169^{ab}	2.393^{a}
	11	138.67^{a}	104.67^{ab}	69.08^{ab}	3.170^{ab}	2.392^{ab}
	12	126.00^{a}	77.33^{b}	58.39^{b}	2.880^{b}	1.768^{b}
	13	142.00^{a}	101.67^{ab}	70.01^{a}	3.246^{ab}	2.324^{a}
	14	159.33^{a}	113.33^{ab}	72.62^{a}	3.642^{ab}	2.590^{a}
Ceylon spinach	15	192.33^{a}	152.67^{a}	77.14^{a}	4.396^{a}	3.139^{a}
	16	162.67^{ab}	118.67^{ab}	72.89^{a}	3.718^{ab}	2.636^{ab}
	17	149.33^{ab}	105.33^{ab}	69.80^{a}	3.413^{ab}	2.385^{ab}
	18	131.33^{b}	91.00^{b}	66.55^{a}	3.002^{ab}	2.301^{ab}
	19	146.67^{ab}	99.33^{ab}	66.55^{a}	3.352^{ab}	2.209^{b}
	20	131.33^{b}	91.00^{b}	66.55^{a}	3.002^{b}	1.989^{b}
	21	171.33^{ab}	130.67^{ab}	73.36^{a}	3.916^{ab}	2.834^{ab}

Means with different letters in each column indicate significant difference at $\alpha = 0.05$.

For lettuce, the actual yield was the highest in treatment 6 using Seaweed organic foliar fertilizer with 2.45 kg/m^2. Treatment with 100 mL of MFB (diluted of water to a total volume of 1 liter) and NPK chemical foliar fertilizer control (treatment 7)

produced similar yields (2.38 and 2.35 kg/m^2 respectively). For mustard spinach, the highest actual yield (2.82 kg/m^2) was recorded when 100 mL of MFB diluted of water to a total volume of 1 liter 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 (Table 4.10). Plant height, number of leaves, canopy diameter, and leaf area index were found to be the highest when MFB was applied at 100 mL diluted of water to a total volume of 1 liter (Table 4.10).

Table 4.10. *Effect of different doses of MFB on the growth of lettuce*

Dose* (mL)	Growth time (day)	Plant height (cm)	Number of leaves (leaves per plant)	Canopy diameter (cm)	Leaf area index
First planting					
100	36	22.9^{a} ± 1.10	12.1^{a} ± 0.51	30.9^{a} ± 1.68	57.65^{a} ± 2.94
50	37	20.3^{ab} ± 1.22	11.2^{ab} ± 1.40	30.8^{a} ± 1.59	55.36^{ab} ± 3.61
33.3	36	20.9^{bc}±0.56	10.8^{ab}±0.40	30.7^{a}±2.31	44.67^{c}±3.42
25	35	19.4^{c}±0.57	10.5^{b}±0.42	27.3^{b}±0.98	45.57^{c}±3.12
20	36	22.0^{ab}±1.26	11.0^{ab}±0.81	29.4^{ab}±1.83	49.43^{bc}±3.17
$LSD_{0.05}$		1.98	1.42	3.34	5.59
Second planting					
100	32	23.0^{a}±1.35	12.2^{a}±1.41	29.6^{a}±0.87	51.30^{a}±2.23
50	33	20.9^{ab}±0.75	10.7^{ab}±1.05	27.3^{b}±1.36	48.42^{ab}±2.85
33.3	34	19.7^{b}±1.06	10.5^{b}±0.62	26.7^{bc}±0.45	45.71^{b}±1.89
25	33	19.5^{b}±1.26	11.5^{ab}±0.53	26.5^{bc}±0.72	45.40^{b}±3.07
20	34	19.7^{b}±1.14	10.2^{b}±0.91	25.7^{c}±1.03	45.92^{b}±1.52
$LSD_{0.05}$		2.02	1.54	1.51	3.41

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

**diluted of water to a total volume of 1 liter*

Foliar application of MFB at 100 mL diluted of water to a total volume of 1 liter significantly increased the fresh mass and estimated yield compared to the lower doses (Table 4.11). The actual yields were comparable between 100 and 50 mL dilutd of water to a total volume of 1 liter treatments and were significantly higher than those of other treatments. Higher ascorbic acid content and Brix were observed in the first planting with 100 and 50 mL diluted of water to a total volume of 1 liter treatments, however, these observations were not reproducible in the second planting.

Table 4.11. *Effect of different doses of MFB on the yield and quality of lettuce*

Dose*	Fresh weight (g per plant)	Estimated yield (ton per ha)	Actual yield (ton per ha)	Ascorbic acid (%)	Brix (%)
First planting					
100	$127.3^{a}\pm9.02$	$33.7^{a}\pm2.40$	$21.3^{a}\pm0.60$	$2.67^{a}\pm0.12$	$5.53^{a}\pm0.25$
50	$108.6^{b}\pm6.43$	$29.0^{b}\pm1.07$	$19.7^{ab}\pm0.95$	$2.57^{ab}\pm0.15$	$5.10^{a}\pm0.15$
33.3	$106.0^{bc}\pm4.01$	$28.0^{bc}\pm1.71$	$18.3^{bc}\pm1.03$	$2.34^{bc}\pm0.21$	$4.53^{b}\pm0.11$
25	$96.0^{c}\pm6.24$	$26.7^{bc}\pm0.53$	$18.2^{bc}\pm0.67$	$2.19^{c}\pm0.07$	$4.47^{b}\pm0.18$
20	$100.0^{bc}\pm2.18$	$25.6^{c}\pm1.66$	$17.7^{c}\pm0.43$	$2.16^{c}\pm0.16$	$4.43^{b}\pm0.24$
$LSD_{0.05}$	10.88	2.95	1.68	0.28	0.43
Second planting					
100	$140.2^{a}\pm8.26$	$34.4^{a}\pm1.83$	$21.7^{a}\pm1.26$	$3.45^{a}\pm0.38$	$5.45^{a}\pm0.15$
50	$117.0^{b}\pm6.15$	$28.7^{b}\pm1.91$	$20.0^{ab}\pm0.95$	$2.94^{a}\pm0.27$	$4.94^{a}\pm0.26$
33.3	$107.3^{bc}\pm5.23$	$27.0^{bc}\pm1.34$	$19.0^{bc}\pm0.78$	$3.01^{a}\pm0.41$	$5.01^{a}\pm0.68$
25	$101.6^{c}\pm2.55$	$26.3^{bc}\pm0.95$	$18.0^{bc}\pm1.14$	$3.07^{a}\pm0.06$	$5.07^{a}\pm0.22$
20	$99.3^{c}\pm4.79$	$25.8^{c}\pm1.06$	$17.3^{c}\pm0.87$	$3.04^{a}\pm0.09$	$5.04^{a}\pm0.17$
$LSD_{0.05}$	10.85	2.54	2.36	0.72	0.71

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

**diluted with water to a total volume of 1 liter*

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 (Table 4.12). Plant height, number of leaves, canopy diameter, and leaf area index slightly changed and tended to decrease with decreasing amounts of MFB.

Table 4.12. *Effect of different doses of MFB on the growth of mustard spinach*

Dose*	Growth time (day)	Plant height (cm)	Number of leaves (leaves per plant)	Canopy diameter (cm)	Leaf area index
First planting					
100	34	35.1^{a}±2.97	11.4^{a}±0.31	31.9^{a}±2.07	46.30^{a}±3.71
50	33	27.2^{b}±3.23	11.3^{a}±0.35	30.9^{ab}±1.58	43.55^{ab}±2.96
33.3	33	31.7^{ab}±4.15	10.2^{bc}±0.50	28.8^{bc}±2.00	40.06^{b}±2.28
25	34	30.7^{ab}±2.24	9.5^{c}±0.45	26.7^{cd}±1.68	39.53^{b}±4.33
20	36	26.8^{b}±3.56	10.3^{b}±0.37	25.8^{d}±1.45	39.09^{b}±2.57
$LSD_{0.05}$		5.76	0.68	2.39	5.22
Second planting					
100	31	29.7^{a}±1.15	11.5^{a}±1.01	31.2^{a}±3.07	44.52^{a}±3.12
50	29	27.1^{ab}±2.24	10.7^{ab}±0.75	29.9^{a}±3.21	40.19^{ab}±1.14
33.3	29	27.8^{ab}±1.63	10.5^{ab}±0.31	31.4^{a}±2.87	39.43^{ab}±2.41
25	28	25.5^{b}±2.41	10.3^{b}±0.54	28.8^{a}±2.12	37.50^{b}±3.97
20	32	24.9^{b}±3.01	10.0^{b}±0.16	29.8^{a}±1.93	37.21^{b}±2.71
$LSD_{0.05}$		3.99	1.17	3.61	5.31

The upper-case letters a, b, c, d, within columns indicate the significant difference at $P \leq 0.05$

**diluted with water to a total volume of 1 liter*

Similarly, fresh mass, estimated yield, and actual yield of mustard spinach also decreased when fewer MFB was applied (Table 4.13). The highest dose of MFB (100 mL diluted of water to a total volume of 1 liter) 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 diluted of water to a total volume of 1 liter) to 5.26 (20 mL of MFB diluted of water to a total volume of 1 liter) in the first planting but it did not significantly change in the second planting.

***Table 4.13**. Effect of different doses of MFB on the yield and quality of mustard spinach*

Dose*	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

The upper-case letters a, b, c, d within columns indicate the significant difference at $P \leq 0.05$

**diluted with water to a total volume of 1 liter*

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 (Table 4.14). 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.

***Table 4.14**. Effect of various foliar fertilizers 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					
MFB	34	25.4^{a}±1.21	12.8^{a}±1.02	23.6^{ab}±1.33	41.9^{a}±2.57
Chitosan fertilizer	33	23.8^{a}±1.83	11.5^{ab}±1.00	24.9^{a}±1.65	38.6^{ab}±4.98
Seaweed fertilizer	35	24.6^{a}±0.92	11.6^{ab}±0.25	24.4^{a}±0.61	38.8^{ab}±2.81
Control	35	18.4^{b}±2.97	10.2^{b}±0.82	21.1^{b}±1.51	34.0^{b}±3.24
$LSD_{0.05}$		3.18	1.48	2.96	5.68
Second planting					
MFB	35	24.3^{a}±0.69	12.1^{a}±0.52	23.9^{a}±1.76	42.2^{a}±3.04
Chitosan fertilizer	36	21.5^{bc}±1.14	11.2^{ab}±0.31	24.9^{a}±0.55	39.0^{a}±2.56
Seaweed fertilizer	35	22.9^{ab}±0.76	11.8^{a}±0.67	25.4^{a}±1.15	40.1^{a}±2.18
Control	35	20.5^{c}±1.41	10.3^{b}±0.71	21.8^{b}±1.37	34.8^{b}±1.19
$LSD_{0.05}$		1.74	0.96	1.84	3.61

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

The yield of lettuce was enhanced by spraying foliar fertilizers at both plantings (Table 4.15). 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. On the other hand, the ascorbic acid content was not influenced by foliar treatments. Lettuce treated with MFB and chitosan fertilizer had higher Brix in the first planting but these results were not reproducible in the second planting seasons.

***Table 4.15**. Effect of various foliar fertilizers on the yield and quality of lettuce*

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

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

Like lettuce, mustard spinach growth was also affected by foliar treatments (Table 4.16). In the first planting, plant height and leaf area index did not vary between different treatments, however, the number of leaves and canopy diameter was found to be higher in plants treated with MFB and seaweed fertilizer. 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. Actual yields of plants treated with MFB were comparable to those treated with seaweed fertilizer and higher than those treated with chitosan fertilizer and the control plants. The ascorbic acid of plants grown in the first planting varied from 3.31% (control) to 5.21% (seaweed fertilizer treated), however, the changes were not significant in the second planting. The Brix of mustard spinach across treatments remained constant (larger than 6.0).

Table 4.16. *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

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

In this study, the effects of MFB, prepared from non-edible parts, on the growth and yield of leafy vegetables was investigated. Results of the study revealed that the composting time impacted the quality of MFB (Table 4.6) and a four-month composting time yielded biofertilizer with the highest Nitrogen content. Further, phosphorus content also slightly increased when the composting time was longer than three months, while the organic matter remained unchanged. Furthermore, the pH of the composite biofertilizer increased from 3.37 to 5.04 with increasing composting time. High Nitrogen content in Moringa foliar biofertilizer was prioritized as Nitrogen is one of the most essential elements to enable fast growth and optimal production of vegetables (Tam and Cong 2018; Hoa and Thanh 2020). Hence, these results suggested that a four-month composting period was suitable to produce biofertilizer from non-edible Moringa plant parts. Apart from macronutrients, Moringa plant extracts also contain various antioxidant compounds like zeatin, ascorbic acid, phenolic, flavonoids, vitamin E, minerals, and many other growth hormones such as

indole-3-acetic acid (IAA), and gibberellins (GAs) (Isman, 1997; Rady & Mohamed, 2015; Latif & Mohamed, 2016). The previous study also indicated that the stem of moringa was found to enrich nutrients such as vanillin, β-sitostanol, 4-hydroxymellin, β-sitosterol, and octacosanoic acid (Faizi et al., 1994). During the application of MFB to the leafy plants, the higher the dose of MFB enhanced fresh mass and yields (Tables 4.11 and 4.13). In both planting seasons, the dose of 100 mL per Litre MFB produced the highest fresh mass and yields in both lettuce and mustard spinach. It had been reported that the concentration of Moringa leaf extract at 200 mg per Liter was sufficient to enhance the quality of baby leaves (Toscano et al., 2021). In this study, the spray of MFB at 25 mL per Liter and 20 mL per Liter did not improve the yields of these vegetables compared to the Control. Similarly, leaf area indices in both lettuce and mustard spinach decreased in these treatments which could be justified by the poor nutrient supply in these treatments (Tables 4.12 and 4.14). Previously, it was demonstrated that the extracts derived from Moringa stem bark enhanced the leaf area and fruit yield of sweet bell pepper fruit (Nwokeji et al., 2022). Taken together, the application of 100 mL of MFB diluted of water to a total volume of 1 liter produced the highest yield and quality vegetables in this study. Different types of foliar fertilizer used in this study had comparable effects on the growth of lettuce. However, the actual yield was higher when treated with MFB compared to the seaweed fertilizer treatment. Since leaf areas and plant sizes were similar in plants treated with different foliar fertilizers, it is suggested that MFB stimulated root formation in lettuce which resulted in the differences in yield. Consistent with this, previous studies (Culver et al., 2012; Yasmeen et al., 2013) had shown that the application of Moringa plant extract increased root dry weight and root length of tomatoes and wheat. Mustard spinach plants grown on the first planting achieved the highest yield when treated with MFB but these results were not reproducible in the second planting. The effects of seaweed fertilizer on the growth and yield of vegetables in this study were similar to those reported by Hoang et al. (2022). In lettuce, the ascorbic acid content was not significantly influenced by spraying different foliar fertilizers. Yaseen and Hajos (2022) found no significant difference in the ascorbic acid content between Moringa plant extract treated and non–treated lettuce in 2019, but Moringa plant extracts were found to improve the ascorbic acid content of lettuce in 2020. This can be explained by the temperature fluctuation in 2020, which caused physical stresses to plants. In this study, lettuce that was grown in the second planting showed a higher percentage of ascorbic acid when treated with foliar fertilizers despite the effect of higher temperatures from February to March (average temperatures at 18.2°C in January, 21.1°C in February and 25.7°C in March, data not shown). Meanwhile, the ascorbic acid content in mustard spinach varied when treated with different foliar fertilizers on the first planting, although no difference was observed in the second planting.

Furthermore, MFB did not affect the percentage of ascorbic acid when various doses (20 mL per Litre to 100 mL per Litre) were sprayed on mustard spinach. These results were contradictory to the findings of Cintya et al. (2018) who found an increase in the content of vitamin C with increasing doses of organic fertilizers in spinach (*Amaranthus tricolor* L.), mustard (*Brassicca rapa* chinensis). Brix of lettuce tended to decrease when the doses of MFB decreased in the first planting; however, there was no significant difference across doses in the second planting. Similarly, the application of MFB and chitosan fertilizer improved the Brix in lettuce, compared to seaweed fertilizer and control treatments only in the first planting. Meanwhile, in mustard spinach, Brix varied greatly (5.26%–8.07%) across the different doses of MFB in the first planting but remained relatively constant in the second planting. The effects of MFB on the quality of lettuce and mustard spinach were consistent with previous studies on kale and broccoli baby leaves (Toscano et al., 2021).

Thus, in this work, Moringa residues including stems, branches, and leaf petioles, were fermented using EM product and molasses to produce MFB. To obtain optimal MFB, the composting should be allowed to continue for four months. MFB application enhanced the growth and yield of both lettuce and mustard spinach grown in January and February but did not affect the ascorbic acid content and Brix consistently. The application of MFB produced similar effects compared to the chitosan and seaweed fertilizers. To the authors' knowledge, this was the first study to investigate the effects of MFB on the growth, yield, and quality of leafy vegetables grown in the tropical.

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

The results presented in Table 4.17 indicated that the Nitrogen contents changed during the incubation period. Moringa organic fertilizer (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 4.17. *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 upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$.

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. 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 4.18).

Table 4.18. *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^{ab}±1.83	10.7^{ab}±1.01	28.3^{ab}±1.66	47.6^{ab}±0.82
R2	29	20.4^{a}±1.27	11.6^{a}±0.12	28.7^{a}±1.34	48.3^{a}±2.52
R3	30	19.5^{ab}±1.17	11.1^{ab}±0.53	26.7^{ab}±0.61	48.2^{a}±2.43
R4	30	17.3^{b}±2.01	9.6^{b}±1.28	25.3^{b}±0.42	45.3^{b}±1.15
$LSD_{0.05}$		2.95	1.62	3.2	2.6
Second planting					
R1	28	24.5^{b}±1.56	12.1^{a}±1.83	24.8^{b}±1.41	41.1^{b}±4.32
R2	28	25.6^{ab}±0.64	14.0^{a}±1.83	28.6^{a}±0.70	49.2^{a}±2.23
R3	29	26.7^{a}±0.92	14.4^{a}±1.83	29.2^{a}±0.57	50.9^{a}±3.71
R4	28	26.5^{ab}±1.96	13.6^{a}±1.83	29.3^{a}±0.69	44.8^{ab}±2.08
$LSD_{0.05}$		2.2	2.9	3.7	6.3

The upper-case letters a, b, within columns indicate the significant difference at $P \leq 0.05$.

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 4.19), the values remained constant across treatments in the first planting. However, the treatment with 15 tons of MOF per ha in the second planting 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 4.19. *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}±6.66	26.7^{b}±0.63	19.0^{c}±1.67	2.767^{a}±0.11	4.93^{a}±0.31
R2	101.7^{b}±4.23	27.0^{b}±1.78	20.3^{bc}±2.01	2.730^{a}±0.14	4.76^{ab}±0.46
R3	123.3^{a}±5.04	32.7^{a}±0.53	23.7^{a}±1.30	2.741^{a}±0.30	5.17^{a}±0.25
R4	125.4^{a}±6.50	33.0^{a}±1.34	22.7^{ab}±1.71	2.693^{a}±0.15	4.90^{a}±0.32
$LSD_{0.05}$	7.89	3.12	2.56	0.41	0.39
Second planting					
R1	99.9^{c}±2.01	25.7^{c}±0.54	20.8^{c}±0.42	2.607^{b}±0.11	4.40^{b}±0.26
R2	110.0^{bc}±5.29	29.3^{b}±1.42	22.9^{bc}±1.10	2.770^{ab}±0.23	4.76^{a}±0.33
R3	122.7^{a}±4.73	31.7^{a}±0.67	25.6^{a}±0.98	2.863^{a}±0.05	5.10^{a}±0.36
R4	117.8^{b}±9.62	30.0^{ab}±0.85	24.5^{ab}±2.00	2.874^{a}±0.07	4.86^{a}±0.29
$LSD_{0.05}$	12.0	2.1	2.5	0.2	0.4

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

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). 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 fresh mass was the highest when 25 tons of MOF per ha were used in both planting times (Table 4.21). 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 4.21). 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.

Table 4.20. *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^{b}±1.36	11.0^{a}±0.64	25.8^{b}±1.51	39.8^{b}±1.79
R2	32	27.2^{a}±2.98	11.9^{a}±1.63	26.9^{ab}±0.64	43.5^{ab}±1.13
R3	33	28.8^{a}±2.65	12.1^{a}±0.99	29.3^{a}±2.09	44.6^{a}±0.86
R4	31	27.9^{a}±1.10	11.3^{a}±0.91	28.1^{ab}±1.33	43.8^{ab}±0.92
$LSD_{0.05}$		2.50	1.80	3.34	4.03
Second planting					
R1	30	24.0^{c}±1.35	11.9^{b}±0.12	27.9^{a}±1.51	39.1^{b}±0.97
R2	32	26.6^{b}±1.04	12.5^{ab}±0.50	29.0^{a}±0.64	43.2^{a}±0.94
R3	31	29.6^{a}±0.50	13.3^{a}±0.84	29.4^{a}±1.39	43.3^{a}±0.97
R4	32	29.1^{a}±0.59	13.2^{a}±0.48	29.2^{a}±0.41	44.7^{a}±1.62
$LSD_{0.05}$		1.9	1.0	1.9	2.4

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

Table 4.21. *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

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

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

Applying organic fertilizers, including MOF, cow manure and bioorganic fertilizer, enhanced the lettuce's performance compared to the control (Table 4.22). Applying organic fertilizers did not affect the 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 4.23). 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.

Table 4.22. 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	$26.4^{a}\pm1.21$	$13.1^{a}\pm0.31$	$23.5^{a}\pm3.52$	$43.4^{a}\pm0.77$
F2	32	$24.2^{b}\pm2.00$	$12.5^{a}\pm0.91$	$24.1^{a}\pm2.33$	$41.8^{a}\pm2.24$
F3	31	$25.3^{ab}\pm3.03$	$13.2^{a}\pm0.69$	$24.9^{a}\pm1.68$	$42.4^{a}\pm1.08$
Control	33	$22.1^{c}\pm2.08$	$11.7^{a}\pm1.02$	$24.2^{a}\pm1.94$	$31.5^{b}\pm4.44$
$LSD_{0.05}$		1.87	1.71	6.26	2.83
Second planting					
F1	33	$25.5^{a}\pm2.12$	$13.4^{a}\pm0.25$	$28.6^{a}\pm0.92$	$41.8^{a}\pm10.64$
F2	32	$24.2^{a}\pm2.07$	$12.3^{a}\pm0.86$	$25.3^{b}\pm1.47$	$41.1^{a}\pm0.97$
F3	33	$25.9^{a}\pm1.16$	$13.0^{a}\pm0.62$	$24.8^{bc}\pm1.69$	$40.3^{a}\pm1.54$
Control	34	$19.8^{b}\pm1.35$	$10.1^{b}\pm0.56$	$23.2^{c}\pm2.62$	$28.9^{b}\pm3.08$
$LSD_{0.05}$		2.7	1.7	1.8	2.2

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

Table 4.23. *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^{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}±3.52	37.4^{a}±0.53	25.5^{a}±0.34	5.6^{a}±0.30	5.1^{a}±0.10
F2	129.6^{b}±4.04	34.0^{b}±0.59	22.8^{b}±0.73	5.7^{a}±0.23	5.0^{a}±0.26
F3	123.5^{c}±4.92	33.5^{b}±1.67	21.7^{b}±1.42	5.7^{a}±0.29	5.1^{a}±0.15
Control	101.7^{d}±5.44	26.2^{c}±1.26	18.1^{c}±0.95	4.7^{b}±0.27	3.9^{b}±0.49
$LSD_{0.05}$	5.99	2.12	1.55	0.3	0.2

The upper-case letters a, b, c, d within columns indicate the significant difference at $P \leq 0.05$

Like lettuce, organic fertilizers enhanced the growth of mustard spinach compared to the control (Table 4.24). 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 4.24. *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^{a}±2.44	12.2^{a}±0.42	32.1^{a}±1.50	42.8^{a}±3.28
F2	27.1^{a}±1.55	11.9^{a}±0.35	33.0^{a}±0.95	42.3^{a}±3.57
F3	27.4^{a}±50.63	12.0^{a}±0.30	30.3^{ab}±2.61	41.7^{a}±3.73
Control	21.6^{b}±3.21	11.6^{a}±0.87	27.4^{b}±1.54	32.0^{b}±4.52
$LSD_{0.05}$	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 upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$

At harvest time, fresh mass and yields of mustard spinach were significantly different across organic fertilizer treatments (Table 4.25). 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.

Table 4.25. *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	$158.0^{a}\pm8.93$	$38.7^{a}\pm0.38$	$25.9^{a}\pm0.51$	$5.7^{a}\pm0.38$	$4.5^{a}\pm1.01$
F2	$140.3^{b}\pm9.14$	$37.3^{a}\pm1.55$	$23.3^{b}\pm1.35$	$5.6^{a}\pm0.56$	$4.4^{a}\pm0.76$
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

The upper-case letters a, b, c within columns indicate the significant difference at $P \leq 0.05$.

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 & Lavanya 2021; Upendri & Karunarathna, 2021). Research on the production of bio-extract or organic fertilizer derived from *Moringa 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 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 vermicompostbased 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.

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.

4.4. Demonstration of Moringa foliar biofertilizer on leafy vegetables

4.4.1. Demonstration of Moringa foliar biofertilizer on lettuce

The evaluation of technical measures for the growth and development of vegetable crops through demonstration models is the basis for confirming more accurately the effectiveness of technical measures applied to production.

The results of some growth and development characteristics of lettuce in Model 1, using Moringa foliar biofertilizer in a ratio 100 mL of MFB diluted of water to a total volume of 1 liter, and Model 2, using the farmer's fertilizer practice, are presented in Table 4.26.

The growth time of lettuce ranged from 33 to 34 days in Models 1 and 2, respectively, with no significant differences between the two models. However, growth characteristics such as plant height, leaf numbers showed significant differences between the two models. In Model 1, which applied Moringa foliar biofertilizer, plant height reached 14.47 cm, significantly higher than that in Model 2. The number of leaves was 8.6, which tended to be higher in Model 1 than in Model 2.

In general, the growth characteristics of lettuce showed better performance in Model 1 than in Model 2.

Table 4.26. *Effect of MFB on the growth characteristics of lettuce in demonstration*

Parameters	Model 1	Model 2	T-test
Growth time (day)	33	34	
Plant height (cm)	14.47	12.98	0.02
Number of leaves (leaves. plant^{-1})	8.60	7.67	0.04
Canopy diameter (cm)	26.87	23.67	0.03

T-test values show a significant difference between models if the values at P≤0.05

Yield and quality are always the top concern of vegetable growers. Through a demonstration model, growers can observe and evaluate the model's productivity, thereby deciding the investment in production. The results on the yield and quality of lettuce are presented in Table 4.27.

Table 4.27*. Effect of MFB on yield and quality of lettuce in demonstration*

Parameters	Model 1	Model 2	**T-test**
Brix	6.63	5.50	0.02
Vitamin C%	5.06	4.56	0.02
Fresh weight/ plant (g)	125.7	105.0	0.01
Theoretical yield (tons/ha)	31.43	29.07	0.01
Actual yield (tons/ha)	21.32	19.45	0.02

T-test values showed a significant difference between the models if the values at P≤0.05.

The fresh weight in Model 1 using moringa foliar biofertilizer was 125.6 g plant^{-1} and it was significantly higher than in Model 2. Higher fresh weight resulted in higher theoretical yield and actual yield of 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 biofertilizer on mustard spinach

The characteristics of mustard geen results are presented in Table 4.28.

Table 4.28. *Effect of MFB on the growth characteristics of mustard spinach in demonstration*

Parameters	Model 1	Model 2	T-test
Growth time (day)	29	29	
Plant height (cm)	39.00	36.13	0.02
Number of leaves (leaves. plant^{-1})	9.13	8.27	0.03
Canopy diameter (cm)	37.20	34.87	0.04

T-test values show a significant difference between models if the values at P≤0.05.

The growth characteristics such as plant height, number of leaves, and canopy diameter tended to be higher in the demonstration model using MFB, except for growth time.

The growth time of mustard spinach was 29 days in both models. The plant height was 39.0 cm and 36.13 cm in Model 1 and Model 2, respectively. The number of leaves in Model 1 was 9.13 and was significantly higher than in Model 2. The same tendency was observed in the canopy parameter.

Table 4.29. *Effect of MFB on yield and quality of mustard spinach in demonstration*

Parameters	Model 1	Model 2	T-test
Brix	7.00	5.70	0.00
Vitamin C%	3.98	2.53	0.04
Fresh weight/ plant (g)	151.67	126.67	0.00
Theoretical yield (tons/ha)	37.92	31.67	0.03
Actual yield (tons/ha)	29.52	25.79	0.04

T-test values show a significant difference between models if the values at P≤0.05.

The yield and quality of mustard spinach in two demonstration models are presented in Table 4.29. The brix values reached 7.00 in Model 1 using moringa foliar biofertilizer and it was higher than that in Model 2 using farmer practice. Using MFB also increased the acid ascorbic content in mustard spinach. The vitamin C values occupied 3.98%, then higher 2.53% in the model using farmer practice.

The fresh weight of mustard spinach was 151.67 g plant^{-1} and was significantly higher than that in the control model. These explained why the actual yield of mustard spinach was significantly higher in the demonstration model using moringa foliar biofertilizer.

We can conclude that applying 100 mL of MFB diluted of water to a total volume of 1 liter 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

Organic fertilizer plays an important role in improving the physical and chemical properties of the soil such as pH, humus, soil nutrients and maintaining microorganism's activities. Therefore, farmers applied organic fertilizer was applied annually in order to promote plant growth.

In the demonstration model using MOF, we applied 25 tons ha^{-1} and compared with the farmer fertilizer practice. The results are presented in Table 4.30.

Table 4.30. *Effect of MOF on the growth characteristics of lettuce in demonstration*

Parameters	Model 1	Model 2	T-test
Growth time (day)	30	30	
Plant height (cm)	20.47	19.33	0.26
Number of leaves (leaves plant^{-1})	9.20	8.47	0.17
Canopy diameter (cm)	28.47	24.87	0.07

T-test values show a significant difference between models if the values at $P \leq 0.05$

The growth parameters of lettuce using moringa organic fertilizer were increased to compare with control model. However, the differences among these characteristics were not significantly. The growth time of lettuce was the same between the two models.

Table 4.31. *Effect of MOF on yield and quality of lettuce in demonstration*

Parameters	Model 1	Model 2	**T-test**
Brix	6.77	5.50	0.01
Vitamin C%	6.28	4.66	0.01
Fresh weight/ plant (g)	120.67	110.06	0.02
Theoretical yield (tons/ha)	30.07	28.61	0.02
Actual yield (tons/ha)	23.62	21.22	0.04

T-test values show a significant difference between models if the values at P≤0.05.

The fresh weight in model 1 using MOF was 120.67 g plant^{-1}, significantly higher than in model 2 (110.06 g plant^{-1}). The higher fresh weight resulted in higher theoretical and actual yields of 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 in Model 1 was higher than those in Model 2.

4.5.2. Demonstration of Moringa organic fertilizer on mustard spinach

The results of Table 4.32 indicated that MOF strongly affected the plant height and canopy diameter of the mustard spinach, except for growth time and the number of leaves.

Table 4.32. *Effect of MOF on the growth characteristics of musstard spinach in demonstration*

Parameters	Model 1	Model 2	T-test
Growth time (day)	31	32	
Plant height (cm)	26.07	19.93	0.03
Number of leaves (leaves. plant^{-1})	8.07	7.80	0.42
Canopy diameter (cm)	31.67	27.13	0.04

T-test values show a significant difference between models if the values at P≤0.05.

The growth time was 31 and 32 days in the two models, and the difference in growth time was unclear. The plant in the model using MOF had longer leaves to compare the farmer's practice (data not shown).

Table 4.33. *Effect of MOF on yield and quality of mustard spinach in demonstration*

Parameters	Model 1	Model 2	**T-test**
Brix	6.60	5.37	0.02
Vitamin C%	8.70	7.37	0.00
Fresh weight/ plant (g)	128.70	104.64	0.00
Theoretical yield (tons/ha)	32.03	28.08	0.02
Actual yield (tons/ha)	22.54	19.12	0.04

T-test values show a significant difference between models if the values at $P \leq 0.05$.

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.

Rachmawatie and co-workers (2022) reported that the amount of N, P, K, and Fe content in rice plants and dry weight were increased when liquid organic fertilizer from Moringa leaves was applied (Rachmawatie et al., 2022). In this study, the demonstrations showed that the growth, quality, and yield of lettuce and mustard spinach were enhanced when MOF and MFB were applied.

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

- Morphological variation was observed amongst 76 SPLs at 40 days. The young shoot color varied from green, greenish purple, light purple, and purple; the stem circumferences ranged from 3.4 to 8.00 cm, the plant height varied from 36 to 132 cm, and the leaf number ranged from 9 to 21 leaves. Genetic diversity was presented amongst 76 using RADP and SRAP markers.

- 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 lines with the lowest phenolic contents were SPL 15 (5.5 mg of GAE/g of dry weight), SPL 2 (11.7 mg of GAE/g of dry weight), and SPL 20 (12.0 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), SPL 2 (11.6 mg/RE/g of dry weight), and SPL 62 (20.9 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 diluted of water to a total volume of 1-liter 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. It 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.

- 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's non-edible parts can make organic fertilizer and foliar biofertilizer to enhance leafy vegetables' growth, yield, and quality.

- Moringa foliar biofertilizer (MFB) and Moringa organic fertilizer (MOF) can produce 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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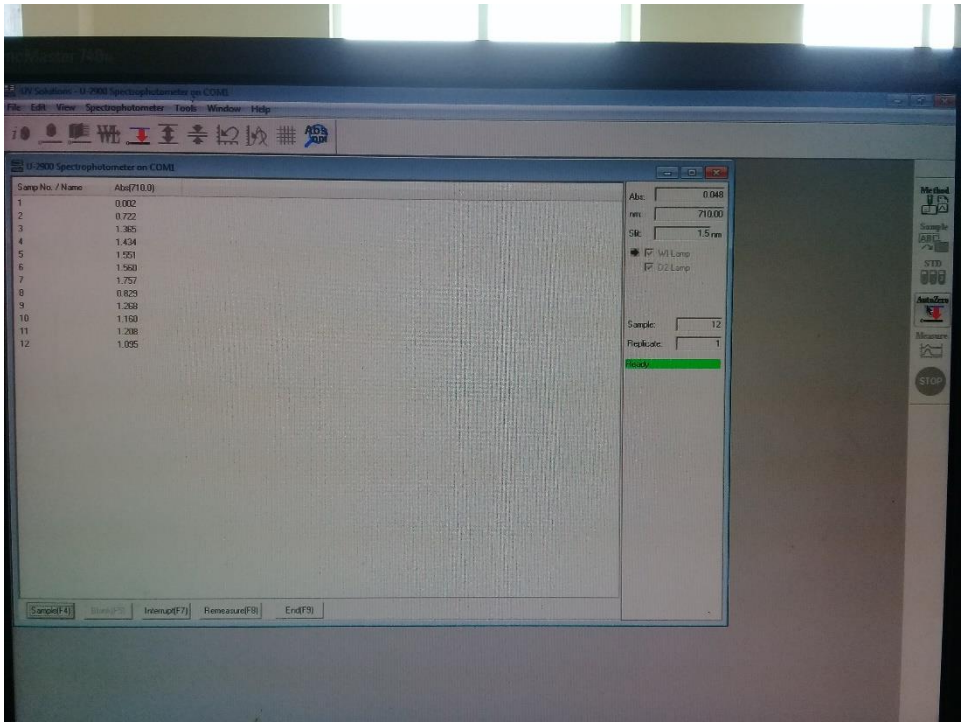
Appendix

Planting moringa and prepare material from moringa residues at HUIB

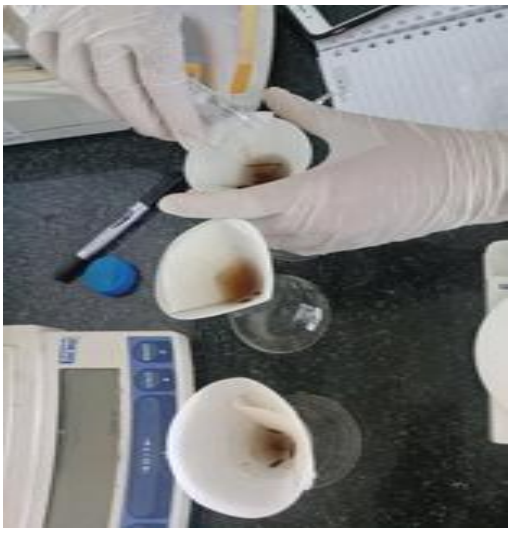
Making for Moringa foliar biofertilizer from Moringa residues at HUIB

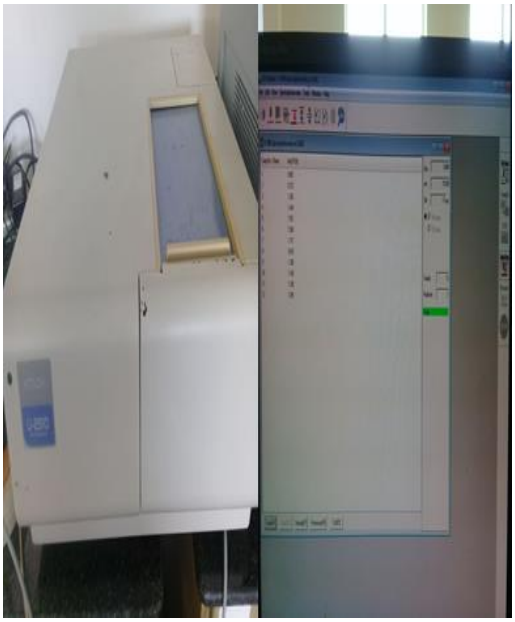
Preparing Moringa organic biofertilizer from Moringa residues at HUIB

Methodology analysis Potassium from Moringa organic biofertilizer

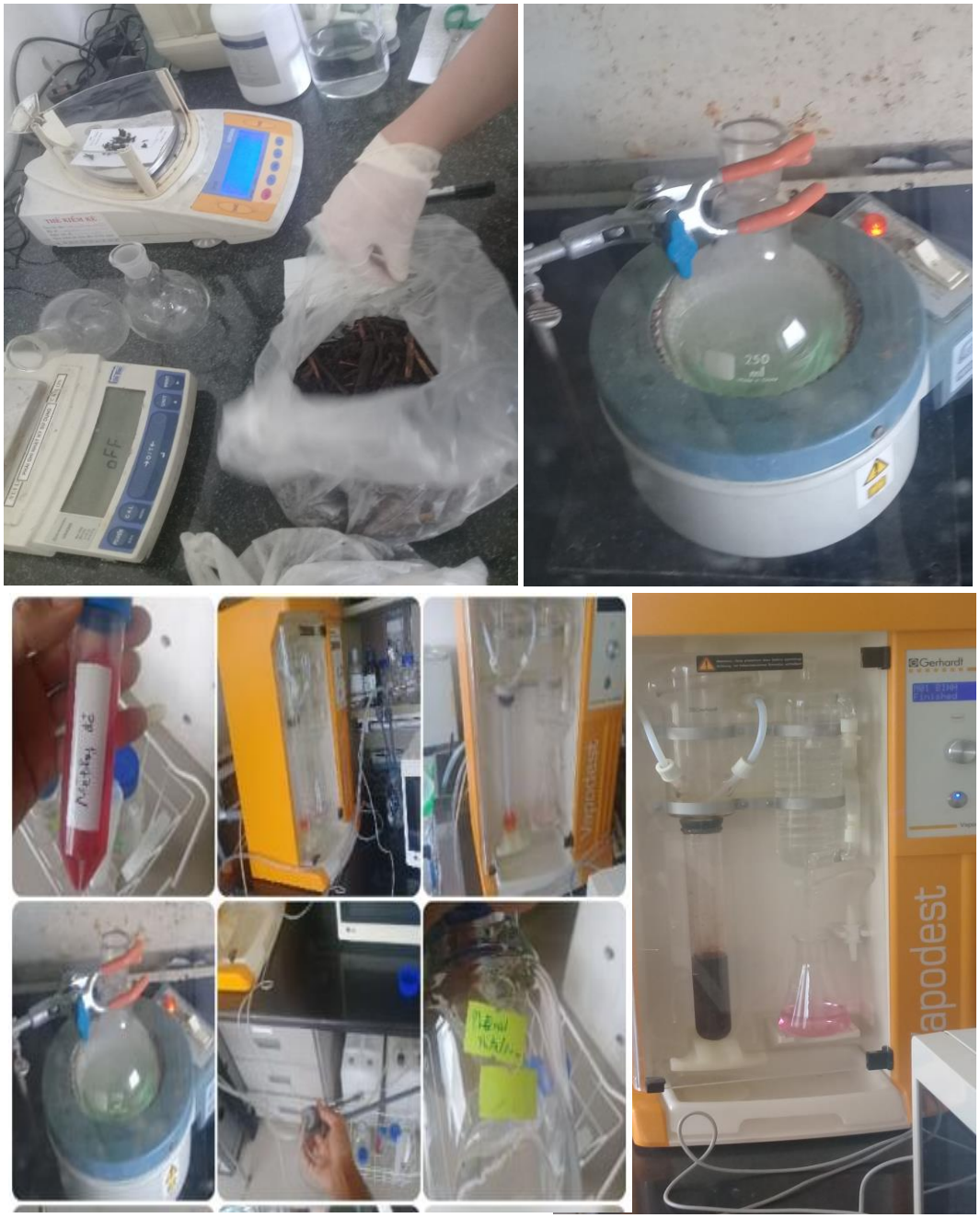


Methodology analysis Phosphate from Moringa organic biofertilizer

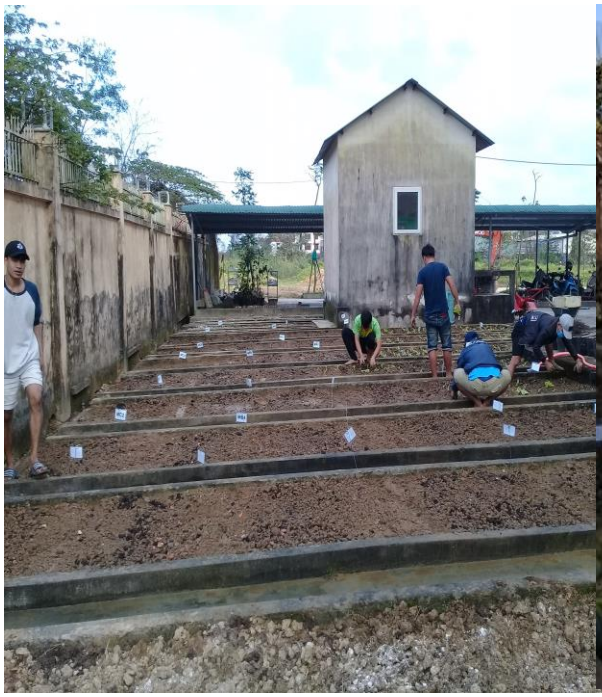




Methodology analysis Nitrogen from Moringa organic fertilizer



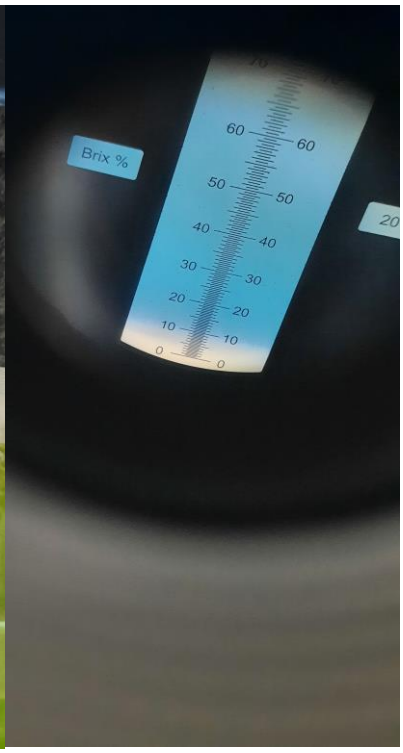
Preparing soil for experimental design layout

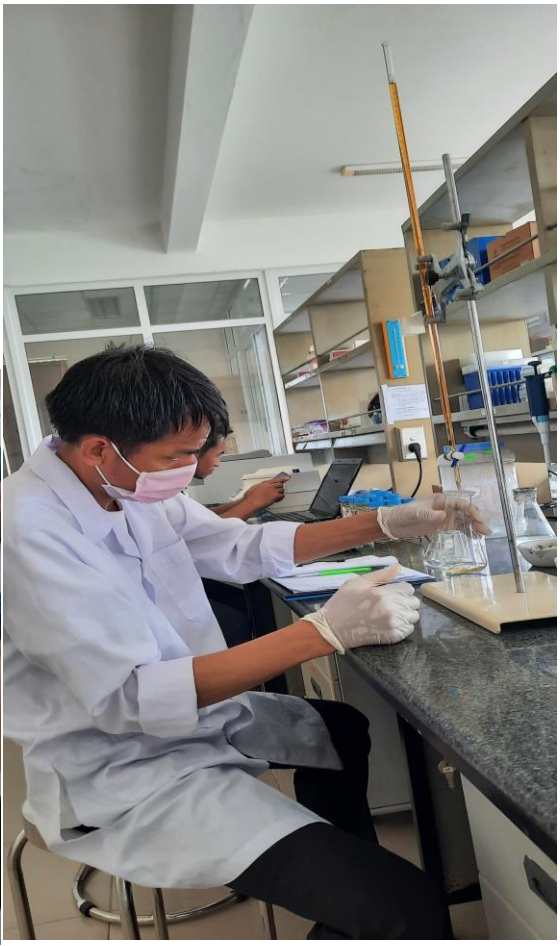


Experiment and data collection of Lettuces

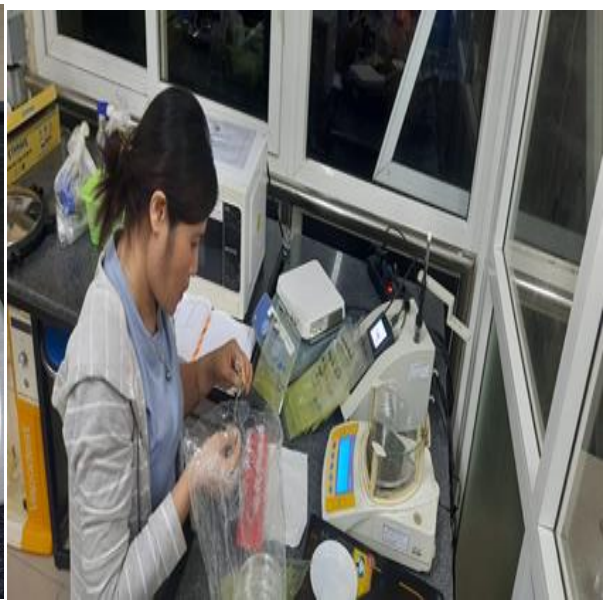
Experiment and data collection of Mustard spinach

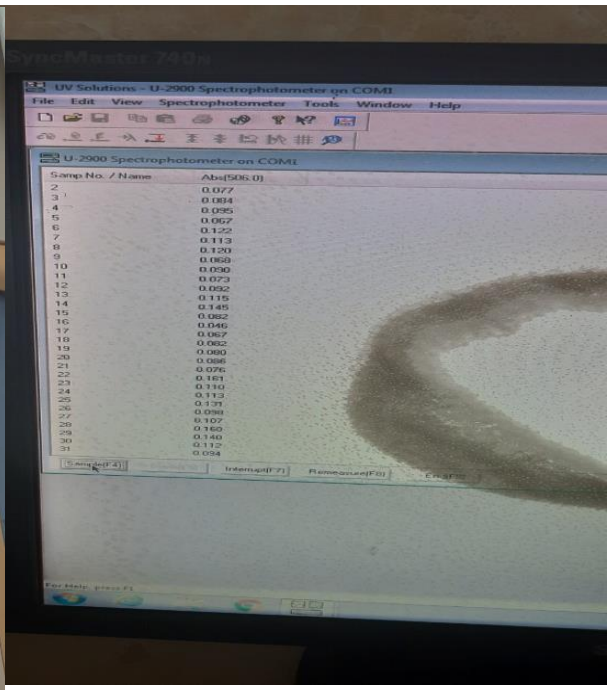
Test Vitamin C and Brix of leafy vegetables





Methodology of analysis Phenolic and Flavonoid from Moringa





Selecting waterlogging tolerance and good characteristics of *M. oleifera* lines for biomass production in Thua Thien Hue and breeding programs.