

**COMPARATIVE ANALYSIS OF DIFFERENT MULCHING MATERIALS ON
SELECTED SOIL PROPERTIES, GROWTH, YIELD, AND QUALITY OF
MORINGA OLEIFERA L. LEAVES**

by

SOKOMBELA ASANDA

THESIS

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SUPERVISOR: PROF AR NDHLALA

CO-SUPERVISOR: PROF MP BOPAPE-MABAPA

CO-SUPERVISOR: DR. K.B. EIASU (UNIVERSITY OF FORT HARE)

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DECLARATION

I declare that the '**COMPARATIVE ANALYSIS OF DIFFERENT MULCHING MATERIALS ON SELECTED SOIL PROPERTIES, GROWTH, YIELD, AND QUALITY OF MORINGA L. OLEIFERA**' thesis hereby submitted to the University of Limpopo, for the degree of **DOCTOR OF PHILOSOPHY (PLANT PRODUCTION)** has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

A handwritten signature in black ink, appearing to read 'A. Sokombela', enclosed within a large, loopy oval shape.

Miss. A Sokombela

27 March 2025

THESIS OVERVIEW

Chapter 1: General Introduction

This chapter provides an overview of medicinal plants, their uses, and their impact on food security. It also includes the study's problem statement, motivation, aim, objectives, and hypotheses.

Chapter 2: Literature Review

This chapter offers a critical and in-depth review of existing literature on the production and origin of moringa. It examines the role of moringa in food security and its benefits, including nutrition, medicine, human food sources, animal feed, biofuel, and water purification. Additionally, it reviews various mulching materials and their effects on soil properties, plant growth and development, yield, and quality.

Chapter 3: Chemical Soil Properties and Enzymes

This chapter explores the effects of different mulching materials on the chemical properties of soil and the activity of soil enzymes.

Chapter 4: Moringa Growth and Yield

This chapter evaluates the impact of different mulching materials on moringa's growth and yield attributes.

Chapter 5: Nutritional Composition of Moringa Leaves

This chapter investigates how various mulching materials affect the nutritional composition of moringa leaves.

Chapter 6: Secondary Plant Metabolites in Moringa Leaves

This chapter provides a detailed analysis of the effects of different mulching materials on identifying and quantifying secondary plant metabolites in moringa leaves.

Chapter 7: General Discussion, Conclusions, and Recommendations

This chapter summarizes the entire thesis, discusses the findings, and offers conclusions and recommendations. Though the chapters have been treated as separate, they are linked studies; therefore, some repetitions between the chapters could not be avoided entirely. All the literature cited in the study is listed in the

reference list. Appendices containing extra but relevant information in the study are presented at the end of the thesis.

ABSTRACT

General Abstract

Food insecurity in Africa is attributed from an underdeveloped agricultural sector heavily dependent on primary agriculture. Agricultural productivity faces diminishing soil fertility, resource constraints, environmental degradation, and insufficient investment in adding value to products and diversifying crops. To improve food insecurity, the production of high valued crops is a necessity. The cultivation of moringa trees in rural communities of South Africa emerges as one of many promising solutions to combat food insecurity and malnutrition, given its nutritional richness and versatility for both dietary enhancement and economic gain. Therefore, development of agronomic techniques that enhance food production is vital. Mulching has been identified as a strategy to reduce weeds and improve crop performance amongst rural smallholder farmers. Plastic mulching has been extensively used to boost the productivity of horticultural crops. Research has primarily concentrated on fruits and vegetables, overlooking other highly nutritious and economically valuable horticultural crops.

A study was conducted to investigate the effects of different mulch materials on soil chemical properties, growth, yield, nutritional quality and metabolite composition of moringa leaves at the University of Fort Hare Research Farm, Alice, and was carried out as a randomized complete block design (RCDB) with six treatments replicated four times with a view to establishing a link between the different treatment and the seasonal variation. The treatment factors included (1) control (NM), (2) plant residues (OG), (3) red plastic mulch (PR), (4) black plastic mulch (PB), (5) green plastic mulch (PG), and (6) white plastic mulch (PW). The results indicated an interaction between treatment and season had no significant effect on the measured soil properties ($p > 0.05$). The treatments showed significant differences in sodium ($p < 0.001$) and potassium ($p < 0.05$), while season was significantly different in exchangeable acidity ($p < 0.001$), sodium ($p < 0.001$), potassium ($p < 0.05$), magnesium ($p < 0.05$), total carbon ($p < 0.001$), total nitrogen ($p < 0.05$) and phosphatase ($p < 0.001$). Black, white, and green plastic mulch exhibited the most significant increase in sodium and potassium compared to the other treatments. Seasonal effects showed that soil properties were higher in summer than autumn except for the phosphatase enzyme.

Plant growth parameters were significantly improved in black plastic mulch compared to other mulch types used in the experiment. For instance, the black plastic mulch resulted in improvements in plant height, stem diameter, chlorophyll content, leaf width, leaf length, petiole width, petiole length, and yield (292 mm, 72 mm, 75.96 mm, 10.7 mm, 24 mm, 35 mm, and 1.84 kg/plot, respectively) compared to un-mulched plots.

Mulching with black plastic significantly enhanced the nutrient composition of moringa leaves, attributed to improved growing conditions facilitated by plastic mulching. For example, the black plastic mulch maintained optimal moisture content (8.29), significantly enhanced proximate nutrients such as ash (14.93%), fat (4.92%), acid detergent fibre (29.87%), neutral detergent fibre (42.99 %), and crude protein (27.93 %) compared to un-mulched plots, and macro-nutrients, including phosphorus, nitrogen, calcium, magnesium, and potassium, were also improved. Conversely, sodium content was higher in red plastic mulch, while copper, manganese, iron, aluminum, and zinc were significantly higher in black plastic mulch. Amino acid analysis revealed that aspartic acid content (2.21%) was highest in black plastic mulch and lowest in red plastic mulch, whereas glutamic acid content (3.56%) was highest in the control and lowest in red plastic mulch. The results demonstrate the potential of plastic mulch technology as a beneficial agricultural practice for improving productivity and optimising nutrient profiles in moringa cultivation, thereby contributing to food security initiatives in African rural communities.

Keywords: Plastic mulch, food security, climate change, smallholder farms, Moringa

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DEDICATION

I would like to dedicate this study to Tanaka Ivanna Sokombela, my baby girl.

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LIST OF ABBREVIATIONS

ADF: Acid detergent fibre

Al: Aluminium

ANOVA: The Analysis of variance

AOAC: Association of Official Analytical Chemistry

Ca: Calcium

CP: Crude protein

Cu: Copper

CV: Coefficient of variance

DM: Dry matter

DMI: Dry matter intake

EA: Exchangeable acidity

Fe: Iron

FMC: Plastic-film mulching

GHG: Greenhouse gas

GMC: Gravel mulching

HIV: Human immune virus

K: Potassium

LDS: least significant

Mg: Magnesium

Mn: Manganese

Na: Sodium

NDF: Neutral detergent fibre

NM: Control

NS: Non- significance

OG: Plant residues

P: Phosphorus

PAR: Photosynthetically active radiation

PB: Black plastic mulch
PG: Green plastic mulch
PM: Plastic mulch
PR: Red plastic mulch
PW: White plastic mulch
RCDB: Randomized complete block design
ROS: Reactive oxygen species
SA: South Africa
SOC: Soil organic carbon
STZ: Streptozotocin
SV: Source of variation
TC: Total carbon
TN: Total nitrogen
Zn: Zinc

CHAPTER 1: GENERAL INTRODUCTION

1.1. Introduction

Indigenous knowledge has been in existence for centuries and adopted by people on how use medicinal plants to fight various diseases and maintain healthy bodies (Roberson, 2008, Balick and Cox, 2020). Hence, harvesting wild medicinal plants is an important economic activity for communities in rural areas and may reduce poverty and malnutrition (Shackleton *et al.*, 2007, Kumar *et al.*, 2021). Due to their important health attributes, medicinal plants growing in the wild have been receiving scientific and commercial attention. As such scientific studies on wild medicinal plants have revealed their unlimited health benefits. As a result, the nutraceutical, pharmaceutical and cosmeceutical industries have intensified utilizing medicinal and food plants in large quantities.

The rise in the need and use of traditional medicine is slowly impacting the growth of some plant species in their wild habitat (Ghimire *et al.*, 2005). Furthermore, a combination of the high frequency of harvesting and climatic changes exacerbates the rate of decline and the possibility of extinction of some plant species used as traditional medicine (Hopping *et al.*, 2018). As a result, the domestication of wild medicinal plants has recently gained momentum. *Moringa oleifera* Lam. (referred to as moringa in the present study) is one of the medicinal plant species that has drawn the attention of nutritionists and health experts (Biswas *et al.*, 2020) while it has also reached an appreciable height in studies related to its domestication and cultivation.

Moringa is a multipurpose tree widely used in traditional medicine, for industrial purposes, home consumption, and animal feed (Ndhlala and Tshabalala, 2023). Foidl *et al.* (2001) reported that every part of the tree is valuable: seeds can be used as a water purification agent; the bark, roots, and flowers contain medicinal properties; and the leaves are the source of vital nutrients that can potentially supplement human food and animal feed. It

is presumed that moringa was first brought to South Africa (SA) during the 1800s through the KwaZulu-Natal port during the development of sugarcane plantations (Ndhlala and Tshabalala, 2023). However, the tree started gaining popularity in SA around 2006 (Lekgau, 2011). Nonetheless, production is still at a developmental stage and is only confined to the backyards (Mabapa *et al.*, 2017). To promote moringa production in South Africa, it is important to establish appropriate agronomic practices like weed, water, and fertilizer management protocols to maximize yield and improve nutritional quality. One sustainable weed management practice involves mulching (Nyambo *et al.*, 2020a). Any physical barrier covering the soil surface can be defined as mulch (Zhang *et al.*, 2021). Mulching suppresses weeds, thus reducing the associated costs such as labour costs, herbicide costs, equipment costs, reduced crop yields, soil health and fertility, environmental costs, training and education (Nyambo *et al.*, 2020b). Furthermore, mulching lowers evaporation, regulates soil temperature, microbes, and fertility, and promotes plant growth and the yield of high-quality produce (Lee and Thierfelder, 2017).

Generally, smallholder farmers use organic materials as mulching material to enhance crop yields with minimum adverse effects on the environment (Raman *et al.*, 2018). Besides reducing soil loss, improving soil fertility, and crop yields, organic mulching has, however, been found to have detrimental effects on the ecosystem and environment by increasing pest incidence and promoting greenhouse gas (GHG) emissions (Daryanto *et al.*, 2018; Nyambo *et al.*, 2020a). Furthermore, adverse effects of surface residue retention on crop performance have been attributed to nitrogen immobilization, waterlogging, and decreased soil temperature, as reported in some environments (Turmel *et al.*, 2014). In addition, organic mulch has been promoted with little success due to the trade-offs associated with the low amount of biomass (Nyambo *et al.*, 2020b).

Most smallholder farmers get low yields of less than 0.6 tons per hectare of maize (Chimonyo *et al.*, 2019). Thus, this translates to low crop biomass of maize stalks that can hardly be enough to cover the soil. On the other hand, the trade-offs associated with

crop residue use in mixed crop-livestock farming systems results in fewer or no crop residues remaining in the field to significantly cover the soil (Nyambo *et al.*, 2020b). Additionally, microbes readily decompose organic mulches, hence the need to continually put in biomass. . Thus, the organic mulches are subjected to decomposition, which is less efficient, labour-intensive, and weather-dependent (Thankamani *et al.*, 2016). In contrast to annual cropping systems, where mulch has an expected longevity of 3–5 months, mulches in perennial systems may be expected to remain in place for several years, depending on the growers' goals. Therefore, it is essential to explore alternative mulching techniques that can potentially boost soil and crop productivity in the Eastern Cape Provinces in South Africa.

There has been a shift from organic mulching materials to polyethylene plastics as mulching materials. Plastic mulching in agriculture has mainly been used in horticultural crop production, focusing on vegetable and fruit cultivation (Kasirajan and Ngouajio, 2012). Different coloured plastic mulches are utilized and have been reported to have potential to improve yields and quality of crops (Amare and Desta, 2021). Different mulch colours vary in their light reflection and transmission properties, thus differ in their moderation of soil and canopy temperatures (Kasperbauer and Loughrin, 2004; Manganelli, 2017). Still, plastic mulch, especially different coloured plastics, is new in moringa production. Therefore, there is a need to explore the benefits and limitations of using plastic mulching in moringa.

The use of plastic mulch has gained attraction in agricultural production (Kasirajan and Ngouajio, 2012, Transparency Market Research, 2013). Plastic mulches are essential in weed management, improving crop quality and yield, regulating soil temperature and moisture regimes, and modifying plant photobiology (Kasirajan and Ngouajio, 2012). Plastic mulching also suppresses weed growth and reduces competition with weeds for water and nutrients (Abouzienna *et al.*, 2008). The water use efficiency (WUE) of potatoes was increased when plastic mulch was used in a study in Northwest China (Qin *et al.*, 2014; 2015). Similarly, plastic mulch was reported to reduce soil bulk density, increase soil porosity, and enhance nutrient contents in a sweet potato study in

Northwest China (Hou

et al., 2015). Zhao *et al.* (2014) noted that plastic mulches decreased soil evaporation, enhanced soil water infiltration, redistributed soil moisture, and relieved water stress (Xiao *et al.*, 2013). However, the plastic colour plays a vital role in regulating the attributes mentioned above. Different coloured plastic mulch differently modifies soil and canopy temperatures due to their light-transmitting or reflecting properties, which can be beneficial, negative, or neutral depending on the crop and environment (Deschamps *et al.*, 2019; Grout and Greig, 2012; Locascio *et al.*, 2005).

Various studies have reported the benefits of coloured plastic mulch on crop and soil properties, but the results are inconsistent and vary from area to area and from crop to crop (El-Beltagi *et al.*, 2022; Zhang *et al.*, 2021). A study conducted in Western Oregon state in the USA revealed black plastic mulch used at pre-planting resulted in improved blueberry yield (Strik *et al.*, 2012a, b). On the contrary, in a study conducted in Georgia, the USA in Bell peppers, the result was significantly higher in silver plastic mulch compared to black plastic mulch during the summer season (Díaz-Pérez, 2010). In another study done in northwest China, black and white plastic mulching increased WUE in potato plants by 31% compared to no mulched land (Li *et al.*, 2018). Fortnum *et al.* (1995) reported that blue plastic mulch had higher soil temperature than red plastic mulch. On the other hand, a study in Syria revealed that transparent plastic mulch increased soil temperature more than black mulch during the initial stages of cucumber growth, whereby the plant has not produced enough leaf canopy to cover the soil (Yaghi *et al.*, 2013). On the contrary, Amare and Desta (2021) reported that black and blue plastics increased soil temperature, while clear and white plastics reduced the temperature. These variations in response to different plastics were only observed in vegetable crops and, therefore signal that it is worth evaluating the reaction of the other coloured plastic mulches in the moringa plantation.

Since ancient times, plants as food and medicinal remedies have been partially attributed to the biological efficacy of secondary metabolites with antioxidant activities such as phenolic compounds, vitamins C and E, and carotenoids (Ndhlala *et al.*, 2010). Phenolic compounds are derived from the shikimate and phenylpropanoid pathways and constitute a diverse and ubiquitous class of plant secondary metabolites characterized by

an aromatic ring and one or more hydroxyl groups (Robards *et al.*, 1999). According to Jahan *et al.* (2018), coloured plastic treatments significantly influenced the phytochemical concentrations and nutritional value of lettuce, resulting in increased chlorophyll, anthocyanin, phenols, and carotenoid contents in lettuce grown in soil covered with black polyethylene mulch.

The inconsistent observations on crop and soil properties are because soil responses to management practices are influenced by soil type, crop rotation, tillage types, and climate (Bescansa *et al.*, 2006). Furthermore, the use of coloured plastic mulch is mainly concentrated in vegetable production; their use in moringa production remains largely unknown. Presently limited studies are focusing on assessing the effectiveness of plastic mulching practices in integrating soil fertility, weed control, and water management for moringa. Furthermore, no study has focused on the effects of different coloured plastic mulches on moringa plant response. With the increasing population in South Africa, it is necessary to devise strategies to fight against poverty, malnutrition, and food insecurity. Therefore, promoting the plantation of moringa could assist in the fight against malnutrition.

1.2. Research problem

There is a need to improve nutrition within the rural communities in South Africa through affordable alternative foods, pharmaceuticals as well as nutraceuticals. Eastern Cape province is characterized by high poverty and malnutrition rates; thus, the government and non-government organizations are driven to promote planting moringa trees in rural households to reduce food insecurity and malnutrition (Pahla *et al.*, 2014, Bell *et al.*, 2024). Moringa is a multipurpose medicinal plant that can potentially be used to reduce food insecurity and poverty. Moringa is consumed as food in the Eastern Cape of South Africa, although it is not as widely recognized or consumed as in other parts of Africa or South Asia (Mashamaite *et al.*, 2021). In the Eastern Cape, moringa is sometimes used in traditional dishes, particularly its leaves, which are known for their high nutritional value (Mashamaite *et al.*, 2023). The leaves are often cooked or dried and used in soups, stews, or teas. Moringa is valued for its rich content of vitamins, minerals, and antioxidants,

making it a popular choice for improving health and nutrition in some communities. However, its usage may not be as prevalent as in other regions where moringa has been traditionally cultivated for food and medicinal purposes (Mashamaite *et al.*, 2023). The tree's production and utilization by smallholder farmers in South Africa are hampered by the lack of knowledge about its potential benefits (Moyo *et al.*, 2011), growth habits, silvicultural management practices, and seed availability (Gadzirayi *et al.*, 2013). To promote moringa production in SA, it is essential to establish appropriate agronomic practices to maximize yield and improve its nutritional quality.

Smallholder farming systems in Eastern Cape, South Africa are plagued by low and declining soil fertility and erratic rainfall, contributing to food insecurity (Phakisi, 2023). Agricultural productivity loss is exacerbated by limited soil water availability caused by prolonged dry periods, low and irregular precipitation, and high potential evapotranspiration, which are common in the semi-arid areas of these provinces (Abaker *et al.*, 2018). Additionally, the constantly increasing global temperatures are predicted to worsen the moisture deficit problems, further affecting agricultural productivity in the region (TerAvest *et al.*, 2015). To attain a sustainable agricultural system, coping strategies that mitigate these problems must be employed in moringa-based cropping systems in the Eastern Cape. Therefore, interventions that seek to enhance and preserve the natural resource base and the environment through improved or appropriate agronomic practices need to be developed.

Amongst the smallholder agronomic practices, weed management is generally one of the most labour-intensive tasks during the cropping season. Farmers tend to remove weeds using a hand hoe, usually more than once in a season. In most cases, they lose the battle against weeds, hence the low crop productivity (Bakare *et al.*, 2021). Mulching has been identified as a strategy to reduce weeds and improve crop performance amongst rural smallholder farmers (Singh *et al.*, 2021). Organic mulch has been vastly promoted, with little success owing to the trade-off issues associated with the small biomass amount at these farms (Nyambo *et al.*, 2020a). Elsewhere, plastic mulching is growing, but mainly in vegetable crops and a few fruit trees (Amare and Desta, 2021). Studies have reported the positive effects of plastic mulch on both crop and soil properties. Therefore, there is a need to undertake this study to evaluate the effects of different coloured plastic mulch on selected soil properties, growth, yield, and quality of moringa under the Eastern Cape soil and climatic conditions.

1.3. Research Aims and Research Objectives

The study was aimed at documenting the influence of different mulching materials on selected soil properties, growth, leaf biomass production, nutrient content, and phytochemical compounds in moringa leaves.

1.3.1. Specific research Objectives

- I. To determine the effect of different mulching materials on selected soil's chemical properties and enzyme activity.
- II. To determine the impact of different mulching materials on the growth and leaf yield of moringa trees.
- III. To determine the effect of different mulching materials on the nutrient content of Moringa leaves.
- IV. To determine the effect of different mulching materials on the phytochemical compounds in moringa leaves.

1.3.2. Research hypotheses

- I. Different mulching materials will not influence the selected soil's chemical properties and enzyme activities.
- II. Mulching will not improve the growth and leaf yield of moringa.
- III. The use of different mulching materials will not improve the nutrient content of moringa leaves.
- IV. The use of mulching materials will not increase the phytochemical compounds of moringa leaves.

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CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

For centuries humankind has relied on medicinal plants to fight various diseases and maintain a healthy lifestyle (Yimer *et al.*, 2019; Fayiah *et al.*, 2024). This is still the case in many under-developed parts of Africa (Mothibe and Sibanda, 2019, Fayiah *et al.*, 2024). Approximately 50% of the African population uses traditional medicine to treat various ailments (Shoko, 2018), while 80% of the southern hemisphere population still relies on the conventional approach to medicine based on herbal drugs (Geck, 2020; Salmon, 2022). According to Peltzer *et al.* (2016), African traditional medicine has treated symptoms of several ailments and conditions such as HIV, diabetes mellitus, hypertension, pain, gynaecological disorders, mental disorders, and asthma (Idm'hand *et al.*, 2022). The World Health Organization (2008) defines traditional medicine as the sum of total knowledge, skills, and practices based on theories, beliefs and experiences indigenous to different cultures, whether applicable or not, that are used to maintain health as well as to prevent, diagnose, improve or treat physical and mental illnesses. Literature postulates that traditional medicine has been on the right track (Geck, 2020; Peltzer *et al.* 2016). Moringa is a multipurpose tree used for various human and animal benefits (Mashamaite *et al.*, 2021); for example, it can be used for industrial purposes, home consumption, animal feed, and traditional medicine (Crini, 2020). The leaves contain essential vitamins and minerals, particularly vitamins A and C. in addition, due to the high nutritional value of Moringa, the leaves could prevent the scourge of malnutrition and related diseases (Trigo *et al.*, 2023).

2.2. Origin and history of Moringa

Moringa, a genus within the Moringaceae family, has gained significant interest from nutritionists and health experts due to its diverse medicinal properties (Chhikara *et al.*, 2021). Among the 13 recognized species in this genus, *Moringa oleifera* is the most widely cultivated (Sujatha and Patel, 2017). Arno (2020) characterizes the plant as a fast-growing, drought-tolerant deciduous tree that can reach heights of up to 3 meters within

its first year and can grow to an average height of 15 meters at maturity (Alavilli *et al.*,

2022). This species is native to the foothills of the sub-Himalayan regions of Northwest India (Singh, 2020) but has been naturalized in various regions, including parts of the Islands and South America (Lenzner, 2022). Historically, the tree has been utilized for medicinal purposes in the Himalayas for at least 5,000 years and has gained recognition as an important dietary supplement globally (Shah, 2019). Furthermore, Mohanty *et al.* (2021) reported that moringa has been widely introduced around the world due to its high nutritional value and commercial significance.

2.3. Physiological attributes of *M. oleifera*

The moringa tree is widely cultivated in tropical and subtropical areas where young seed pods and leaves are used as vegetables. Studies have confirmed that every part of the moringa tree has beneficial properties that can serve humanity (Patil, *et al.*, 2022). In addition, Tshabalala *et al.* (2020) reported that the leaves and roots are a good source of natural antioxidants and contain significant amounts of total phenols, tannins, and flavanols. Jegede (2020) reported that the moringa could propagate through either seeds or cuttings. Moreover, the seeds can be planted directly in the field or seedlings raised in nurseries. According to FAO (2015), moringa thrives best in arid and semiarid environments and still provides versatile, nutritious food throughout the year, however, they can survive in poor soils. They require little water, which implies that they can thrive in arid environments that are unsuitable for increasing other nutritionally rich plants (Mashamaite *et al.*, 2021).

In the first year of the plantation, the tree can produce pods (Hossain *et al.*, 2022). However, on average, the tree can start producing seeds in the second year of planting. Moringa thrives well under temperatures between 25- 30°C (Akintola, 2023), although the plant can withstand temperatures up to 48°C. It grows well in soils that are well-drained and sandy loam soil. The moringa plant can grow in soils with acidic and basic pH status, with pH levels ranging from 5.0 to 9.0. The plant can withstand a range of climatic conditions. However, it cannot thrive in flood- prone areas or areas with poor drainage.

Thus, 250 to 300 mm of rainfall is sufficient for

moringa oleifera water requirements (Mashamaite *et al.*, 2021). The flowers of moringa are fragrant, bisexual (perfect), and characterized by five unequal, thinly veined, yellowish-white petals. Each flower measures approximately 1.0–1.5 cm in length and 2.0 cm in width. The inflorescence develops on slender, hairy stalks, exhibiting spreading or drooping patterns, with flower clusters ranging from 10 to 25 cm long (Dehgan, 2023). Moringa exhibits rapid growth, allowing for the harvesting of leaves a few weeks after planting, with the potential for up to seven harvests per year thereafter (Makkar and Becker, 1997; Alavilli *et al.*, 2022). Since the leaves are produced year-round, they provide essential nutritional support during dry seasons when other crops may be scarce. Additionally, the roots of the plant can be shredded and used as a condiment, imparting a sharp flavour due to their polyphenol content (Bajwa, 2020). The moringa seeds are notable for their richness in essential oils and their potential applications as a biofuel (Dzuvor *et al.*, 2022).

2.4. Production of Moringa in South Africa

It is presumed that moringa was first brought to South Africa (SA) during the 1800s through the KwaZulu-Natal port during the development of sugar cane plantations (Ndhlala and Tshabalala, 2023). Up until this moment, production is still at a developmental stage and is only confined to backyards (Rajkumar, *et al.*, 2021; Sokombela *et al.*, 2022). South Africa has nine provinces; moringa is produced in six regions out of the nine, namely are Limpopo, Gauteng, Mpumalanga, KwaZulu-Natal, Free State, and Northwest (DAFF, 2013; Mashamaite *et al.*, 2023). Among these provinces, Limpopo Province is the pivotal province where moringa was initially introduced, and massive production occurs.

According to Rajkumar *et al.* (2021), commercial production of moringa in South Africa is still at an infant stage, making it difficult to quantify the hectares under production and the volume and value of the commodity. Some farmers, mainly at house-hold level, produce moringa in provinces highly affected by poverty and malnutrition, such as Limpopo, KwaZulu-Natal, and Mpumalanga (Mashamaite *et al.*, 2021). In these provinces, moringa

is used for fighting malnutrition among children and households whose members have ailments such as cancer, constipation, diabetes, chronic headaches and arthritis (Mashamaite *et al.*, 2023). A few companies trade in moringa seeds in the country, but getting data on imports and exports is a challenging for comparisons with other countries. South African seed suppliers import moringa seeds from India, making them available to the producers. Some South African nurseries nurture seedlings for export to countries such as Botswana and Namibia. Based on the preceding, there is a need to utilize the moringa tree beyond the existing cultural boundaries to boost nutrition, fight food insecurity, improve greater profitability, and reduce reliance on expensive imported animal feed.

2.5. Potential of moringa as a food security agent

Globally, agriculture plays a crucial role in improving livelihood, especially in rural communities. As such, moringa could play a significant role in solving malnutrition problems in some parts of the African continent where there is a high percentage of resource-poor communities. With its high nutritive value, every part of the tree is suitable for either nutritional or commercial purposes. The leaves of this tree are worthy of special attention. The leaves are rich in minerals, vitamins, and other essential phytochemicals (Ndhlala and Tshabalala, 2023). Minakshi *et al.* (2021) reported that moringa is considered one of the world's most useful trees because almost every part of the tree is valuable. Milla *et al.* (2021) stated that all moringa food products have high nutritional value. Moreover, the protein content of moringa leaves is high, 20-35% (on a dry weight basis), with significant quantities of all the essential amino acids (Milla *et al.*, 2021). As a traditional item, the moringa plant in Africa can potentially improve nutrition, food security, and rural development by enhancing and sustaining rural households and thus supporting land use (Horn *et al.*, 2022).

Moringa can further be used as an alternative crop for biofuel production. The crop can be grown in any marginal soil, and it contains oil, which can be used for biofuel production (Carrino *et al.*, 2020). Therefore, the promotion of moringa for industrial use can help to solve problems relating to food security, where fertile soil could only be used for crops such as maize and sunflower while marginal soils can be used for moringa production. Moringa has various reported health benefits and has the potential to create employment and income in rural areas through primary and secondary production/processing.

Every part of the plant is beneficial, and both rural and urban people depend on it for their livelihood. The plant can be used as a windbreaker, for erosion control, as a live fence, as an ornamental, or intercropped to provide semi-shade to species requiring less direct sunlight (Mashamaite *et al.*, 2021). Therefore, sustainable management of moringa must balance the short-term needs of the people for their social and economic development and the protection of the natural resource base (Talucder *et al.*, 2024). Protection is a longer-term goal for ensuring that the resource base is utilized wisely. This will ensure that it can continue to provide benefits for improving people's livelihoods and quality of life, reducing poverty, and fostering economic growth into the future on a sustainable basis (Carrino *et al.*, 2020). Moreover, it contributes to a more balanced diet for many people and significantly improves food security for the community at large. It also offers a significant opportunity for the poorest people to earn a living as producers and traders without requiring large capital investments (Audi *et al.*, 2022)

2.6. Commercialization of moringa

Moringa has also been receiving increasing attention from the food processing industries. According to the Global Facilitation Unit (GFU) (2008), African companies manufacturing cereals are interested in adding moringa leaf powder to enrich their products with a low-cost, local source of vitamins and minerals (Singh *et al.*, 2018). Moringa has the potential to save millions of lives in poor countries where malnutrition is prevalent because of its high nutritional value. According to Audi *et al.* (2022), moringa is increasingly becoming

an important source of livelihood for a number of people as several villagers in parts of Matabeleland in Zimbabwe have started nurseries to grow moringa in large quantities for sale to other villagers and other provinces.

Commercial production of immature pods for processing is large in India, with about 1.3 million metric tons produced on 38 000 ha (Siddiq *et al.*, 2022). Commercial production of moringa in South Africa is still emerging, thus, it is difficult to quantify hectares under production, volume, and value of the commodity. However, there are a few farmers who are producing the commodity as food supplements (Lencucha *et al.*, 2020). In South Africa, there are a few companies that trade moringa seeds.

2.7. Attributes of moringa

2.7.1. Medicinal properties of moringa

According to Gupta (2022), moringa is often regarded as a panacea for various health issues, with the potential to cure over 300 diseases. In regions such as India and Africa, various moringa species have been utilized in herbal medicine due to their rich phytochemical contents, which confer medicinal uses on the (Rawat *et al.*, 2024). The phytochemical metabolites present in Moringa are recognized as sources of anti-diabetic, and anticancer compounds, and antiproliferative agents that inhibit the growth of cancer cells (Gopalakrishnan *et al.*, 2016). For instance, Oguntibeju *et al.* (2020) demonstrated that feeding Streptozotocin (STZ)-induced diabetic rats with moringa seed powder resulted in a rapid decrease in blood glucose levels. In their study, rats administered approximately 500 mg of moringa seed powder per kg of body weight exhibited increased levels of antioxidant enzymes in the serum. This suggests that the antioxidants in moringa may reduce reactive oxygen species (ROS) generated in beta-

cells as a result of STZ induction (Oguntibeju *et al.*, 2020). Furthermore, antioxidants derived from moringa have been noted to diminish ROS, thus offering neuroprotective effects (Ghimire *et al.*, 2021). A study conducted in Nigeria found that incorporating Moringa into the diet of rats enhanced protein content while reducing blood urea and creatinine levels, thereby preventing renal dysfunction (Al Muhaimeen, 2022; Afolabi *et al.*, 2024).

Moringa also exhibits potential in treating ulcers, with research indicating reductions of over 80% in gastric acidity and ulcer incidence when administered doses of 500 mg and 350 mg, respectively (Choudhary *et al.*, 2013; Kashyap *et al.*, 2024). Additionally, certain attributes of Moringa suggest its utility as a source of antiviral agents for HIV/AIDS treatment. Gambo *et al.* (2021) reported that Moringa may enhance immune function in HIV/AIDS patients when used as a dietary supplement. However, they emphasized the need for further investigation to confirm its effects on antiretroviral drugs (Twinomujuni *et al.*, 2022).

The seeds of Moringa demonstrate antimicrobial properties, making them effective against bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Vibrio cholera* (Chen and Verdes, 2009; Viera *et al.*, 2010; Al-Jadabi *et al.*, 2023). Every part of the Moringa plant possesses medicinal attributes; for example, the juice from the leaves is known to stabilize blood pressure (Kashyap *et al.*, 2022). A review of the literature indicates that Moringa leaves hold the potential for treating numerous conditions, including asthma, diarrhea, epilepsy, malaria, headaches, flu, and skin diseases (Gambo *et al.*, 2021). Moreover, the roots and bark of Moringa have been identified as cardiac, circulatory, and anti-inflammatory agents (Alia *et al.*, 2022).

2.7.2. Nutritional importance of moringa

Moringa is a nutrient-dense tree, rich in proteins, vitamins, and essential minerals, including calcium, zinc, magnesium, potassium, and iron, all of which are crucial for human growth (Gambo *et al.*, 2021; Patil *et al.*, 2022; Islam *et al.*, 2021; Hossain *et al.*, 2022).). Additionally, Moringa leaves are abundant in ascorbic acid, niacin, β -carotene (vitamin A), and several B vitamins, such as folic acid, pyridoxine, and nicotinic acid (Mbikay, 2012; Shivangini *et al.*, 2022). Vitamin A is crucial for various physiological processes, including vision, reproduction, embryonic growth, and development (Carazo *et al.*, 2021). The β -carotene content in Moringa leaves ranges from 6.6 to 6.8 mg/100 g, comparable to the levels found in carrots (6.9 mg/100 g), pumpkins (3.6 mg/100 g), and apricots (2.2 mg/100 g) (Tohouenou, 2019). The protein content of Moringa leaves constitutes approximately 20–35% of their dry weight, primarily comprised of essential amino acids (Su and Chen, 2020; Cao *et al.*, 2023). These leaves can be consumed directly as food and preserved as dry leaves with minimal nutritional loss over several months (Su and Chen, 2020). Their low caloric value also makes them suitable for individuals who are obese (Kahn *et al.*, 2020).

Moringa is also an important source of vitamin C, with fresh leaves containing approximately 200 mg/100 g of dry mass (Peñalver *et al.*, 2022). This concentration exceeds that of vitamin C in oranges and plays a vital role in the synthesis and metabolism of various compounds in the human body, including tyrosine, folic acid, and tryptophan, as well as the hydroxylation of glycine, proline, lysine, carnitine, and catecholamines (Sarode *et al.*, 2023). Furthermore, vitamin C facilitates the conversion of cholesterol into bile acids, which can lower blood cholesterol levels and enhance iron absorption in the gut by reducing ferric to ferrous iron (Sarode *et al.*, 2023). According to Chambial *et al.* (2013), vitamin C acts as an antioxidant, protecting the body from the harmful effects of free radicals, pollutants, and toxins. However, vitamin C is sensitive to heat and oxygen; thus, its concentration in dried Moringa leaves decreases from 18.7 to 140 mg/100 g of dry weight (Chhikara *et al.*, 2021).

Fenech *et al.* (2019) noted that variations in environmental conditions, genetic factors of the plant, and differences in drying methods, extraction, and analysis techniques may account for the wide range of vitamin C content reported in the literature. Notably, freeze-drying has been shown to better preserve vitamin C from oxidation, with concentrations ranging from 271 to 920 mg/100 g of dry weight in freeze-dried leaves (Hutasingh, *et al.*, 2023).

Fresh Moringa leaves are also a good source of vitamin E, specifically α -tocopherol, containing approximately 9.0 mg/100 g (Özcan, 2020), which is comparable to the vitamin E content in nuts (Ribaud *et al.*, 2019). Vitamin E primarily functions as a liposoluble antioxidant and is involved in regulating gene expression, inhibiting cell proliferation, platelet aggregation, monocyte adhesion, and bone mass regulation (Fenech *et al.*, 2019). The drying procedure can enhance vitamin E concentration, reaching 74.4 and 122.16 mg/100 g of dry weight (Morbiato *et al.*, 2019). Among vitamins of group B, *Moringa oleifera* leaves contain only thiamine, riboflavin and niacin. These vitamins mainly act as cofactors of many enzymes involved in the metabolism of nutrients and energy production. As observed in Table 2.1, their concentration in fresh leaves ranges between 0.06 and 0.6 mg/100 g, 0.05 and 0.17 mg/100 g and 0.8 and 0.82 mg/100 g for thiamine, riboflavin and niacin, respectively (Milla *et al.*, 2021).

Table 2.1: Vitamin and mineral content of moringa leaves per 100 grams of edible portion

Nutrient component	Fresh leaves	Dried leaves
Carotene (Vit. A)	6.78 mg	18.9mg
Thiamin (B1)	0.06 mg	2.64 mg
Riboflavin (B2)	0.05 mg	20.5 mg
Niacin (B3)	0.8 mg	8.2 mg
Vitamin C	220 mg	17.3 mg
Calcium	440 mg	2,003 mg
Calories	92 cal.	205 cal.
Carbohydrates	12.5 g	38.2 g
Copper	0.07 mg	0.57 mg
Fat	1.70 g	2.3 g
Fibre	0.90 g	19.2 g
Iron	0.85 mg	28.2 mg

Magnesium	42 mg	368 mg
Phosphorus	70 mg	204 mg
Potassium	259 mg	1,324 mg
Protein	6.70 g	27.1 g
Zinc	0.16 mg	3.29 mg

Source: Gopalan *et al.* (1989)

Among the B vitamins, moringa leaves contain thiamine, riboflavin, and niacin, which serve as cofactors for many enzymes involved in nutrient metabolism and energy production. Their concentrations in fresh leaves range from 0.06 to 0.6 mg/100 g for thiamine, 0.05 to 0.17 mg/100 g for riboflavin, and 0.8 to 0.82 mg/100 g for niacin (Morbiato, *et al.*, 2019), similar to levels found in various fruits and vegetables (Sarode *et al.*, 2023). Only one study has reported on the concentrations of vitamin B1, B2, and B3 in dried leaves of *Moringa oleifera*, with values of 2.85, 22.16, and 8.86 mg/100 g of dry weight, respectively (Patil *et al.*, 2022).

Notably, the riboflavin concentration in dried leaves appears significantly higher than in fresh leaves, warranting further investigation to confirm these findings. Moringa leaves are recognized as a significant source of sulfur-containing amino acids, such as methionine and cystine, which are often limited in other food sources (Castro *et al.*, 2023). These leaves can be dried and processed into powder, serving as a nutritional additive in soups and meat dishes (Sokombela and Eiasu 2022). Although some nutritional content may be lost during processing, the powder retains a good concentration of vitamin A. An earlier study by Abdoun *et al.* (2022) demonstrated that moringa has low toxicity in animals, which has heightened interest in its utilization as a protein source for livestock. Su and Chen (2020) also identified that Moringa leaves possess high nutritional value essential for animal growth and health, functioning as a galactagogue in dairy production and aiding in parasite load reduction (Prajapati *et al.*, 2022). Consequently, the high concentration of essential amino acids, mineral ions, and vitamins in Moringa makes it an ideal nutritional supplement.

Nutritional analysis reveals that moringa leaves are particularly rich in protein and contain all essential amino acids, including arginine and histidine, which are critical for infants who may not synthesize sufficient protein for their growth needs (Ray and Chaudhary, 2022; Fenton *et al.*, 2020). This is noteworthy, as such comprehensive amino acid profiles are not commonly found in plant foods. The aforementioned research indicates that approximately 30% of children in sub-Saharan Africa experience protein deficiency,

highlighting the potential of Moringa as a valuable food source.

In addition moringa leaf powder effectively prevented and treated malnutrition among pregnant and breastfeeding women, as well as their children, in southwestern Senegal (Idohou-Dossou *et al.*, 2011). Malnutrition is a significant issue in this Senegal, with over 600 malnourished infants assessed annually (Ibrahim *et al.*, 2022). The study revealed that children supplemented with moringa leaf powder maintained and increased their weight, exhibiting overall health improvements. Additionally, pregnant women showed recovery from anaemia and gave birth to infants with higher birth weights. Breastfeeding women also experienced an increase in milk production (Ibrahim *et al.*, 2022). Table 2.2 outlines the recommended daily allowance of moringa intake for parents and children to support healthy bodies.

Table 2.2: Percentage of the recommended daily allowance (RDA) of various nutrients supplied to nursing mothers and a 1–3-year-old child by *Moringa oleifera* leaf powder (6 Tablespoons per day for a nursing mother, 1 Tablespoon three times per day for a 1–3-year-old child).

Nutritional component supplied	RDA	
	Parent (%)	Child (%)
Protein	21	42
Calcium	84	125
Magnesium	54	61
Potassium	22	61
Iron	94	71
Vitamin A	143	272
Vitamin C	9	22

Source: Agbogidi and Ilondu (2012)

2.7.3. Moringa as a human food source

Moringa exhibits significant nutritional value, with nearly every part of the plant recognized for its food potential (Prajapati *et al.*, 2022; Patil *et al.*, 2022). Rawat and Vimal (2023) noted that Moringa seeds can be eaten like peanuts, while, the leaves are acknowledged as a highly nutritious vegetable. Immature seeds can be consumed in a similar way like to green

peas, whereas mature seeds are often fried, added to curries, and sold as essential side dishes or vegetables in retail outlets throughout India (Dhakad *et al.*, 2019). Additionally, moringa flowers are edible and can be prepared by cooking them in combination with other foods or by frying them in a flour batter. Notably, moringa flowers, leaves, and seeds are excellent sources of essential minerals, such as potassium and calcium, which are particularly vital for populations in developing countries (Sokombela and Eiasu, 2022). The literature indicates that young moringa plants can also be utilized as tender vegetables, contributing further to their dietary significance (Abdoun *et al.*, 2022).

In many cultures, staple diets predominantly consist of starchy foods, such as cornmeal, cassava, and millet-based porridges (Oladiran and Emmambux, 2022). Therefore, accompanying side dishes or sauces are crucial from a nutritional standpoint, as they frequently represent the primary sources of protein, vitamins, and minerals in these diets (Rawat and Vimal, 2023). Moringa leaves can be readily integrated into such sauces as a potherb or incorporated in their dried form. In addition, Sokhela *et al.* (2023) noted that moringa presents a cost-effective and accessible alternative food source, demonstrating the substantial nutrient value that can enhance the effects of unbalanced diets, particularly in regions affected by malnutrition (Li *et al.*, 2020). Furthermore, dried moringa leaves are commonly processed into herbal tea, capsules, and powders, which can be ingested to enhance energy levels.

2.7.4. Moringa as an animal feed source

A potential strategy for enhancing the quality and availability of feeds for smallholder ruminant animals during the dry season involves the utilization of fodder trees and shrub forages (Amole *et al.*, 2022). Many of these trees and shrubs can be easily propagated and do not require high management inputs such as fertilizers, pesticides, or advanced technologies. One such promising forage tree is the moringa. The leaves, stems, and twigs of moringa are rich in protein and can serve as a highly nutritious fodder source for cows, sheep, pigs, and goats (Minakshi *et al.*, 2021, Prajapati *et al.*, 2022).

Moringa has been shown to improve the digestibility of other feed consumed by animals, contributing positively to their overall health (Jahni *et al*, 2020). According to Shea (2024), when moringa leaves constituted 40-50% of the feed, milk yields for dairy cows increased by 43-65%, and daily weight gains for beef cattle rose by 30%. Supporting this, Mendieta-Araica (2011) reported that milk production increased to 10 litres per day for cows fed moringa, compared to 7 litres per day for those not receiving moringa supplementation. Additionally, beef cattle fed moringa exhibited an increased daily weight gain of 1,200 grams, in contrast to 900 grams per day for cattle not fed with moringa.

Shea, (2024), emphasizes the importance of balancing the high protein content of moringa leaves with other energy-rich feeds. For instance, cattle feed containing 40-50% moringa leaves should be combined with molasses, sugarcane, young elephant grass, sweet sorghum, or any other locally available energy sources (Chharang, 2022). The maximum protein and fibre content of livestock feed is summarized in Table 2.3.

Table 2.3: Highest amount (%) of protein and fibre in livestock feed considered safe for cows and pigs

Animal	Protein (%)	Fibre (%)
Lactating cow	18	26-30
Beef cow	12-14	36
Lactating sow	16-18	5-7
Meat pig	12-14	5-7

Source: Fuglie (2001)

As indicated in Table 2.3, it is important to recognize that different animal species require specific maximum allowances of Moringa in their diets. For instance, when pigs consume excessive protein, muscle development may occur at the expense of fat production. In cattle, a high protein intake can disrupt the nitrogen cycle and potentially lead to fatal consequences (Nieder and Benbi, 2022). Kassymbek *et al.* (2023) reported that ruminants require nitrogen to support the bacteria in their stomachs; however, excess nitrogen can impair liver and kidney function and may ultimately result in death (Fu *et al.*, 2022).

To enhance the nutritional value of moringa leaves for poultry and swine, the incorporation of the enzyme phytase is recommended, as it facilitates the breakdown of phytates and improves the absorption of phosphorus present in moringa (Abdoun *et al.*, 2022). When moringa constitutes 40-50% of the feed, the average birth weight of local Jersey cattle, typically around 22 kg, can increase by 3-5 kg. However, it is important to note that a higher birth weight may pose challenges for smaller cattle (Kassymbek *et al.*, 2023). To mitigate potential complications, inducing birth approximately 10 days early may be advisable. Additionally, the incidence of twin births has been reported to rise significantly with moringa feed, with occurrences of 3 twin births per 20 births, compared to the usual average of 1 twin birth per 1000 births (Monden *et al.*, 2021; Mutwedu *et al.*, 2022).

Moringa exhibits a high concentration of fibre, specifically acid detergent fibre (ADF) and neutral detergent fibre (NDF), which are critical indicators of animal feed digestibility. ADF and NDF are essential chemical properties that reflect the fibrousness and energy value of feed (Sokombela *et al.*, 2022). The National Research Council of the National Academies recommends a minimum ADF content of 19% and an NDF range of 25-35% for animal feed. Additionally, Janhi *et al.* (2020) suggest that recommended forages should contain ADF and NDF levels below 45% and 65%, respectively. Sokombela *et al.* (2022) reported that dried moringa leaves have ADF and NDF contents of 23.90% and 30.71%, respectively, which fall within the recommended range. Similarly, Moyo *et al.* (2011) reported even lower values, with NDF at 11.40% and ADF at 8.49%. Consequently, the presence of NDF and ADF in animal feed serves as an indicator of feed quality and energy content (Habte *et al.*, 2020).

2.7.5. Water purification attributes of moringa

The World Health Organization has estimated that up to 80% of all diseases and sicknesses worldwide are caused by inadequate sanitation, polluted water and/or unavailability of water (WHO, 2021). In many parts of the world, river water, which can be highly turbid, is used for drinking purposes. According to Okoro *et al.* (2021), moringa has proven

to be one of the most effective plants as a primary coagulant for water treatment and can be compared to that of alum. This turbidity is conventionally removed by treating the water with expensive chemicals, many of which are imported at a huge expense. Natural coagulants have been used for centuries in traditional water treatment practices in certain areas of the developing world (Okoro *et al.*, 2021). There has been great interest in finding suitable inexpensive materials that can be used in water purification systems (Singh *et al.*, 2015). The current methods used in the wastewater treatment plants showed shortcomings including incomplete removal of metal ions, high cost, and requiring a lot of energy.

Powdered moringa seed can be used in the treatment of water because it is rich in antibacterial properties, which make it an essential alternative to toxic chemicals such as aluminum sulfate used in the purification of water (Krupińska, 2020). The use of moringa seeds can be advantageous because they are effective sorbents for the removal of metal volatile compounds in aqueous solutions (Gomes *et al.*, 2022). The use of moringa on a large scale was tested in a drinking water treatment plant in Sri Lanka (Andrade *et al.*, 2021). However, strict procedure must be followed, as several studies have shown that improper use of chlorine treatment combined with Moringa seed powder may result in negative effects such as chemical imbalance, smell and taste on water quality and human health (Simpson and Mitch, 2022).

The moringa seedcake left after oil extraction can be used as fertilizer or in the treatment of turbid water where it is applied not more than 250 mg/l of water (Merwad, 2019). Moringa seed powder can be used as a quick and simple method for cleaning dirty river water. Studies by Saravanan *et al.* (2021) showed that this simple method of filtering not only diminishes water pollution but also harmful bacteria. The moringa powder coagulates the solids matter in the water and suspended bacteria, and these impurities then dissolves to the bottom (**Figure 2.1**). Water from varying sources will need different amounts of moringa seed powder because the impurities present will not be the same.



Figure 2.1: A comparison of non-treated (glass on left) vs. Moringa-seed-treated (glass on right) pond water (WHO, 1985).

Research shows that there are many natural materials available in many communities in the world that can be used to treat water for drinking. Additionally, literature has demonstrated that plant and geological materials can be used to treat any type of polluted water (Delelegn *et al.*, 2018). Thus, it is clear, moringa seeds are ecological, and low cost when compared to the application of synthetic chemicals currently used in water pollution management.

2.7.6. Moringa as a biofuel source

Due to the increasing energy demand and environmental problems associated with fossil fuels, the improvement of alternative fuels and renewable sources of energy is required. Biodiesel can replace petroleum-derived oil, without any sulfur or aromatic compounds and with lower emission of monoxides, hydrocarbons, and particulates. Furthermore, biodiesel can reduce dependence on imported fuels: a crucial problem in developing countries (Saravanan *et al.*, 2021).

The use of moringa seed as a sustainable source of renewable energy for the production of biodiesel is promising. This is because it contains 38-40% of oil and 25-30% moisture with a high quality (Mohammed *et al.*, 2020). Some of the research studies show that moringa seed oil samples have comparable values with oil-rich seed samples used in biodiesel production (Zhao *et al.*, 2019; Mohammed *et al.*, 2020). The foregoing scholars

further state that the fatty acid profile of moringa oil is high in oleic acid. In addition, moringa show resistance to oxidative stress.

These properties make moringa oil a good choice to produce biodiesel after transesterification. Conversion of biodiesel from vegetative oil by transesterification reaction is one of the effective methods of conversion (Kayode and Hart, 2019). In a study conducted in Australia by Singh *et al.* (2020), it was reported that approximately 3 030 kg of oil is required to produce 1000 liters of biodiesel. Furthermore, an equivalent of 3.03 tons/ha of oil seeds can be harvested from dry land, and 6.06 tons/ha can be harvested from irrigated land. Biodiesel production with moringa seed oil is a second-generation production and moringa can grow on degraded land. Therefore, studies suggest that moringa biodiesel is an acceptable substitute to fossil fuels, even when compared to biodiesel derived from vegetable oil of other species (Tabatabaei *et al.*, 2019).

2.8. Types of mulching materials

Many cropping systems are practiced, to improve the yield and quality of crops. Mulching is one of the cropping systems that plays a significant role. It has many advantages related to crop yield and quality attributes. Mulching can be defined as an effective method of manipulating crop growing conditions to enhance yield and improve quality by controlling weed growth, soil temperature modification, conserving soil moisture, reducing soil erosion, improving soil structure, and enhancing the organic matter content accumulation of the soil (Mbow *et al.*, 2020). Lee and Thierfelder (2017) described the mulching practice as an important technique that enhances crop development and yields improvement as it results in suppressing weed growth, nutrient addition, soil structure improvement, and crop quality control. Therefore, it facilitates soil moisture retention, improves soil physical, chemical, and biological properties, and supports the management of temperature fluctuations, as it supplements nutrients to the soil (Awopegba *et al.*, 2017).

Conservation of soil moisture and improving soil nutrition through mulching may be effective tools to save water, and increase production in dryland farming. Mulching materials improve soil physicochemical properties, regulate soil temperature and evaporation, and increase soil moisture content, thus creating favourable soil microclimatic conditions for crop growth (Wong *et al.*, 2021). Amare and Desta (2021) reported that mulches protect the soil from physical, chemical, and biological degradation and reduce irrigation requirements by conserving water. The selection of mulching material is important for crop type, management practices, and climatic conditions. Mulches are categorized according to two mulching types: namely organic mulch and inorganic mulch. An organic mulch consists of natural substances that are easily decomposed with the help of worms, insects, and slugs (Prem *et al.*, 2020) and add nutrient benefits to the soil. Examples of organic mulch include but are not limited to bark, wood chips, pine needles, dry grasses, paddy straw, dry leaves, sawdust, grass clipping, compost, and vermicompost.

Inorganic mulches on the other hand, consist of synthetic materials such as plastics, geotextiles, stones, and rocks. They are generally used because the materials are resistant to decomposition and can stay for an extended period while they add no nutrients to the soil (Prem *et al.*, 2020). In addition, mulches can be further classified as surface mulching, vertical mulching, polythene mulching, pebble mulching, dust mulching, live vegetative barriers, and straw mulching (Patil *et al.*, 2013).

2.8.1 Organic mulching material

Organic mulch can further be categorized into two groups based on its nutrient composition (Chopra and Koul, 2020). These two types of organic mulches are non-nutritional and nutritional. These are classified according to their ability to add nutrition to the soil. According to Muñoz *et al.* (2022), different mulches will affect growing substrates differently due to the differences in nutrient makeup. Organic mulches generally reduce

the soil temperature and conserve soil moisture by slowing evaporation while allowing rainfall to better penetrate the soil (Prem *et al.*, 2020).

2.8.1.1. Non- nutritional organic mulch

Non-nutritional organic mulches include mostly cellulose and contain no significant amount of nutrients. These mulches include, wood chips, pine needles, paper, and pine bark mulches. Bucki and Siwek, (2019) alluded that organic materials such as sawdust, bark, and peat exhibit a low-bulk density of about 0.06-0.30 g cm⁻³ which implies that they are a good source of organic matter. Literature on non-nutritional organic mulches indicates that these mulches can be used and are beneficial to crop growth (Khamare and Marble, 2023). Non-nutritional organic mulch has been reported to improve water status, reduce soil temperature fluctuations, and suppress weed growth (Begna and Yali, 2021). This can potentially enhance root growth and indirectly improve vegetative growth, which can result in increased yields.

Stratton and Rechcigl, (2020), reported that pine bark mulch consistently improved root shape and promoted an increased plant population in comparison with oat straw mulch in White pine (*Pinus strobus L.*) thus, resulting in increased yields. According to Hussain and Luqman, (2022), a pine bark mulch improved the yield of two-year-old apple trees. Similar beneficial effects of mulching through the improvement of the soil environment, resulting in better plant growth and tuber yield of potatoes were observed (Li and Chang, 2021).

Jehan *et al.* (2023) reported that a wood chip mulch applied to one-year-old pecan trees had a significant height increase. According to Jehan *et al.* (2023), soil moisture was notably higher in wood chip mulch areas in comparison to control which were unmulched. Barzegar *et al.* (2002) reported that organic mulch is used to enhance the accumulation of soil organic matter. According to the literature, wheat straw mulches improve the

porosity of the soil by reducing the bulk density. Higher soil porosity may be associated with a higher water infiltration rate. Shaheb *et al.* (2021) reported that higher soil porosity is an important attribute for commercial crop production because it results in improved root growth and subsequent higher nutrient uptake.

Non-nutritional organic mulches can be used to achieve improved growth and increase soil moisture retention, although they do not add nutrients to the soil. However, it can be detrimental to the soil. For instance, paper mulch does not have the attributes of suppressing weed development for up to 25 weeks. When the paper mulch becomes wet it is easily ripped apart and blown by the wind and thereby resulting in the bare soil (Prem *et al.*, 2020). Paper mulch can also trap moisture, thus resulting in a waterlogged growing environment which may restrict air circulation in the soil. Furthermore, straw and paper can pose a fire hazard. Literature that addresses the use of non-nutritive mulches in the production of Moringa is limited, therefore there is a need to explore the influence of non-nutritive effects in the production of moringa. Mulching has been identified as a strategy to reduce weeds and improve crop performance amongst rural smallholder farmers (Singh *et al.*, 2021). However, the effectiveness of the practice is site-specific and depends on the type of material used for mulching.

2.8.1.2. Nutritional organic mulch

Nutritional organic mulch is the type of mulch that can be decomposed and add nutrients to the soil (Larkin, 2020). Straw is the most easily decomposed substrate, and the addition of straw is beneficial to the improvement of soil enzyme activity and the growth of fungi and bacteria (Zhao *et al.*, 2019). Compost consisting of animal excrement has been described as nutrient-rich mulch due to the quantity of organic matter found in the compost (Ayilara *et al.*, 2020). Secondly, vermicompost is another type of nutritional organic mulch that adds nutrients to the soil. Edwards and Arancon (2022) defined vermicomposting as a waste management technique that facilitates the decomposition of

organic materials by earthworms into vermicompost that can be applied as soil amendment substances. Lirikum *et al.* (2022) described vermicomposting as a biotechnological process whereby organic waste is converted into nutrient-rich vermicompost through earthworms. In addition, biochemical degradation of organic matter is facilitated by microbes while earthworms condition substrate and alter biological activity.

Nutritional organic mulches contribute to soil water management, weed control, and fertilization and simultaneously show promise as an eco-friendly approach to crop production. According to Teame *et al.* (2017), organic mulches conserved soil moisture due to increased infiltration and better retention of water and suffocating weed growth, thus enhancing crop growth and development and resulting in a higher yield of *Sesamum indicum* L. In addition, Zhou *et al.* (2019) reported that when wheat straws were applied as a mulch at a rate of 10 Mg ha⁻¹, soil organic matter increased by 19%. Zhou *et al.* (2019) reported that when wheat was grown under a straw mulch in a semi-arid region, the yield increased on a dry weight basis. Okra production was significantly higher under straw mulch, followed by dust mulch over control (Abiodun *et al.*, 2022). Application of straw mulch at 6 t/ ha increased the yield of tomato and okra by 100 and 200 percent, respectively over control (Tanveer *et al.*, 2022). The yield of potato was the highest under paddy straw mulch (27.9%), and also starch content was higher in paddy straw mulch (18.18%) than in no mulch plot.). Even though organic mulches add nutrition to the soil as they decompose, they require systematic replenishment. A detrimental factor that limits the widespread use of organic mulch could be attributed to the feasibility of delivering them to the field and evenly distributing them therein.

Organic mulch has been vastly promoted, with little success owing to the trade-offs associated with the low amount of biomass at these farms (Nyambo *et al.*, 2020b). In annual cropping systems, organic mulching material has an expected longevity period of 3-5 months, while in perennial systems like moringa production, it may be anticipated to endure for several years. However, the organic mulches are readily decomposed by

microbes, hence the need to put in biomass continually. Therefore, it is important to evaluate the use of other types of mulching material.

2.8.2. Inorganic mulch

Inorganic mulches are substrates of synthetic material such as plastics, geotextiles, stones, and rocks. They are generally used because the materials don't decompose, or only break down after extended periods. Tanveer *et al.* (2022) described plastic mulch as a substrate that provides vast positive advantages for the user, for example, they result in increased yields, earlier-crop maturity, higher-quality produce, insect management, and weed control. Coloured plastic mulches are widely used for the production of horticultural crops. The reason for such extensive use of these mulches is mostly because of plastic thermal and light properties.

Intensive vegetative crop production needs more care to combat disfavored environmental factors and biotic and abiotic stresses. Plastic mulches are used to cover horticultural crops to modify the soil temperature and moisture regimes, prevent the entry of rainwater and possibly amend the photobiology of the plant (Sharmila-Bharathi *et al.*, 2018). The phytochrome system is a significant plant detector of environmental light's level, intensity, duration, and colour to adjust its physiology. Plastic mulches primarily affect the field microclimate by altering the surface radiation budget, which may influence plant growth and productivity (Ochege *et al.*, 2022). Sharmila-Bharathi *et al.* (2018) indicated that mulching increases soil temperature, thereby providing a conducive environment for nutrients, metabolites, and energy.

Traditionally, smallholder farmers use organic materials as mulch. However, due to the drawbacks associated with organic materials, it is crucial to explore the different coloured plastic mulch as an alternative mulching material that can potentially boost soil and crop productivity. Sharmila-Bharathi *et al.* (2018) also discovered that black polythene plastic

mulches used in moringa resulted in early growth, development, and advanced flowering. however, the most commonly used plastic mulch is black. However, different coloured plastic mulches are also used and have been reported to improve the yield and quality of crops by various researchers. Manganeli (2017) reported that the use of coloured plastic mulches has the same advantages as black plastic mulches. Furthermore, Zahed *et al*, (2022) explained that coloured plastic mulches contain additional benefits with regards to the distribution of light reflected sun-grown plants, which is related to the quality and quantity of reflected light and modifies gene expression enough to improve the yield and nutrient content of food crops.

Different types of polyethylene-coloured plastic mulches have been used in the farming community for different purposes. Literature reports that previously commonly used plastic mulch colours are black, clear, and white for vegetable production (Choudhary *et al.*, 2022,). With the evolution of advances in research, the plastic colours known currently are mainly, Black, white, green, brown, red, silver, and blue (Amare and Desta, 2021). These colours are consequent with their ability to absorb light and enhance crop physiology. Lamont (2017) reported that black plastic mulch is the most commonly used mulch over the other colours. Black is known for the ability to efficiently absorb the Ultraviolet, visible, and infrared wavelengths of solar radiation released from the sun (Salama and Geyer, 2023) while on the other hand, the white plastic mulch is the opposite of black plastic mulch.

The white plastic mulch reduces the soil temperature and is particularly used in crops that require cooler growing conditions (Lamont, 2017). The red plastic mulch showed superior characteristics of global solar radiation when compared to black, blue, green, and yellow plastic mulches. Variation in the colour of plastic mulches affects spectral balance, quality, and quantity of light that affects many plant growth and developmental patterns including the yield of plants (Paradiso and Proietti, 2022). Literature indicates that different coloured plastic mulch resulted in a significant increase in maturity, total yield, and quality of vegetables (Choudhary *et al.*, 2022).

Mulch film is nearly impervious to carbon dioxide released by roots or decomposition of organic matter in the soil, so it accumulates beneath the plastic mulch (Salama and Geyer, 2023). In addition, the film does not allow the gas to penetrate; it has to escape through the holes punched into the plants (Manzoor *et al*, 2023). Black, white-on-black, reflective, and wavelength-selective mulches will reduce light penetration into the soil. Furthermore, Tadayon and Hosseini (2022) reported that mulching resulted in a 21.7 to 29.8% increase in fruit yield when compared to bare soil. Even though many researchers have investigated mulch application on different vegetable species across the world, the cultivation of moringa under different plastic mulch types is not popular among South African farmers. Therefore, there is a need to conduct a study to determine the effect of using different coloured plastic mulch materials on nutritional and phytochemistry of moringa.

2.9. The effect of mulching on the physical and chemical properties of soil

Cropping season, root zone temperature, crop type, insect pest infestation, and water use efficiency considerations require careful consideration when using coloured plastic mulches (Amare and Desta, 2021). Ultraviolet (UV), visible, and infrared wavelengths of solar radiation are efficiently absorbed, significantly increasing soil heat due to the absorption of a large amount of radiation. Black plastic mulch is the most commonly used mulch, whereas white plastic mulch is the polar opposite of black plastic mulch. It cools the soil and is commonly employed in crops that do not require much heating (Ma *et al*, 2023).

2.9.1. Soil temperature as affected by coloured plastic mulch.

Soil temperature is described as the primary soil attribute affecting crop production. The other soil temperature-dependent factors are nutrient uptake, absorption of water, root growth, and life of soil microorganisms (Sabri *et al.*, 2018). Coloured plastic mulches significantly alter the soil temperature. Amare and Desta (2021) reported the colour of

the plastic mulch contributes significantly to the soil temperature when compared to no mulch. Soil temperature varies considerably with the type of mulching material used, time of the day, and soil depth. The colour of the plastic mulch affects the surface and underlying temperature of the soil. Jones *et al.*, (2021), reported that the soil surface beneath red polyethylene mulch had the highest mean temperature, followed by surface soil on bare plots and beneath the black and silver mulches.

Job (2017) alluded that the colour of the plastic mulch regulates energy radiation behaviour and impacts the microclimate around the vegetable plant. The soil temperature under a plastic mulch depends on the thermal properties such as reflectivity, absorptivity, and transmittance of a particular material to incoming solar radiation (Jones *et al*, 2021). Jones (2019) reported that polythene mulches act as barriers to outgoing thermal radiation while allowing a portion to escape. Black plastic mulch is an opaque black body absorber and radiator, absorbing most ultraviolet, visible, and infrared wavelengths of incoming solar radiation and re-radiating absorbed energy in the form of thermal radiation or longwave length infrared radiation. Much of the solar energy absorbed by black plastic mulch is lost to the atmosphere through radiation and forced convection (Jones *et al*, 2021). El-Beltagi *et al.* (2022) recorded an increase in soil temperature under the different coloured mulches between 3 to 6 °C warmer compared to bare soil. The soil that was mulched with blue plastic recorded the highest soil temperature of 30.7 °C, followed by red 30.1 °C and clear 29.5 °C plastic. However, the silver/black plastic mulch recorded the lowest soil temperature El-Beltagi *et al.* (2022).

The efficiency with which black mulch increases soil temperature can be improved by optimizing conditions for transferring heat from the mulch to the soil. El-Beltagi *et al.* (2022) reported that polythene mulch increased the soil temperature by about 6°C at 5 cm depth and by 4°C at 10 cm depth in groundnut. By contrast, clear plastic mulch absorbs little solar radiation but transmits 85% to 95%, with the relative transmission depending on the thickness and degree of opacity of the polyethylene (Erdmann *et al.*, 2019). Maida *et al.* (2019) reported that transparent plastic mulch used in Chilli pepper

production resulted in higher soil temperatures when compared to no mulch. Contrary to that, Sarkar *et al.* (2019) reported that black polythene film resulted in an increased soil temperature of 2.65°C in comparison to control, olive, silver, white and blue coloured polythene film. Black plastic mulch recorded the highest surface temperature, followed by the blue and transparent plastic mulches in a study on chilli pepper (Maida *et al.*, 2019). The increased soil temperature in black and blue coloured polythene film could be associated with higher heat conductivity and high light absorbent capacity that heated more rapidly.

2.9.2 Soil moisture fluctuations under coloured plastic mulches

The use of mulches for early crops significantly influences moisture conservation and improves soil temperature. The transparent plastic mulch increased soil moisture, while black and blue polythene mulch recorded lower soil moisture content (Sarkar *et al.* 2019). The silver-coloured polyethylene film retained an appreciable amount of soil moisture content than the control (Sarkar *et al.*, 2019). On the other hand, the Olive and blue polythene film conserved similar amounts of soil moisture. The soil moisture retention capacity variation could be attributed to reduced soil evaporation.

Since the soil vapours are trapped within the mulches, thus results in fog formation, which is deposited into the upper layer of the soil. Choudhary Wang *et al.* (2022) reported that the use of different polythene mulch resulted in to increase in the soil moisture content in the chilli field compared to control. The plastic mulch resulted in reduced evaporation partly due to plastic's high degree of impermeability to water vapour. Therefore, soil water evaporative loss is reduced (Tugwell-Wootton *et al.*, 2020). Plastic-film mulch is a globally applied agricultural practice, mainly in vegetable production (Ding *et al.*, 2022). However, its effects on soil physical and chemical properties in relation to moringa plant production have been hardly studied.

2.9.3. Coloured plastic mulches influence the soil pH

Soil pH is a vital chemical attribute that influences plant growth by regulating physiological activities during seed germination and root growth. It affects ion mobility, precipitation and dissolution equilibrium, microbial activity, and soil nutrient availability (Faulkner and Richardson, 2020). The highest values of soil moisture and pH were found when covered with a silver film (Sarkar *et al.*, 2019), while black film provided the higher temperature among other types of film. El-Beltagi *et al.* (2022) reported that the usage of plastic mulch resulted in an increased soil temperature and moisture while improving productivity thereby maintaining the soil organic carbon (SOC) level in temperature- and rainfall-limited semiarid regions by balancing the increased Soil organic carbon mineralization with increased root-derived carbon input.

2.9.4. Different coloured plastic mulch influences the soil bulk density attributes

The use of black plastic mulch in tomatoes resulted in increased bulk density, soil pH, and infiltration rate. However, the straw mulch resulted in a significantly greater infiltration rate and lower pH, bulk density, and surface evaporation. On the contrary, Blanco-Canqui and Ruis (2018), reported a significantly lower soil bulk density range of 1.10 and 1.26 Mg m⁻³ in cultivated plots mulched with clear and black plastic in cocoyam. However, the uncultivated plots with clear and black plastic-film mulch increased bulk density by 1.40–1.45 Mg m⁻³. Zhao *et al.* (2023) reported that incorporating plastic-film mulch in a maize field increased soil bulk density, whereas straw mulch decreased.

The reduced bulk density of straw mulch could be associated with a higher soil organic carbon concentration (Zhou *et al.*, 2019). Soil organic matter has a lower density and thus occupies a larger volume than soil minerals. Only few studies have been published related to the effects of plastic mulches on soil quality and plant growth. Coloured polythene mulches would generate an amiable environment around the moringa plant including pH, moisture, and nutrient accessibility, which will result in

improved crop management for increased productivity. An intervention is required for a better understanding of the physiological knowledge of moringa plant growth as influenced by coloured plastic mulches in comparison to bare soil.

Moisture, temperature, precipitation, and other soil conditions limit crop productivity in arid and semi-arid regions in the tropics and subtropics. Cropping systems are determined by low root zone temperatures, high evaporation rates, and insufficient rainfall. The use of coloured plastic mulches to remedy the concerns mentioned above is generally acknowledged and practiced in the farming community (El-Beltagi *et al.*, 2022). However, their effect is predominantly applied to vegetables. Their impact on medicinal plants such as moringa remains unknown. Coloured plastic films have impacted plant root zone temperature, microbial life, soil characteristics, and moisture levels. Coloured plastic mulches promote plant growth, development, quality, and yield by improving the plant microclimate (Amare and Desta, 2021). Even though coloured plastic mulches have sound effects on some crops, their application has a negative impact on productivity and insect pest management. Even if the use of plastic mulch improves crop yield and quality, it requires careful consideration of factors such as cropping season/mulch interaction, root zone temperature/mulch interaction, plastic mulch colour/crop interaction, coloured plastic mulch/insect pest interaction, and crop type/water use efficiency factors (Amare and Desta, 2021).

2.10. The impact of different coloured plastic mulch on growth and yield of horticultural crops.

Different coloured plastic mulches have been produced and used in various crop production systems to improve crop growth and yield. According to (Amare and Desta, 2021), the primary goal of using coloured plastic mulches is to change the radiation budget and reduce soil water loss. The spectral balance, quality, and quantity of light emitted by plastic mulches influence plant growth and developmental processes, including plant yield (El-Beltagi *et al.*, 2022). Amare and Desta (2021) revealed that the

effectiveness of plastic mulches in reducing the adverse effects of environmental stress

on crops is greatly dependent on the colour of the plastic mulch. The utilization of coloured plastic mulch to improve the microclimate around plants contributes to the improvement of cell expansion and elongation, boosting plant growth and development (Getachew and Bizuayehu, 2021). The impact of coloured plastic mulches on producing an optimal growth environment, such as adequate moisture and root zone temperature, increases plant growth and development. The colours of plastic mulches and seasonal weather conditions considerably impact the pepper plant's fruit yield.

Specifically, black polyethylene plastic mulch resulted in an increased number of leaves, longleaf, and wider leaf breadth in lettuce, followed by white, blue, silver, and olive film (Shah *et al.*, 2018). Plastic mulch has the ability to reflect light, is excellent in weed suppression, and absorbs a large percentage of sunlight and heat during the day (Telkar *et al.*, 2017). As a result, its use eliminates the competition of nutrients by crops and weeds and promotes normal development of the root areas, ensuring good plant growth. Furthermore, Prem *et al.* (2020) agreed that mulching with black plastic is an excellent alternative to hand hoeing, even though it does not necessarily influence the plant height of lavender and thyme. On the other hand, other investigations claimed that black plastic mulch reduced thyme yield compared to no mulch regions (Nejatzadeh-Barandozi, 2020). Black plastic mulch produced the maximum yield in tomatoes, whereas white plastic mulch and no mulch areas produced the lowest (Miles *et al.*, 2019).

Plants on black plastic mulch had the maximum number of fruits and the highest yield. Thus, El-Beltagi *et al.* (2022), reported that plastic mulching appears to be a viable tool to increase chilli production under tropical conditions. Black plastic mulch was observed to produce significantly higher plant fruits of chilli plants in comparison to blue and transparent plastic mulch (El-Beltagi *et al.*, 2022). However, the control which had no mulch resulted in fewer fruits per plant. The increase in fruit yield per plant in mulched plots is attributed to the moisture conservation and improved microclimate beneath and above the soil surface. The improved growing conditions enhance the plant growth and development and produced an increased fruit-bearing environment compared to no

mulched plots. McIntosh *et al.* (2022) reported that mulching significantly improved the number of fruits per plant and reduced the percentage of fruit death compared to unmulched control.

Silver mulching employed in onions increased plant height, leaves, and fresh root biomass, whereas black plastic mulching increased root length more than silver polythene film (Sarkar *et al.*, 2019). Silver plastic film mulching produced better yields in the hot season than black plastic film mulching, but in the cold season, the opposite is true (Yadav *et al.*, 2023). Silver plastic with black strip mulch produced the most fruits per plant during two seasons. On the other hand, silver plastic mulch had the most fruit per plant. Plastic mulching has been reported to reduce the yield capacity of shrubs such as hyssop, garden thyme, lemon balm, and coneflower (Jason and Adams, 2023). Plant yield is directly proportional to crop growth and development performance. As a result, the usage of plastic mulches increased the yield of vegetable plants (Lamont, 2017). This effect is associated with the influence of plastic mulching on the microenvironment and root zone temperature, which promotes cell expansion and growth. Additionally, the active role of enzymes in enhancing plant development contributes to improved plant yield (Wang *et al.*, 2023).

Mulching treatment significantly influences the nutrient content of leaves. The plastic mulch contributes to nutrient retention within the root zone, thus promoting efficient nutrient use by the vegetable crop (El-Beltagi *et al.*, 2022). Otuario *et al.* (2024), reported that black plastic mulch resulted in higher proximate nutrient contents of maize than white plastic mulch. Vitamin C was recorded as the highest in fruits that were mulched using black plastic at the mature green stage. The evaluation and understanding of the potential application of coloured plastic mulches to enhance the crop growth, quality, and yield of Moringa remains largely unknown, despite the plant's high nutritional and economic attributes to society.

2.11. The effect of mulching materials on biological and phytochemical compounds in horticultural crop

Plants have long been used as a source of food and medicine. Farmers are driven to produce healthy food containing appreciable amounts of antioxidant compounds, mainly phenolics, and flavonoids, which can potentially serve human and animal health. The beneficial effects of plant products can be associated with the biological activities of their phytochemical and antioxidant constituents (Jucá *et al.*, 2020). Studies indicated that phenolics observed in fruits and vegetables are essential compounds responsible for antioxidant capacity (Munteanu and Apetrei, 2021). According to Su *et al.* (2019), phenolic compounds can extend shelf life and enhance the quality attributes of fresh fruits, thus delaying senescence induced by oxidative degradation. Jucá *et al.* (2020) reported that moringa leaves are a good source of antioxidants such as ascorbic acid, flavonoids, and phenolics. These bioactive molecules protect radical and reactive oxygen cells and decrease oxidative damage of molecular compounds such as lipids, proteins, and nucleic acids (Tshabalala *et al.*, 2020).

Plants phenolics are described as a defense mechanism against herbivory by animals or micro-organisms containing at least one benzene ring (C6) and one or more hydroxyl groups (Abdelaziz *et al.* 2022),. Rana and Gulliya, (2019) described flavonoids as poly compounds consisting of a group of benzo-γ-pyrone structures occurring in every plant part. They are accountable for antimicrobial, anticancer, and antiaging activities. The flavonoids found in moringa include myricetin, quercetin, kaempferol, isorhamnetin, and rutin (Liu *et al.*, 2022). According to Abdelaziz *et al.*, (2022) quercetin has been advocated as an antioxidant that promotes a scavenging effect on reactive oxygen species released from mitochondria, protecting the beta cells and controlling hyperglycemia ailments.

According to Amare and Desta, (2021), coloured plastic treatments significantly influenced the phytochemical concentrations and nutritional value of lettuce, resulting in increased chlorophyll, anthocyanin, phenols, and carotenoid contents in lettuce grown in soil covered with black polyethylene mulch. Morra *et al.* (2022) reported that the Anthocyanin and flavonoid content of strawberries planted in red plastic mulch was significantly higher than in plots without mulch, and black plastic mulch. However, Morra *et al.* (2022) further alluded to the fact that anthocyanin was not significantly different in white plastic. Amare and Desta, (2021) reported that basil leaves grown over yellow and green plastic mulches contained significantly higher concentrations of phenolics than those produced over the other colours. The inconsistent observations on the phytochemical constituents are because the soil responses to management practices are influenced by soil type, crop rotation, tillage types, and climate (Passaris *et al.*, 2021). Furthermore, coloured plastic mulch is mainly concentrated in vegetable production; their use in moringa production remains largely unknown. Presently there is limited literature focusing on assessing the effectiveness of coloured plastic mulching practice in the phytochemical composition of moringa.

2.12. The effect of weed density and species occurrences under different mulching materials

The ability of plastic mulch to control weed infestation largely depends on the colour of the plastic used. The infrared-transmitting (IRT) mulches afford the weed control of black mulch but are intermediate in increasing soil temperature between black and clear mulch. These mulches warm the soil like clear mulch but without the accompanying weed problem. Transparent and blue plastic mulches encouraged the weed population, which was suppressed under black plastic (Jabran and Jabran, 2019). Amare and Desta, (2021) reported coloured plastic mulch to improve weed control compared to no mulch and clear plastic. For example, a study in California on strawberries revealed that black, yellow, green, and white plastic mulches resulted in 76-100% weed control. Transparent plastic mulch recorded the highest number of weeds (186.5) per m², while black plastic mulch recorded the lowest weed (54.25) per m² (Amare and Desta, 2021).

Getachew and Bizuayehu, (2021) reported that the silver/black mulch provided 98% weed control, followed by black (95%), blue (89%), clear (85%), and red (84%) plastic. In addition, Getachew and Bizuayehu, (2021) reported that silver/black polyethylene mulch eliminated weed development, whereas, in non-mulched plots, weeding was done manually. Zhang *et al.* (2019) also reported the complete elimination of weeds using black polyethylene. According to Getachew and Bizuayehu, (2021), the silver/black and the black plastic mulch produced weeds only through the punch. The high light transmission increased soil temperature and created a microclimate conducive to weed germination. (Amare and Desta, 2021). Moreover, clear plastic mulch can improve soil temperature but suppresses poor weeds. In contrast, white and silver plastic mulches decrease soil temperature but have an excellent weed suppression ability. This is attributed to the low capacity of white, clear, and silver plastic mulches to absorb light (Reddy *et al.*, 2020). Therefore, these variations in weed control are worth exploring in semi-arid regions of South Africa.

2.13. The influence of different mulching materials on soil microbial biomass carbon and enzyme activity

Changes in levels of soil fertility can be measured using active fractions in the soil organic pool, such as soil microbial biomass. An immense diversity of microbes (bacteria, archaea, fungi, insects, annelids, and other invertebrates as well as plants and algae) lives in the soil and are crucial for its biogeochemical processes and function because of exoenzymes (Liu *et al.*, 2021). Microbes are responsible for the recycling of soil nutrients through the breakdown of organic materials and opening channels through which the gas and water exchange occurs. Various studies have reported that microbial community structure and function are sensitive to changes in the soil microenvironment (Malobane *et al.*, 2020; Malobane *et al.*, 2022; Zhang *et al.*, 2022). Total solids, reducing sugar, non-

reducing sugar, vitamin C, and pyruvates were higher in onion crops planted with silver polyethylene film. This aspect was linked to the ability of coloured plastic films to reflect light, which regulates microbial activity by increasing S and K activity photosynthesis (Sarkar *et al.*, 2019). The impacts of low-cost, eco-friendly, and biodegradable mulching materials on soil microbes, nutrient balance, plant growth, and soil erosion should be explored in marginal land. Therefore, management that aims to alleviate water and nutrient limitations in drylands could probably alter soil microbial diversity, community composition, and inter-species interaction, thereby affecting soil productivity and sustainability.

The use of mulch causes changes to soil ecosystems and microbial community functioning through modification of soil microclimate and atmosphere (Bandopadhyay *et al.* 2018). When applied to the soil surface mulch limits the direct effects of rainfall and the sun thus controlling the microbial and enzyme activity (Nyambo *et al.*, 2022). Amongst the different mulching materials available on the market, research has shown that the use of plastic mulch has an influence on the structures and diversity of soil microbial community, and soil micro-ecological environment (Zhu *et al.*, 2018; Bandopadhyay *et al.*, 2018; Wang *et al.*, 2021). However, the results are not consistent with other studies in other climes. For example, Aslam and Haider (2022) in their study conducted in northwestern China found that white plastic mulching increased microbial biomass carbon, bacterial diversity, dehydrogenase activity, and urease activity (**Figure 2.2**).

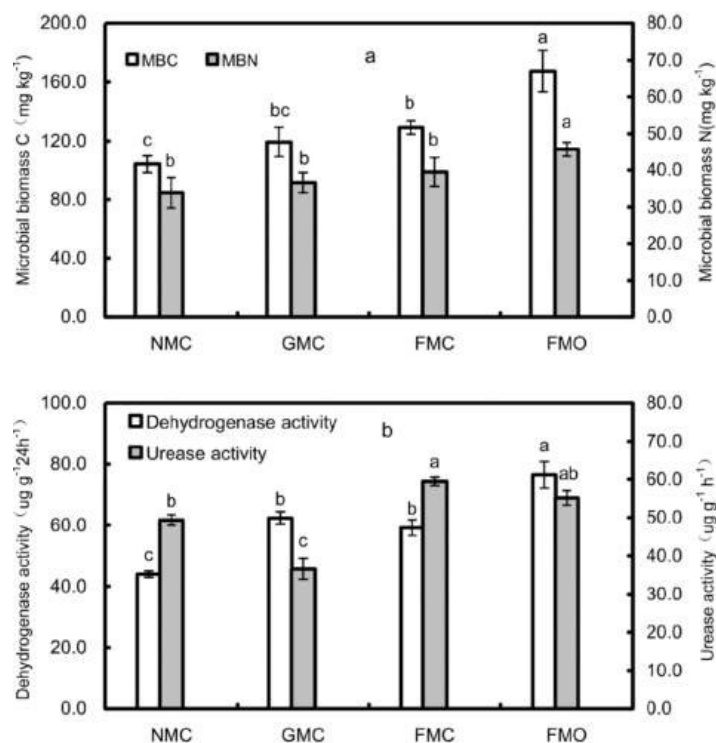


Figure 2.2: The microbial biomass C and microbial biomass N (a) and dehydrogenase activity and urease activity (b) in each treatment. NMC, no mulching with inorganic N fertilizers; GMC, gravel mulching with inorganic N fertilizers; FMC, plastic-film mulching with inorganic N fertilizers; FMO, plastic-film mulching with chemical N fertilizers and organic manure addition.

Similarly, Zhang et al. (2022) reported that when fertilizer is applied, plastic cover increased microbial biomass carbon compared to no cover. On the contrary, a study by Xavier et al, (2020) in Southern Brazil reported that organic mulching increased microbial carbon, microbial nitrogen, and the ratios C_{mic}: C_{org}, N_{mic}: N_{org} and microbial C: N than black plastic mulch. The differences can be due to environmental conditions, type of plastic and the colour of plastic used. Therefore, it is important to evaluate the effect of different coloured plastic mulches under semi-Arid conditions in the Eastern Cape Provinces in South Africa.

One sustainable approach to weed management is the use of mulching (Nyambo et al., 2020a). In addition, mulching helps conserve moisture, regulate soil temperature, enhance microbial activity, improve soil fertility, and promote plant growth, resulting in

higher yields of quality produce. The effectiveness of mulching, however, depends on the type of material used, with organic materials such as crop residues being the most common choice among farmers. There has been a shift towards using polyethylene plastics, particularly in horticultural crop production such as fruit and vegetables (Kasirajan and Ngouajio, 2012). Different colored plastic mulches have also been shown to improve crop yield and quality by altering light reflection and transmission properties, which in turn affect soil and canopy temperatures (Manganelli, 2017). However, the use of plastic mulching, especially colored plastics, remains relatively new in moringa cultivation. Further research is needed to investigate the potential benefits and limitations of plastic mulching for moringa production.

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CHAPTER 3: THE INFLUENCE OF DIFFERENT COLOURED PLASTIC MULCH ON CHEMICAL SOIL PROPERTIES AND SOIL ENZYME ACTIVITY

ABSTRACT

The use of plastic mulch, specifically in climate change-prone semiarid and arid regions, has become a beacon of hope for improving crop productivity. However, research has been devoted to how plastic mulch influences plant growth and yield while neglecting how different colours of plastic mulch affect soil properties. This study examined the impact of different mulch colors on various soil properties, including pH, potassium, phosphorus, calcium, sodium, total carbon, total nitrogen, magnesium, exchangeable acidity, phosphatase, and glucosidase enzymes. The research was conducted over two growing seasons, summer and autumn, at the University of Fort Hare Research Farm in Alice. The experiment was designed using a randomized complete block design (RCBD) with six treatments, each replicated four times. The mulching treatment factors included (1) control without mulch (NM), (2) plant residues mulch (OG), (3) red plastic mulch (PR), (4) black plastic mulch (PB), (5) green plastic mulch (PG), and (6) white plastic mulch (PW). Soils were collected during two seasons, (a) summer and (b) autumn, making the season another factor. The interaction between treatment and season had no effect on the measured soil properties ($p > 0.05$). The treatments showed significant differences on sodium ($p < 0.001$) and potassium ($p < 0.05$), while season played a major role as observed through the significant difference on exchangeable acidity ($p < 0.001$), sodium ($p < 0.001$), potassium ($p < 0.05$), magnesium ($p < 0.05$), total carbon ($p < 0.001$), total nitrogen ($p < 0.05$) and phosphatase ($p < 0.001$). Black, white, and green plastic PM exhibited the largest increase in sodium and potassium compared to the other treatments. Seasonal effects showed that soil properties such as macro and micro soil nutrients were higher in summer than in autumn, except for the phosphatase enzyme. However, this study is one of a few that have investigated the effects of different mulch colours on soil properties, and more studies are needed for a better understanding.

Keywords: Climate change, plastic mulch colour, semi-arid soil properties.

3.1. Introduction

Smallholder farming practices in the Eastern Cape Province of South Africa are plagued by low and declining soil fertility, and erratic rainfall, which contribute to food insecurity. According to Malobane *et al.* (2020) smallholder farms are characterized by degraded soils with soil organic carbon (SOC) less than 1% which results in a decline in agricultural productivity. This situation is further exacerbated by limited soil water availability caused by long dry periods, insignificant and erratic rainfall, and increased potential evapotranspiration, which is common in the semi-arid areas of the Eastern Cape Province (Du Preez and Van Huyssteen, 2020). Furthermore, most smallholder farmers use inappropriate cultivation techniques on already vulnerable marginal soils, exacerbating SOC exhaustion, deteriorating soil quality, and increasing food insecurity (Malobane *et al.*, 2020). The potential economic returns and soil productivity of smallholder farmers may be negatively affected by soil degradation, which leads to increased food insecurity (Kopittke *et al.*, 2019). Therefore, identifying and developing agricultural practices that are suitable for conserving soil and improving its nutritional status is imperative.

The conservation of soil moisture and improvement of soil nutrition through mulching may be an effective way to save water and increase production in dryland farming. Mulching materials improve soil physicochemical properties, regulate soil temperature and evaporation, and increase soil moisture content, thus creating favorable soil microclimatic conditions for crop growth (SK *et al.*, 2020). Amare and Desta, (2021) reported that mulches protect the soil from physical, chemical, and biological degradation and reduce irrigation requirements by conserving moisture. The selection of mulch material is important with respect to the crop type, management practices, and climatic conditions. Therefore, it is important to investigate alternative mulching techniques that can improve soil and crop productivity.

Largely, smallholder farmers use organic mulch materials to improve soil and crop productivity, with minimal adverse effects on the environment (Ngosong *et al.*, 2019). Most

smallholder farmers produce low yields of less than 0.6 tons per hectare (Chimonyo *et al.*, 2019). Therefore, this implies that the low yield results in limited plant residues, which can hardly be used for mulching. On the other hand, the trade-offs associated with crop residue use in mixed crop-livestock farming systems result in fewer or no crop residues remaining in the field to use for mulching purposes (Nyambo *et al.*, 2020). Currently, there has been a shift from organic mulching materials to polyethylene plastics as mulching materials. Studies have reported positive effects of plastic mulch (PM) on both crop and soil properties. Therefore, there is a need to evaluate the effects of different coloured plastic mulches on selected soil properties and enzyme activity.

Soil pH is a vital chemical attribute that influences plant growth by regulating physiological activities during seed germination and root growth. Moreover, it affects ion mobility, precipitation and dissolution equilibrium, microbial activity, and soil nutrient availability (Penn and Camberato, 2019). The highest values of soil moisture and pH were found when covered with a silver film (Sarkar *et al.*, 2019), while black film provided the highest temperature among other types of films. Wang *et al.* (2016) reported that the usage of PM resulted in an increased soil temperature and moisture while improving productivity, furthermore, maintaining the SOC level in temperature- and rainfall-limited semiarid regions by balancing the increased SOC mineralization with increased root-derived carbon input. The impact of low-cost, eco-friendly, and biodegradable mulching materials on soil microbes, nutrient balance, plant growth, and soil erosion should be explored in marginal land. Therefore, management that aims to alleviate water and nutrient limitations in drylands could alter soil microbial diversity, community composition, and interspecies interactions, thereby affecting soil productivity and sustainability.

Soil enzymes indicate the health status of the soil and act as catalysts in organic matter decomposition, nutrient cycling, and availability (Neemisha and Sharma, 2022). Microbial enzymes are affected by climatic conditions in the microenvironment (Zheng *et al.*, 2019), and high soil temperatures are most likely to kill cells, denature, and cause partial sterilization of enzymes, resulting in decreased enzymatic activity (Bharti *et al.*, 2022;

Martinez, 2023). The enzymes β -glucosidase, phosphatase, and urease that occur broadly in the environment, are highly susceptible to alterations in the soil ecological unit and are essential for the cycling of growth nutrients such as nitrogen (Daunoras *et al.*, 2024).

The use of mulch causes changes in soil ecosystems and microbial community functioning by modifying the soil microclimate and the atmosphere (Bandopadhyay *et al.*, 2018). When applied to the soil surface, mulch limits the direct effects of rainfall and the sun, thus controlling microbial and enzymatic activity (Nyambo *et al.*, 2022). Among the different mulching materials available on the market, research has shown that the use of PM influences the structure and diversity of soil microbial communities and the soil micro-ecological environment (Zhu *et al.*, 2018; Bandopadhyay *et al.*, 2018; Wang *et al.*, 2021). However, these results are not consistent. For example, Zhang *et al.* (2021) reported that white plastic mulching increased microbial biomass carbon, bacterial diversity, dehydrogenase activity, and urease activity in northwestern China. On the contrary, the differences could be due to environmental conditions, type of plastic, and the colour of the plastic used. Therefore, it is important to evaluate the effects of different coloured plastic mulches under semi-arid conditions in the Eastern Cape Province of South Africa.

The literature indicates that the benefits of PM have been used predominantly in soil properties, such as temperature and moisture (Mendonça *et al.*, 2021). However, few studies have investigated soil properties and plant parameters in more detail, particularly vegetables, fruits, and medicinal plants. Therefore, there is an urgent need to focus on more detailed soil properties and plant parameters such as pH, nutrients, enzyme activity, microorganisms, and yield after mulching in medicinal plants of high economic and nutritional value. This can be used as a basis for evaluating the combined effects of plastic film mulching and revealing the relationship between soil properties and moringa plants in semiarid areas. The aimed to investigate the effects of different mulch colours on pH, nutrients, and enzyme activity during the cultivation of moringa plants over two climatic/ weather seasons.

3.2. Materials and methods

3.2.1. Description of the experimental site

The study was conducted at the University of Fort Hare Research Farm (32° 46' 282" S and 26° 50' 067" E, at an altitude of 508 m above sea level). The experimental site receives an average annual rainfall of 535 mm and has a mean annual temperature of 18.7°C (Nyambo *et al.*, 2020). The dominant natural vegetation is the *Acacia* species. The temperature and rainfall recorded during the study period are shown in Figure 3.1.

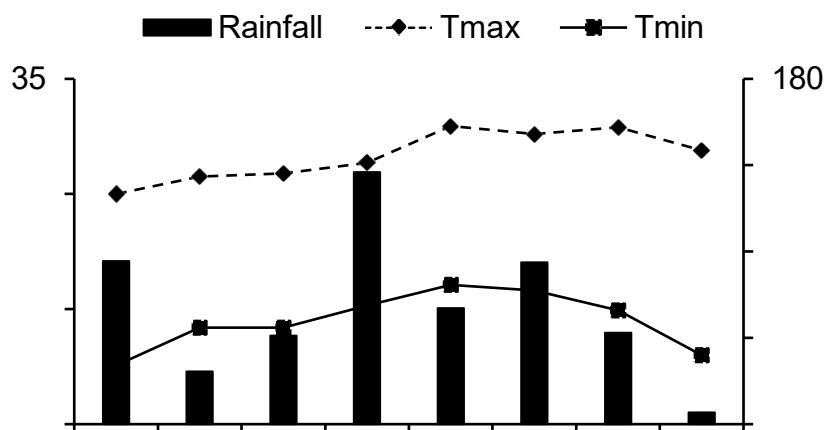




Figure 3.1: Average monthly rainfall, Temperature minimum (Tmin) and maximum (Tmax) readings over the study period of September 2022 to April 2023

3.2.2. Experimental design and treatments

The experiment investigated the effects of various mulching materials on selected soil properties, growth, yield, and quality of moringa leaves. It was arranged in a randomized complete block design (RCBD) with four replications (**Figure 3.2**). The study compared three types of mulching treatments: (1) plant residues, (2) different colored plastic mulches, and (3) no mulch (control). The treatments included: (1) control (NM), (2) plant residues (OG), (3) red plastic mulch (PR), (4) black plastic mulch (PB), (5) green plastic mulch (PG), and (6) white plastic mulch (PW). All plastic mulches had a thickness of 50 μm .

Moringa seeds were planted directly in size 3 black polythene bags filled with Hygromix at a depth of 2 cm, and seedlings were nurtured until they reached a height of 30 cm. Only those seedlings that reached 30 cm in height were transplanted into the experimental field, which was covered with the respective mulch treatments. During the seedling establishment stage, irrigation was applied every 7-10 days. After transplanting, the trees were irrigated three times per week using drip irrigation. Each plant received 10 kg of farmyard manure as a basal application at sowing.

A composite soil sample was collected prior the application of mulch to determine the basic properties of soil before the application of the mulch, and oven dried at 65°C for 24 hours. The experimental field's basic soil properties before mulch application are provided

in **Table 3.1**. Each plot consisted of three rows, with ten plants per row, resulting in thirty plants per plot. The field was designed with an inter-row spacing of 2 m and an intra-row spacing of 2 m, with each plot measuring 18 m in length and 2 m in width. Pathways between plots were 2 m wide, and the distance between blocks was 3 m. The total experimental area was 486 m². Growth data was collected weekly from the middle row of each plot, commencing at 4 weeks after transplanting over the period of 8 weeks and was terminated flowering. In the plastic mulch treatments, cross slashes (approximately 5 cm) were made in the polyethylene sheets through which the seedlings were planted.



Figure 3.2: Indicating the field demarcation and application of plastic mulches.

Table 3.1: Chemical properties of soil before the application of different mulching materials.

pH	P	K	Ca	Na	Mg	Exchangeable acidity	Sand	Silt	Clay	β -glucosidase	Phosphatase
KCl mg kg ⁻¹						%				(μg p-nitrophenol/g)	
5.67	102.7	215	937	34.3	212	0.8	64	14	22	12.82	74.62

3.3. Measurements

3.3.1. Chemical soil parameters

Soil samples were taken after the application of mulch four times during each season and were oven dried at 65°C for 24 hours and stored in tight container until the laboratory analysis. The soil samples were collected from 0-10 cm depth from each plot using a spade and auger. In this study, the soil pH was determined using the 2.5:1 (water: soil) ratio described by Okalebo *et al.* (2006). A sample of 10 g of sieved dry soil was weighed into a 50 ml beaker with 25 ml of deionized water, mixed thoroughly and set aside for 50 minutes. After 50 minutes, the solution was mixed again and allowed to stand for 10 minutes before the pH was measured with a glass electrode pH meter (Crimson Instrumental, Spain).

Macro and micro-nutrients analysis

The molybdenum blue method was used to determine the amount of accessible P after extracting it with 0.03 M NH₄F in 0.10 M HCl (Bray II solution) (Angelova *et al.*, 2022) . Exchangeable K, Ca, and Mg were extracted with 1 M NH₄OAc at pH 7.0. A flame photometer was used to determine the concentration of K. An atomic absorption spectrophotometer (AAS) was used to measure Ca and Mg concentrations.

Soil enzymes

C.β-Glucosidase enzyme activity was measured using the colourimetric method described by Tabatabai (1994). C. Acid phosphatase activity was measured using the colourimetric method described by Kandeler and Gerber (1988). The samples were analyzed at the University of Fort Hare Soil Science Laboratory.

3.4 Statistical analysis

Analysis of variance for was performed using R version 4.3.2 software (R Core Team, 2023) for the data obtained. Where statistical significance was observed, the means were separated using Tukey's test at $p < 0.05$. In addition, a regression analysis was carried out to determine the relationship between soil pH, total carbon, and other soil properties, and only soil properties that showed significant differences were included.

3.5. Results

Table 3.2 presents the Physio-chemical and enzyme results of the sampled points and soils during the study. Significant differences were evident only on sodium ($p < 0.001$) and potassium ($p < 0.05$) with regards to the treatments, while pH, exchangeable acidity, phosphorus, calcium, magnesium, total carbon, total nitrogen, β-glucosidase and

phosphatase did not show significant differences ($p > 0.05$) (**Table 3.2**). However, with regards to seasons, significant differences were observed in exchangeable acidity ($p < 0.001$), sodium ($p < 0.001$), potassium ($p < 0.05$), magnesium ($p < 0.05$), total carbon ($p < 0.001$), total nitrogen ($p < 0.05$) and phosphatase enzyme ($p < 0.001$) (**Table 3.2**). No significant differences were obtained in pH, phosphorus, calcium and β -glucosidase ($p > 0.05$). Lastly, the interaction of season and temperature did not show any significant difference in any soil properties ($p > 0.05$) (**Table 3.2**).

Table 3.2: The Analysis of variance (ANOVA) of chemical soil properties

SV		pH	EA	P	Na	K	Ca	Mg	TC	TN	β-glucosidase	Phosphatase
			%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	µg p-nitrophenol/g	µg p-nitrophenol/g
Treatment (T)	p	ns	ns	ns	***	*	ns	ns	ns	ns	ns	ns
	f	0.65	1.21	1.58	6.44	2.87	0.39	0.69	1.23	1.04	1.47	0.28
Season (S)	p	ns	***	ns	***	*	ns	*	***	*	ns	***
	f	0.22	13.20	3.23	93.01	6.38	1.89	6.76	19.28	6.06	1.92	25.19
T*S	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	f	0.84	0.14	0.86	0.61	1.02	0.57	0.54	2.39	0.33	0.73	0.49
CV												

***, **, * and ns mean statistical significance at $p < 0.001$, < 0.01 , 0.05 and nonsignificant, respectively, SV: Source of Variation, EA: Exchangeable acidity, P: Phosphorus, Na: Sodium, K: Potassium, Ca: Calcium, Mg: Magnesium, TC: Total Carbon, TN: Total Nitrogen.

The exchangeable acidity was significantly ($p < 0.05$) higher in the summer season compared to the autumn season (**Figure 3.2**). The mean exchangeable acidity for both the summer and autumn seasons were 0.75 % and 0.68 %, respectively.

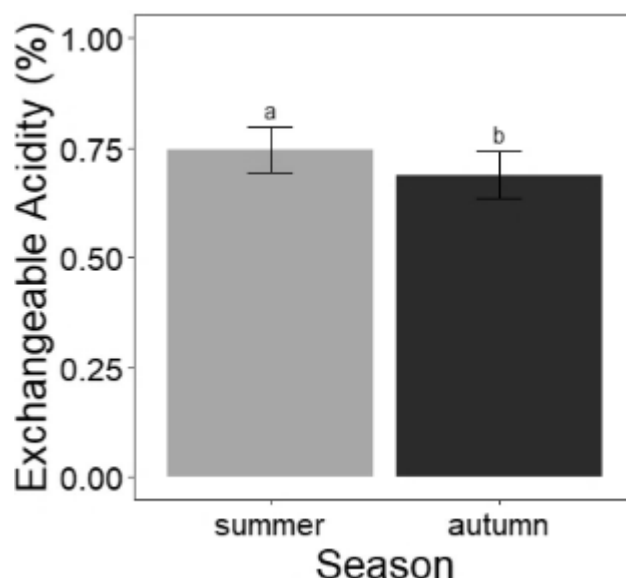


Figure 3.2: Effects of different seasons on exchangeable acidity. Values followed by the same letter indicate no significant differences at 5%. Error bars indicate standard deviations.

Sodium significantly ($p < 0.05$) increased the most in summer compared to autumn (**Figure 3.3a**). The sodium content in the summer season was 76 mg/kg, while in autumn, sodium decreased to 48.6 mg/kg. Among the treatments (**Figure 3.3b**), the highest sodium content was observed under the white PM (70 mg/kg), which did not differ significantly from the black PM, green PM, and plant residues which had a sodium content of 66.7 mg/kg, 66.8 mg/kg, and 56 mg/kg respectively. Conversely, the lowest sodium content was observed when no mulch was added (41.6 mg/kg) which also did not differ significantly from the sodium content under red PM (49.3 mg/kg).

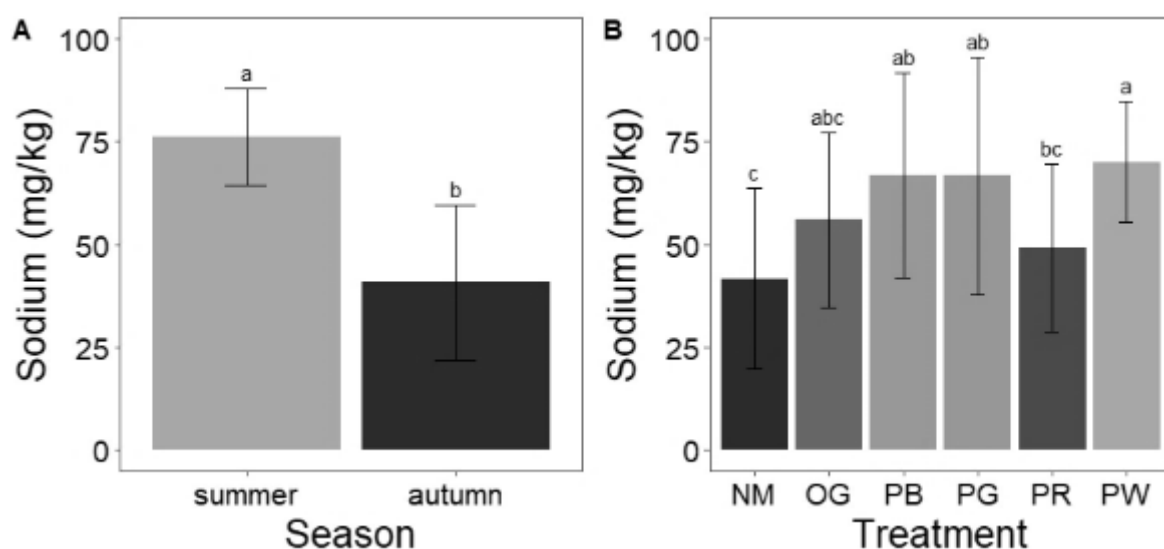


Figure 3.3: Effects of different (a) seasons and (b) mulch treatments on sodium. Values followed by the same letter indicate no significant differences at 5%. Error bars indicate standard deviations. NM: No Mulch, OG: Plant Residues, PB: Black PM, PG: Green PM, PR: Red PM. PW: White PM.

Similarly, sodium and potassium levels were significantly higher in the summer season as compared to the autumn season (**Figure 3.4a**). Specifically, the potassium content in the summer was 186 mg/kg, which decreased to 163 mg/kg in the autumn season. About the treatments (**Figure 3.4b**), the highest potassium content was observed in the black PM treatment (203 mg/kg), which was only significantly different from the control (151 mg/kg). However, no significant differences were observed between the black PM treatment and the other treatments, which had potassium contents of 192 mg/kg, 170 mg/kg, 167 mg/kg, and 164 mg/kg on green PM, white PM, plant residues, and red PM, respectively.

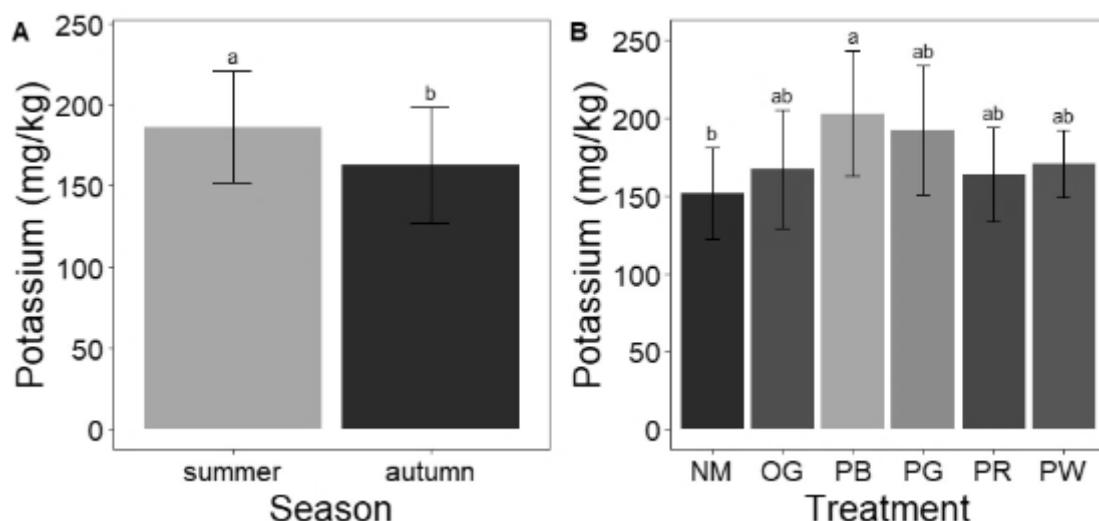


Figure 3.4: Effects of different (a) seasons and (b) mulch treatments on potassium. Values followed by the same letter indicate no significant differences at 5%. Error bars indicate standard deviations. NM: No Mulch, OG: Plant Residues, PB: Black PM, PG: Green P, PR: Red PM. PW: White PM.

The concentration of magnesium was significantly higher during the summer season as compared to the autumn season (**Figure 3.5**). Specifically, the summer season witnessed an increase in magnesium content of 213 mg/kg, whereas the autumn season recorded an increase of 194 mg/kg.

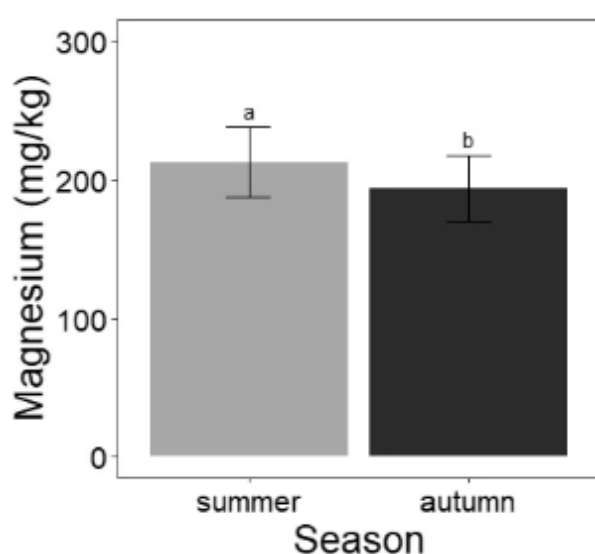


Figure 3.5: Effects of different seasons on magnesium. Values followed by the same letter indicate no significant differences at 5%. Error bars indicate standard deviations.

The levels of total carbon were significantly higher during the summer season as compared to the autumn season (**Figure 3.6**). Specifically, the summer season exhibited a total carbon content of 0.75%, while the autumn season had a lower total carbon content of 0.67%.

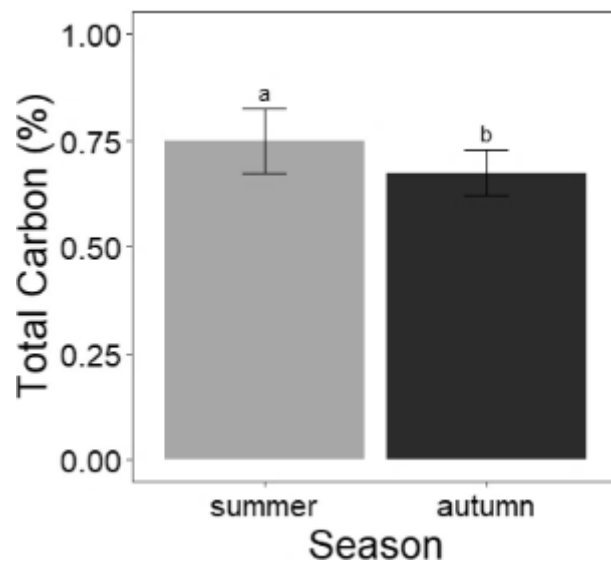


Figure 3.6: Effects of different seasons on total carbon. Values followed by the same letter indicate no significant differences at 5%. Error bars indicate standard deviations.

Similarly, the summer season also demonstrated a significant increase in total nitrogen content as compared to the autumn season (**Figure 3.7**). The summer season had a total nitrogen content of 0.084%, while the autumn season recorded a total nitrogen content of 0.076%.

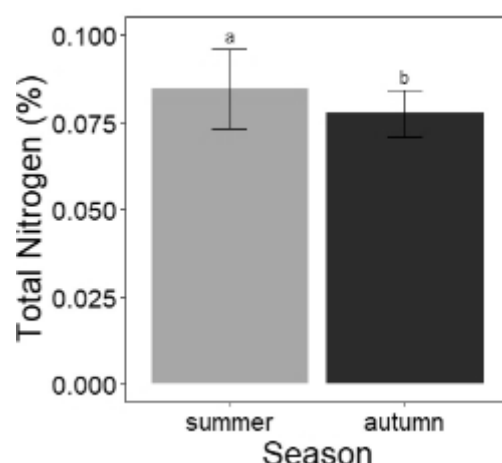


Figure 3.7: Effects of different seasons on total nitrogen. Values followed by the same letter indicate no significant differences at 5%. Error bars indicate standard deviations.

Unlike other soil properties, the phosphatase enzyme exhibited a significant decrease during the summer season compared to the autumn season (**Figure 3.8**). The phosphatase enzyme demonstrated a level of 76.8 $\mu\text{g p-nitrophenol/g}$ during the autumn season, whereas it recorded at 71.4 $\mu\text{g p-nitrophenol/g}$ during the summer season.

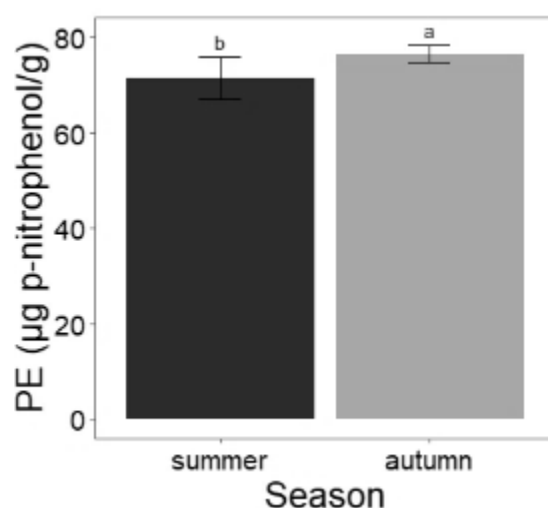


Figure 3.8: Effects of different seasons on phosphate enzyme. Values followed by the same letter indicate no significant differences at 5%. Error bars indicate standard deviations.

The regression results indicated that there was a significantly positive relationship between pH and β -glucosidase enzyme (**Figure 3.9a**), magnesium (**Figure 3.9b**), calcium (**Figure 3.9c**) in the autumn season. Specifically, an increase in soil pH resulted in an increase in these properties by 42%, 49%, 59%, and 55%, respectively.

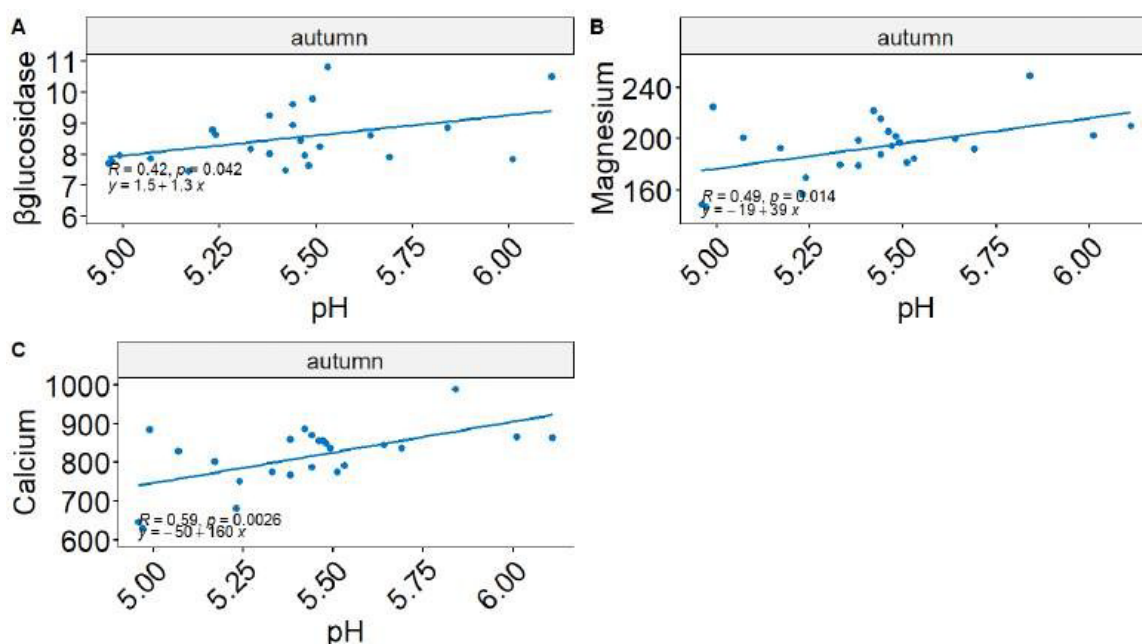


Figure 3.9: Regression relationship in autumn between pH and (a) β glucosidase enzyme, (b) magnesium, and (c) calcium.

However, the relationship between total carbon and other soil properties was significant only in the autumn season for total nitrogen (**Figure 3.10a**), calcium (**Figure 3.10c**), and exchangeable acidity (**Figure 3.10d**), and only for total nitrogen (**Figure 3.10b**) in the summer season. This suggests that an increase in total carbon leads to an increase in total nitrogen (autumn) by 46%, total nitrogen (summer) by 50%, calcium by 41%, and exchangeable acidity by 58%.

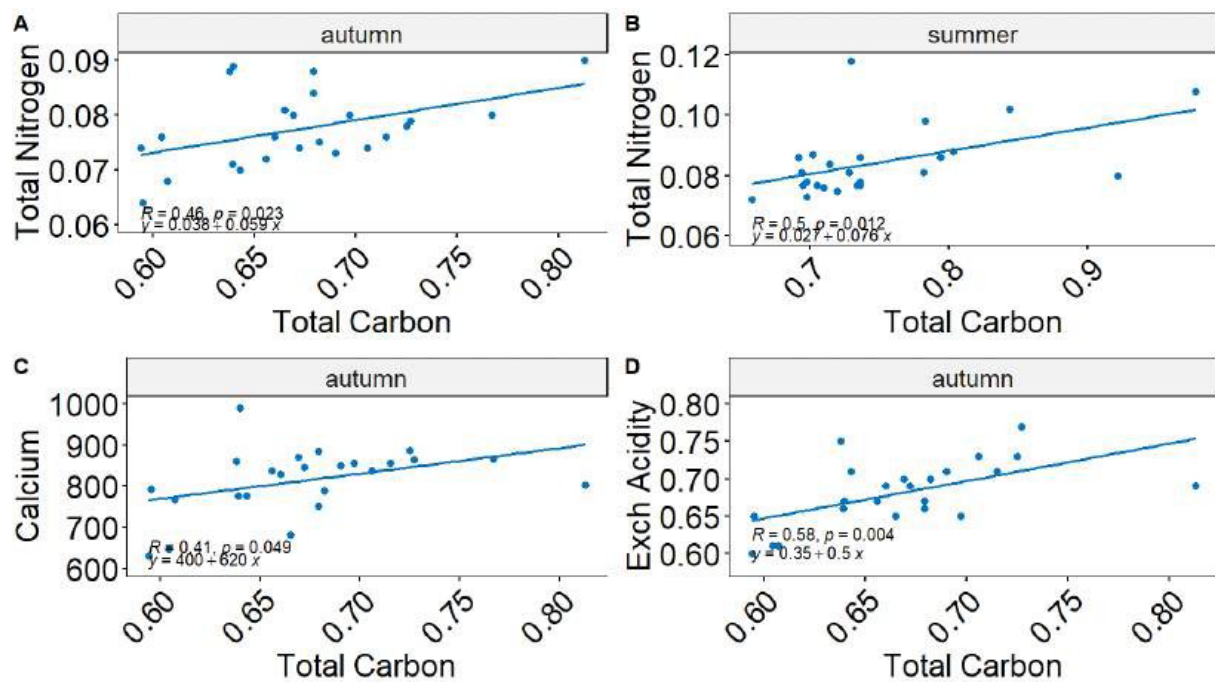


Figure 3.10: Regression relationships between total carbon and (a) total nitrogen (autumn), (b) total nitrogen (summer), (c) calcium, and (d) exchangeable acidity.

3.6 Discussion

The inclusion of PM in agriculture has played a critical role in improving production efficiency, especially in low-rainfall regions where fruit and vegetable cultivation is practiced. Nevertheless, the efficiency of PM largely depends on several factors, and among them, the colour of the PM is critical, and its effects on soil properties has been barely discussed in the literature. Thus, the current study investigated the effects of different PM colours on soil properties, using data collected during different seasons.

The results of this study revealed unexpected findings, where the plastic mulch (PM) treatment factor, with the exception of sodium and potassium, and the interaction between season and temperature did not show significant differences (**Table 3.2**). The trend of some soil properties being significant and others not significant conflicts with previous studies. For instance, Sarkar *et al.* (2019) investigated black, white, blue and silver PM relative to no mulch on soil properties and observed significant differences in all soil properties measured (N, P, K, S and pH). Amare and Desta (2021) observed significant effects on N, while no significant effects on available P and available K

during a comparison

study between black and white PM relative to the control. The researchers did not report any significant differences in soil C levels between the two seasons, but pH, available K, available P, and N exhibited varying levels of significance across different collection dates.

PM are expected to enhance soil chemical properties primarily due to their capacity to prevent leaching and evaporation, which are primarily influenced by high rainfall and elevated ambient temperatures. For the current study, the rainfall received during the experiment may have had less impact on leaching in the control treatment, making no difference from the PM treatments. The temperature range of 10.1 – 30.9°C with an average of 21.1°C rainfall recorded during the period in which the study was conducted may not have had a significant effect on evaporation. Additionally, as the crops grow and provide shade from their canopy, they tend to mitigate the effects of high rainfall and elevated temperatures on the soil. Furthermore, in this study, the impact of soil chemical properties was restricted due to the absence of fertilizer application. Other authors have also reported that during the later stages of growth, demands for crop nutrient increases, which reduce their presence in the soil after plant uptake (Sintim *et al.* 2019; MingFu *et al.* 2022).

The significant differences observed on sodium and potassium with regards to the treatments was unique, in that many of the chemical properties followed a non-significant trend. However, the interesting part was the dominance of black PM on both sodium and potassium (though it was slightly lower than white PM) relative to other mulch treatments. This may confirm the general argument in the literature of the dominance of black PM on high yield increase as a result of soil improvement. Additionally, plant residue treatment was also inferior to other treatments. A study by Pandey *et al.* (2016) reported that rice husks (plant residue) had relatively lower available N, P, and K than white and black PM, although it had higher pH, electrical conductivity and OC than these PM. In the current study,

river grass was used as an organic plant residue, which might have less effect in influencing soil properties.

Unlike treatments, several soil properties were significantly influenced by the season as a factor (exchangeable acidity, sodium, potassium, magnesium, total carbon, total nitrogen, and phosphatase). These properties increased during the summer season than during the autumn season, except for the phosphatase enzyme activity which was *vice versa*. Although the study was unable to find the exact explanation for the increase in nutrients during summer than autumn, it may be attributed to the time of data collection. The data was collected under during one growing season i.e. once at summer and once at autumn. Therefore, as the demand for nutrients typically increases during the latter stages of crop growth, there was a greater decrease in nutrient levels during the autumn compared to the summer period when crop demand was still relatively low. The rise in phosphatase enzyme levels could be attributed to the moderate temperatures experienced during the autumn compared to the summer season. Thus, autumn is more favourable for both enzymatic and microbial activities. It is anticipated that litter production will be higher in autumn than in summer which is a significant stimulator for enzyme activities. However, these results require further investigation to fully understand how soil properties differ seasonally under different PM colours.

The purpose of the regression analysis was to understand if there can be a relationship between soil pH and total carbon on other soil chemical properties and only a few properties showed a relationship from the current study. Soil pH plays a critical role in determining nutrient availability, with lower pH levels reducing nutrient availability and higher pH levels improving it. In this study, it was discovered that only β -glucosidase enzyme, magnesium, and calcium exhibited a significant linear relationship, while other nutrients did not show any significant relationship. The observation of no relationship between other nutrients and soil pH is unexpected and difficult to explain, but we hypothesize that this may be due to the pH measurement in this study (<6) as a result of the acidic nature of the soil. In addition, carbon showed significant effects on properties such as total nitrogen,

calcium, and exchangeable acidity. The increase in total nitrogen because of total carbon alludes to the fact that both are part of the soil organic matter complex and during decomposition, their content increases. The increase in the exchangeable acidity (H^+ and Al^{3+}) can be explained by the presence of negative functional groups that interact with H^+ and Al^{3+} in the exchangeable complex.

3.7. Conclusion

Despite the extensive use of PM to enhance crop productivity in arid and semiarid regions, the influence of different PM colours on soil properties remains unknown. This study aimed to understand how different PM colours affect soil properties, using data collected over two different seasons. The results showed that PM colour had limited effects on soil properties, which was evidenced by no significant differences observed except for sodium and potassium. In properties that showed an effect, black, white, and green PM showed a higher effect in increasing these properties than the other treatments. In addition, the results also showed a seasonal influence on soil properties under PM treatments, with the summer season having increased soil properties relative to the autumn season. Overall, this study is one of the few to focus on the effects of different PM colours on soil properties, and more studies are needed for a better understanding.

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CHAPTER 4: THE IMPACT OF DIFFERENT MULCHING MATERIALS ON THE PLANT GROWTH DEVELOPMENT AND LEAF YIELD OF MORINGA TREES.

ABSTRACT

Food insecurity in Africa persists due to an underdeveloped agricultural sector, reliance on primary agriculture, declining soil fertility, limited resources, environmental degradation, and insufficient investment in value addition. While plastic mulching has improved productivity in horticulture, research has mainly focused on fruits and vegetables, overlooking other valuable crops. Moringa production in South African rural communities offers a promising solution to food insecurity and malnutrition due to its nutritional benefits and commercial potential. This study investigated the impact of different coloured plastic mulches on the growth, development, and yield of moringa at the University of Fort Hare Research Farm, Alice, South Africa. Results indicated that different coloured plastic mulches significantly enhanced the growth and yield of moringa. Specifically, trees mulched with black plastic were notably taller (292 mm) compared to the shortest trees observed in the control (79 mm) after 8 weeks of transplanting. Additionally, black plastic mulch resulted in improvements in stem diameter, chlorophyll content, leaf width, leaf length, petiole width, petiole length, and yield (72 mm, 75.96 mm, 10.7 mm, 24 mm, 35 mm, and 1.84 kg/plot, respectively) compared to un-mulched plots. Mulching with black plastic, among the treatments tested, significantly enhanced the growth and yield of moringa. These findings underscore the potential of plastic mulching as a beneficial agricultural practice for improving productivity in moringa cultivation, thereby contributing to food security initiatives in African rural communities.

Key words: Colour, Food insecurity, Growth, Horticultural crops and yield.

4.1. Introduction

The increase in the need and use of traditional medicine is slowly affecting the growth of some plant species in their wild habitat (Chen *et al.*, 2019). A combination of the high frequency of harvesting and climatic changes exacerbates the rate of decline and the possibility of extinction of some plant species used as traditional medicine (Groner *et al.*, 2022). As a result, the domestication of wild medicinal plants has recently gained momentum. Moringa is one of the medicinal plant species that have drawn the attention of nutritionists and health experts (Biswas *et al.*, 2020) and it has also reached a height in studies related to its domestication and cultivation. Production of moringa by smallholder farmers in rural communities is essential due to its substantial economic and nutritional benefits, which help mitigate food insecurity and malnutrition (Sokombela *et al.*, 2022). However, smallholder farmers face challenges in moringa production, particularly with weed management, due to the plant's slow germination and growth characteristics (Mashamaite *et al.*, 2024).

Mulching has been identified as a potential strategy to enhance crop performance and control weeds (Singh *et al.*, 2021). It influences soil moisture, temperature, fertility, and impact plant growth and phytonutrient contents (Ngosong *et al.*, 2019). Smallholder farmers use organic materials as mulching material to enhance crop yields with minimum adverse effects on the environment (Raman *et al.*, 2018; Ngosong *et al.*, 2019). Besides reducing soil loss, improving soil fertility, and crop yields, organic mulching has, however, been found to have detrimental effects on the ecosystem and environment by increasing pest incidence and promoting greenhouse gas (GHG) emissions (Daryanto *et al.*, 2018; Nyambo *et al.*, 2020a). Furthermore, adverse effects of surface residue retention on crop performance have been attributed to nitrogen immobilization, waterlogging, and decreased soil temperature, as reported in some environments (Setia *et al.*, 2020). In addition, organic mulch has been promoted with little success due to the trade-offs associated with the low amount of biomass (Nyambo *et al.*, 2020b). Therefore, it is essential to explore alternative mulching techniques that can potentially boost soil and crop productivity.

Plastic mulching has been widely used to enhance crop productivity of horticultural crops, but research has predominantly focused on fruits and vegetables, neglecting other high-nutritional and economically valuable horticultural crops. According to Amare and Desta (2021), the primary goal of using coloured plastic mulches is to change the radiation budget and reduce soil water loss. The spectral balance, quality, and quantity of light emitted by plastic mulches influence plant growth and developmental processes, including plant yield (Ding *et al.*, 2021). Amare and Desta (2021) revealed that the effectiveness of plastic mulches in reducing the adverse effects of environmental stress on crops is greatly dependent on the colour of the plastic mulch. The utilization of coloured plastic mulch to improve the microclimate around plants contributes to the improvement of cell expansion and elongation, boosting plant growth and development (Getachew and Bizuayehu, 2021). The impact of coloured plastic mulches on producing an optimal growth environment, such as adequate moisture and root zone temperature, increases plant growth and development in moringa production is under-studied.

Existing research on colored plastic mulches in some horticultural crops mainly focused on fruits and vegetables. For example, black plastic mulch improved blueberry plant growth (Strik *et al.*, 2012a), Shah *et al.* (2018) in lettuce, (Karki *et al.*, 2020b) in potatoes and cucumber, while red plastic mulch enhanced growth in red bell peppers and lettuce (Manganelli, 2017). Conversely, black plastic mulch reduced thyme yield (Taylor *et al.*, 2008), and silver plastic mulch increased bell pepper yields compared to black. Manganelli (2017) reported that tomato plants grown with red and black mulch had increased fruit yields, while plants grown with white and silver-coloured mulch had more foliage.

Adekaldy *et al.* (2021), reported that plastic mulching is an effective method for boosting chilli production in tropical climates. For instance, black plastic mulch resulted in a significantly higher fruit count for chilli plants compared to blue and transparent mulches. In contrast, plants without any mulch produced fewer fruits. The increased fruit yield in mulched plots is attributed to improved moisture retention and improved microclimate conditions both above and below the soil. These enhanced conditions

support better plant growth and development, leading to more fruit production than in no mulched plots. The positive impact of plastic mulches on the plant's microenvironment and root zone temperature facilitates cell expansion and growth (Wen *et al.*, 2023). The study aimed to examine how various mulching materials affect the growth and leaf yield of moringa trees.

4.2. Materials and Methods

4.2.1. Description of the experimental site

The experiment in this section was conducted in the same field as described in Chapter 3 using the same design.

4.3. Data collection

4.3.1. Plant growth parameters

Parameters such as plant height, stem diameter, chlorophyll content, dry and fresh leaf biomass were measured to determine the growth and yield performance of moringa plants. Root collar diameter was measured using a Vernier caliper, and plant height was measured using a meter ruler. Chlorophyll content was measured using a chlorophyll (SPAD) meter (Konica Minolta model SPAD-502). The SPAD N readings were obtained from the youngest fully extended leaves (Donnelly *et al.*, 2020) of three randomly selected Moringa plants inside the net plot. The data was collected bi-weekly, 4 weeks after transplanting. Biomass yield was determined by destructively sampling the moringa leaves before the flowering stage. Fresh biomass was determined by weighing the leaves soon after harvesting, while dry biomass yield was determined after weighing the harvested dried leaves. The leaves were oven dried at 65 °C for 48 hours.

4.4 Statistical analysis

Data collected were subjected to statistical analyses using JMP 15.0 statistical software (SAS Institute, Inc., Cary, NC, USA). Means were separated using Turkey's

test and significant differences were identified at three probability levels; $p < 0.05$, $p < 0.01$ and $p < 0.001$. The study was done over eight months; therefore, time was introduced as an extra factor when analyzing for growth. A two-way ANOVA was carried out to determine significant influence of mulching on growth and yield of moringa

4.5. Results

Application of mulching treatments showed significant differences, the significant differences were identified at three probability levels; $p < 0.05$, $p < 0.01$ and $p < 0.001$ in plant growth (**Table 4.1**). The growth of moringa was significantly influence by the application of the mulch over time. However, there was no significant interaction between the different mulching types studied on growth parameters over time.

Table 4.1: Analysis of variance (ANOVA) for plant growth parameters as influenced by mulch over time.

Source of variation	Plant height	Stem diameter	Chlorophyll	Leaf width	Leaf length	Petiole diameter	Petiole length
Mulch	***	***	***	***	***	***	***
Time	***	***	***	***	***	***	***
Mulch x Time	***	*	ns	ns	ns	ns	ns
CV (%)	8.99	17.03	9.56	17.76	16.27	18.18	13.49

*, **, *** Significance at 0.05, 0.001 and 0.01 probability level, respectively; CV: coefficient of variance.

Photographs taken at various stages of moringa growth to document morphological development and assess the impact of mulch on plant growth (Figure 4.1). Each photo was systematically annotated to track changes in plant height, leaf number, stem diameter, and general plant vigor. In addition to visual documentation, growth data such as plant height, and biomass were regularly collected to provide quantitative support for the photographic observations. This comprehensive approach allowed for a detailed analysis of moringa's growth patterns and the relationship between mulch variables and plant development. The combination of photographic evidence and growth measurements provides a robust dataset for evaluating the effectiveness of different mulching material and the optimization of growth conditions.



Figure 4.1: A pictorial illustration of the researcher during data collection

4.5.1. The effect of different mulching materials on plant height of Moringa tree

Statistical analysis showed that the different mulching materials resulted in significant differences ($p < 0.05$) in plant height of moringa tree (**Table 4.2**). The tallest trees of moringa were observed in plots that were covered by black plastic mulch. At week 8, the tallest trees of 292 mm were observed from those that were on a black plastic

mulch treatment and they were significantly different at $p < 0.05$ from the rest of the other mulching materials used in the study. The shortest trees averaging 79 mm were observed in plots that had no mulch (control) and were significant at $p < 0.05$ from the rest of the treatments. The black plastic mulch resulted in the tallest trees from week 1 to week 8. Moreover, black plastic was significantly different from white, red plastics, organic and no mulch.

Table 4.2: Plant height (mm) of moringa as influenced by different mulching materials.

Treatment	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Plastic black	178a	187a	193a	215a	242a	247a	250a	292a
Plastic green	136ab	146ab	150b	164b	196b	198b	202b	207b
Plastic red	125b	130b	141c	159b	195b	198b	205b	211b
Plastic white	66c	79c	86c	100c	124c	129c	134c	139c
Organic mulch	78c	84c	87c	101c	115c	118c	125c	130c
No mulch	35c	41c	47c	52d	60d	68d	74d	79d
Grand mean	67.04	111.08	117.29	131.91	155.33	159.50	164.91	176.08
CV (%)	13.88	17.07	15.13	13.94	10.49	9.56	8.76	8.99
LSD $p < (0.05)$	14.02	28.57	26.74	24.71	24.56	22.99	21.77	23.87

Figures in the same column with the same letters are not significantly different from one another. CV= coefficient of variance, LSD= least significant Difference

4.5.2. The effect of different mulching types on stem diameter of Moringa tree

The emerging result showed that the stem diameter of moringa increased as the growth season progressed, irrespective of the type of mulching material used (**Table 4.3**). In all sampling weeks, it can be deduced that the highest stem diameter was recorded from black plastic mulch throughout the growing period. At week 8 (72 mm) stem diameter recorded was significantly different ($p < 0.05$) from white plastic, organic mulch, and no mulch. The lowest stem diameter of 25 mm was observed in the control, which had no mulch and was not significantly different from organic mulch and white plastic.

Table 4.3: Stem diameter (mm) of Moringa as influenced by different mulching materials.

Treatment	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Plastic black	40 ^a	45a	48a	51a	64a	65a	69a	72a
Plastic red	32 ^{ab}	42ab	44ab	50ab	57ab	58ab	60ab	64ab
Plastic green	24b	30ab	34ab	41ab	56ab	58ab	60ab	63ab
Plastic white	24b	29abc	31ab	35ab	38bc	39bc	42bc	45bc
Organic mulch	18bc	23c	25ab	29ab	31cd	33cd	36cd	40c
No mulch	6c	7c	17b	17b	18d	18d	21d	25c
Grand mean	24.31	29.30	23.97	25.54	44.14	44.87	47.79	51.25
CV (%)	23.33	32.61	32.68	37.56	19.81	19.14	18.08	17.03
LSD $p < 0.05$	8.54	14.40	11.80	14.04	13.17	12.94	13.02	13.15

Figures in the same column with the same letters are not significantly different from one another. CV= coefficient of variance, LSD= least significant difference

4.5.3. The effect of different mulching types on chlorophyll content of moringa tree

The results showed significant differences amongst the treated means (**Table 4.4**). The highest chlorophyll content was observed in trees that were mulched with black plastic. At week 8 the highest chlorophyll content of 75 SPAD units was recorded in trees that were mulched by black plastic and was significantly different ($p < 0.05$) from trees that were mulched with organic mulch and no mulch. While on the other hand, the lowest chlorophyll content of 59 SPAD units was recorded from trees that had no mulch treatment even though the control was not significantly different ($p < 0.05$) from trees that were mulched with organic mulch material. The trees that were mulched with red, green, and white plastic were not significantly different ($p < 0.05$) from trees that were mulched with black plastic.

Table 4.4: Chlorophyll (SPAD units) content of Moringa tree as influenced by different mulching materials

Treatment	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Plastic black	62a	63a	65a	68a	70a	72a	75a	75a
Plastic green	49a	54bc	55bc	58ab	60ab	63ab	66ab	66ab
Plastic red	50ab	55ab	58c	61ab	63ab	65ab	69ab	69ab
Plastic white	50ab	53bc	54c	56b	58b	59ab	62ab	62ab
Organic mulch	51ab	53c	56ab	58b	58b	60b	60b	60b
No mulch	40b	45bc	46bc	53b	51b	56b	59b	59b
Grand mean	50.50	52.58	55.25	56.25	58.83	61.50	64.96	64.96
CV (%)	10.20	7.22	6.80	12.04	11.22	10.43	9.56	9.56
LSD (p< 0.05)	7.76	5.72	5.66	10.21	9.95	9.67	9.36	9.36

Figures in the same column with the same letters are not significantly different from one another. CV= coefficient of variance, LSD= least significant difference

4.5.4. The effect of different mulching types on leaf width of moringa tree

The size and shape of the leaves significantly influence the photosynthetic efficiency and plant growth which hitherto impacted plant growth potential, nutrient supply, yield, quality, and resistance of plants (**Table 4.5**). The analysis of variance detected differences between the treated means. At week 8 in the black plastic mulch, the moringa tree's highest leaf width of 96 mm was recorded and it was significantly different ($p < 0.05$) from white plastic and no mulch treatments. The lowest leaf width of 40 mm of the moringa trees was noted in trees that had no mulch even though the trees that were mulch with white plastic were not significantly ($p < 0.05$) different from the trees that had no mulch.

Table 4.5: Leaf width (mm) of Moringa tree as influenced by different mulching materials

Treatment	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Plastic black	69a	78a	84a	84a	88a	90a	96a	96a
Plastic green	62ab	69ab	68ab	69ab	72ab	76ab	79ab	79ab
Plastic red	63ab	57ab	60b	65ab	68abc	71abc	74b	74b
Organic mulch	43bc	42bc	54b	55b	58bc	63bc	65b	65b
Plastic white	35cd	38bc	45bc	49bc	52cd	54cd	58bc	58bc
No mulch	18d	17c	25c	30c	35d	38d	40c	40c
Grand mean	48.08	50.25	56.04	58.63	62.00	65.21	68.91	68.92
CV (%)	19.02	29.02	17.76	16.33	13.96	13.37	13.88	13.88
LSD (p<0.05)	13.78	21.9	15.00	14.43	13.05	13.14	14.42	14.42

Figures in the same column with the same letters are not significantly different from one another. CV= coefficient of variance, LSD= least significant difference

4.5.5 The effect of different mulching types on leaf length of moringa tree

The results of the ANOVA indicated that the colour of the plastic plays a significant role in the leaf length development (**Table 4.6**). The black plastic mulch had the highest leaf length development from weeks 6, 7 and 8. Meanwhile, the green plastic mulch recorded the highest leaf length development from week 1 up to week 5. The black plastic mulch overtook the leaf development over green plastic mulch. However, both black and green plastic mulch were not significantly different ($p < 0.05$) from each other. At week 8 the black plastic mulch had the longest leaves of 108 cm and was highly significant from the trees that had organic mulch and no mulch as treatment. On the other hand, the trees that had no mulch at week 8 had the lowest leaf length development of 49 cm and were not significantly different ($p < 0.05$) from white plastic mulch and organic mulched plots.

Table 4.6: Leaf length (cm) of Moringa as influenced by different mulching materials

Treatment	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Plastic black	53ab	69a	75a	78a	90a	101a	104a	107a
Plastic green	62a	72a	78a	80a	84a	87ab	92ab	96ab
Plastic red	52ab	60ab	64ab	68ab	74ab	83abc	89abc	93abc
Plastic white	37bc	43b	50b	54b	61b	64bc	68bcd	71bcd
Organic mulch	40ab	49b	52b	55b	60b	62cd	64cd	69cd
No mulch	17c	21c	25c	31c	36c	39d	44d	49d
Grand mean	43.20	52.21	57.21	61.04	67.29	76.50	76.75	80.75
CV (%)	23.06	16.27	13.58	11.38	13.38	14.30	15.27	13.74
LSD ($p < 0.05$)	15.02	12.78	11.71	10.47	13.57	15.62	17.66	16.72

Figures in the same column with the same letters are not significantly different from one another.
CV= coefficient of variance, LSD= least significant difference

4.5.6. The effect of different mulching types on petiole diameter of moringa tree

Petiole plays a pivotal role in providing mechanical support to self-hold and adjust leaf position towards the sun, improving light harvesting ability and enabling the transportation of nutrients and water from the roots to the leaf. The trees that were mulched with black, green, and red plastic showed no significant difference ($p < 0.05$) from each other (**Table 4.7**). However, from week 1 to week 2 the plots that had white plastic mulch, organic mulch and no mulch were significantly different ($p < 0.05$) from black, green, and red

plastic mulched plots. At week 8 no significant differences ($p < 0.05$) were detected amongst the treated means. However, black, green, red, organic mulched plots had the thickest petiole diameter in comparison with white and no mulched plots. The thinnest petiole diameter was recorded in plants that had no mulch. Even though they were all but significantly different ($p < 0.05$).

Table 4.7: Petiole diameter (mm) of Moringa as influenced by different mulching materials.

Treatment	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Plastic black	11a	16a	18a	19a	20a	21a	23a	24a
Plastic green	11a	13abc	17a	18ab	19ab	21a	23a	24a
Plastic red	10ab	12ab	18ab	18ab	20a	21a	23a	24a
Organic mulch	7bc	8bc	17b	18ab	19ab	20a	22a	24a
Plastic white	6c	7bc	18a	18ab	19ab	20a	22a	23a
No mulch	4c	4c	17b	17a	18b	19a	20a	22a
Grand mean	8.07	9.47	17.65	18.09	19.07	20.12	21.71	23.46
CV (%)	1.18	2.54	1.64	2.40	4.46	5.58	5.74	5.31
LSD ($p < 0.05$)	2.21	4.64	0.44	0.65	1.28	1.69	1.87	1.87

Figures in the same column with the same letters are not significantly different from one another.
CV= coefficient of variance, LSD= least significant difference

4.5.7. The effect of different mulching types on petiole length of Moringa tree

The ANOVA detected some significant differences between the treated means (**Table 4.8**). Whereby, at week 8 the black plastic mulched plots recorded the highest petiole length of 35 cm and was significantly different ($p < 0.05$) from the plots that were mulched with white plastic, organic mulch, and no mulch. The lowest petiole length of 25 cm was recorded in plots that had no mulch, even though. it was no significant difference ($p < 0.05$) from plots that had white plastic mulch.

Table 4.8: Petiole length (cm) content of Moringa as influenced by different mulching materials.

Treatment	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Plastic black	16a	20a	22a	24a	27a	28a	32a	35a
Plastic green	15a	18ab	20ab	23ab	25ab	27a	30ab	32ab
Plastic red	14a	17ab	19ab	22ab	25ab	27a	29ab	32ab
Organic mulch	12ab	14bc	16bc	19abc	21abc	24ab	26bc	30b
Plastic white	10b	13cd	16bc	18bc	20bc	24ab	27bc	28bc
No mulch	6c	10d	12c	15c	16c	20a	22c	25c
Grand mean	12.12	14.95	17.62	20.12	22.37	24.87	27.71	30.25
CV (%)	13.49	10.93	11.33	11.91	10.80	9.71	8.22	6.37
LSD (p<0.05)	2.46	2.46	3.01	3.61	3.64	3.63	3.43	2.90

Figures in the same column with the same letters are not significantly different from one another.

CV= coefficient of variance, LSD= least significant difference

4.5.8. The effect of different mulching materials on the yield of moringa

The colour of the plastic had a significant influence on the yield of moringa trees (**Table 4.9**). After the analysis of variance was carried out, it was evident that black plastic mulch was the most preferred by moringa. The highest fresh weight of 13.16 kg/ha and dry biomass of

1.84 kg/plot was obtained from plots that were mulched with black plastic and were highly significant from the rest of the treatments. The lowest fresh biomass of 3.45 kg/ha and dry biomass of 0.43 was obtained from the plots that had no mulch. The variation in plant biomass could be associated with moisture conservation, nutrient availability, nutrient uptake, and moisture near the root zone.

Table 4.9: Yield of Moringa as influenced by different mulching materials

Treatment	Fresh mass (kg/ha)	Dry mass (kg/ha)	Dry matter content (%)
Plastic black	13.16a	1.84a	14.00a
Plastic green	7.72b	1.02b	13.20b
Plastic red	5.63c	0.71c	13.18b
Plastic white	4.66cd	0.57d	13.65b
Organic mulch	3.65d	0.48de	13.15b
No mulch	3.45d	0.43e	12.40c
Mulch	***	***	*
Grand mean	6.38	0.85	12.93
CV (%)	9.53	6.79	5.00
LSD (p<0.05)	0.92	0.08	0.97

Figures in the same column with the same letters are not significantly different from one another. CV%= coefficient of variance, LSD= least significant difference

4.6 Discussion

The colour of plastic mulch has a significant influence on crop growth performance. Dubey *et al.* (2020) reported that mulching has a significant impact on the increase of crop/fruit production and improving quality. The role of coloured plastic mulches in producing an optimal growth environment, such as adequate moisture and root zone temperature, increases plant growth and development by enhancing water retention, reducing soil temperature fluctuations, and promoting better root establishment, ultimately leading to improved nutrient uptake and higher overall yields. Utilizing coloured plastic mulch to improve the microclimate around plants improves cell expansion and elongation, boosting plant growth and development (Getachew and Bizuayehu, 2021). The mean soil temperature affect plant growth characteristics such as stem diameter, plant height, leaf length, leaf dry weight, stem dry weight, and plant dry weight (Gheshm and Brown, 2020). According to Shah *et al.* (2018), the black polyethylene resulted in an increased number of leaves, longleaf, and wider leaf breadth in lettuce, followed by white, blue, silver, and olive film. On the contrary, Mendonça *et al.* (2021) reported that plants grown in red plastic mulch showed high performance in comparison to plants grown in black plastic mulch. However, this was not the case for the current study. High crop performance was observed in plants grown in black plastic mulch.

Changes in the plant height of moringa have been observed by using different mulches and plastic mulch increased the plant height than other mulches. The black plastic mulch produced the tallest moringa plants in the current study. Similarly, (Bhatta, 2020) reported that the application of black plastic in potatoes resulted in increased plant height. The maximum cucumber height was obtained at 60 days after transplanting in silver on black mulching, while the lowest height was observed in the no mulch (Karki *et al.*, 2020). Similar results were reported where black mulching yielded the highest height while control or no mulch yielded the lowest height in cucumber (Bhandari and Bhandari, 2021). Similar findings were also reported in watermelons, by Ansary and Roy (2005), and in tomatoes, by Sharma *et al.* (2023).

Awasthi *et al.* (2022), reported that the application of black polythene mulching has significant results for plant growth parameters such as plant height, number of leaves, leaf area, number of flowers, fruits, and yield, while on the other hand, no mulching was less effective for obtaining plant growth parameters. According to (Parmar *et al.* 2013), mulching conserves soil moisture, thus, the available moisture promotes root development and minimizes evaporation loss and ultimately results in higher nutrient availability in the soil, significantly improving plant growth which is associated with our findings. The increased plant height in mulched plants was possibly due to better availability of soil moisture and optimum soil temperature provided by the mulches. Ratio of Red to far red (FR: R) is important in phytochrome regulation of plant physiological processes, and can affect internode length and stem elongation, chloroplast ultrastructure, photosynthetic efficiency, and photosynthate partitioning among leaves, stems and roots (Franquera, 2015). The colour of plastic had no significant influence on the chlorophyll content of moringa plants. However, plastic mulches in comparison with organic mulch significantly influenced the chlorophyll content of moringa leaves.

Moringa's increased vegetative growth parameters could be linked to soil moisture retention and availability. The use of mulch indicated an improvement in growth attributes, which might be due to the enhancement of photosynthesis and other metabolic activities (Bhatt *et al.* 2011; Parmar *et al.* 2013). The extended retention of moisture and moisture availability also leads to higher uptake of nutrients for plants' proper growth and development, resulting in higher plant growth than bare soil (Mahadeen, 2014). Plant yield is directly proportional to crop growth and development performance. As a result, using plastic mulches increased vegetable plant yield (Lamont, 2017). This effect is linked to the impact of plastic mulching on the microenvironment and root zone temperature of plants, which enhances cell expansion and enlargement activities. The improved growing conditions enhanced the plant growth and development and produced an increased fruit-bearing environment compared to no mulched plots. The chlorophyll content is a key indicator of a plant's photosynthetic efficiency. According to Zhang *et al.* (2022), higher SPAD values reflect better chlorophyll content and enhanced photosynthetic capability. In the current study, plants grown under black plastic mulch exhibited significantly higher chlorophyll

content, suggesting improved photosynthetic performance and potentially better yields

for moringa. These findings are consistent with Fu *et al.* (2022), which reported that maize grown in black plastic mulch also had higher chlorophyll content compared to those grown in white plastic mulch and no mulch plots.

Amare and Desta (2021) revealed that the effectiveness of plastic mulches in reducing the adverse effects of environmental stress on crops is greatly dependent on the colour of the plastic mulch. On the other hand, Taylor *et al.* (2008) reported that black plastic mulch reduced thyme yield compared to no mulch. Contrary to that, the finding of the current study obtained higher yields in plots that were mulched with black plastic than in no mulched plots. Similar studies were reported in chilli plants by Ashrafuzzaman *et al.* (2011), and in tomatoes by Miles *et al.* (2019,) that the black plastic mulch resulted in maximum yield, whereas white plastic mulch and no mulch areas produced the lowest yield. All the above-mentioned reports align with the findings of our study.

Plant yield is directly proportional to crop growth and development performance. As a result, the usage of plastic mulches increased the yield of vegetable plants (Lamont, 2017). This effect is linked to the impact of plastic mulching on the microenvironment and root zone temperature of plants, which enhances cell expansion and enlargement activities. Furthermore, the enzymes' active role in improving plant growth development enhanced plant yield (Li *et al.*, 2004). The improved growing conditions enhanced the plant growth and development and produced an increased fruit-bearing environment compared to no mulched plots. Also, covering the soil has an effect on the lack of evaporation from the surface of the soil, which reduces the rise of water to the top by capillary action and thus prevents the accumulation of salts in the root area of the plant and the increase in the moisture stock of the root zone due to coverage. It may cause a decrease in the salt concentration in the surface layer of the soil and thus allow growth, division and elongation of cells and in turn positively reflects on improving the traits of vegetative growth and affecting the increase in yield traits. Plastic mulching has the potential to improve soil temperature, reduce evaporation from soil, increase dioxide concentration and nutrient, control weeds, protect plants from pests and reduce fruit rotting.

4.7. Conclusion

The effectiveness of coloured plastic mulches, particularly black, red, and green, in enhancing the growth and yield of moringa can be attributed to their distinct light-modifying properties. Black mulch absorbs light, altering the soil microclimate, while red mulch reflects light and modifies the red to far-red ratio. These modified growing conditions significantly improve moringa's growth and development, leading to increased yields, particularly evident in plots mulched with black plastic compared to un-mulched plots.

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CHAPTER 5: THE INFLUENCE OF DIFFERENT MULCHING MATERIALS ON THE NUTRITIONAL CONTENT AND AMINO ACIDS OF DRIED MORINGA LEAVES

ABSTRACT

Soil fertility is a critical determinant of plant growth and productivity in agriculture, influenced by land degradation, which diminishes nutrient availability and uptake by plants. Climate-smart technologies are essential for mitigating soil fertility issues and improving growing conditions. Plastic mulch is one of such technologies that enhances nutrient retention within the root zone, thereby optimizing nutrient utilization by plants. Moringa, recognized for its high nutritional value, presents an affordable solution to supplement unbalanced diets in malnutrition-affected countries. This study evaluated the impact of different mulching materials on the nutrient composition and amino acids profile of moringa leaves cultivated and harvested from the University of Fort Hare research farm, Alice, South Africa. Results indicated that black plastic mulch maintained optimal moisture content (8.29%), falling within the recommended ranges. Furthermore, black plastic mulch significantly enhanced proximate nutrients such as ash (14.93%), fat (4.92%), acid detergent fibre (29.87%), neutral detergent fibre (42.99%), and crude protein (27.93%) compared to un-mulched plots. Macro-nutrients, including phosphorus, nitrogen, calcium, magnesium, and potassium, were also notably improved in plots mulched with black plastic. Conversely, sodium content was higher in red plastic mulch, while copper, manganese, iron, aluminium, and zinc were significantly higher in black plastic mulch. Amino acid analysis revealed that aspartic acid content (2.21%) was highest in black plastic mulch and lowest in red plastic mulch, whereas glutamic acid content (3.56%) was highest in the control and lowest in red plastic mulch. In conclusion, mulching with black plastic significantly enhanced the nutrient composition of moringa leaves, and it was attributed to improved growing conditions facilitated by plastic mulching. These findings underscore the potential of plastic mulch technology to optimize nutrient profiles in moringa cultivation, thereby supporting efforts to combat malnutrition in resource- constrained countries.

Key words: malnutrition, moringa, micro and macro nutrients proximate nutrients.

5.1. Introduction

As climate change intensifies and global food insecurity becomes a pressing concern, the exploration of resilient crops that could survive the harsh climatic condition is crucial for sustainable agricultural systems. Moringa is characterized by excellent nutritional value and adaptability, and emerges candidate plant with huge resilience potential to survive such harsh climate. The nutrient composition and amino acid profile of moringa leaves are profoundly influenced by agronomic practices, particularly the selection of mulching materials (Odokonyero *et al.*, 2024). Effective mulching can enhance soil health by improving moisture retention, suppressing weeds, and facilitating nutrient availability, thus ensuring optimal plant growth even under climatic stress (Iqbal *et al.*, 2020; El- Beltagi *et al.*, 2022). According to Prem *et al.* (2020), mulches are essential for maintaining healthy plant growth and supporting environmental sustainability. Additionally, mulches increase germination rates by maintaining consistent moisture levels, suppress weed growth by blocking sunlight, and prevent erosion by stabilizing the soil (Adnan *et al.*, 2020). They also enhance the physical and chemical properties of the soil, promoting beneficial microbial activity (Wang *et al.*, 2019). Furthermore, the use of mulch can help improve the yield and quality of crops.

This study particularly focus on plastic mulches, which are widely utilized in commercial farming for several key reasons. Plastic mulches provide a barrier to soil pathogens, play a significant role in the soil solarization process, and create a microclimate beneath the sheet that is enriched in carbon dioxide due to increased microbial activity (Wang *et al.*, 2019). Plastic mulches are available in various colours such as black, red, silver, and white and are designed with specific functions related to light absorption and crop physiology. The primary factor influencing photosynthetically active radiation (PAR) in plastic mulches is their colour, which affects the transmittance, absorption, and reflectance of both short- and long-wavelength radiation (Rylander *et al.*, 2020). For example, variations in the colour of plastic mulches can significantly impact the PAR reaching the plant root zone. Greater PAR reflection generally leads to lower soil temperatures and higher moisture content (Yang *et al.*, 2022). Specifically, silver plastic mulches reflect more PAR than black and white mulches, resulting in cooler root zone temperatures and reduced water loss (Jones *et al.*, 2021).

Black plastic mulch reflect light, it is excellent in weed suppression and absorbs a large percentage of sunlight and heat during the day (El-Beltagi *et al.*, 2022). As a result, the use of this plastic not only eliminates competition for nutrients by crops and weeds but also promotes regular development of the root areas, which ensures good plant growth. Furthermore, Rajamani *et al.* (2019) agreed that mulching with black plastic is a good alternative to hand hoeing, even though it does not necessarily influence the plant height of lavender and thyme.

According to Yadav *et al.* (2023) white, clear and silver plastic mulches have a low light absorbing ability. Moreover, clear plastic mulch can increase soil temperature but has a very poor weed suppression Thakur and Kumar, (2021), while white and silver plastic mulches decrease soil temperature but have excellent weed suppression ability. Amare and Desta (2021) summarized the effectiveness of using plastic mulch on crop production, highlighting its positive influence on plant growth for various vegetables and medicinal plants by increasing branching, root length, plant height, leaf area, and overall plant length. However, they also noted that plastic mulches can have negative effects under certain conditions. Specifically, Thakur and Kumar (2021) found that plastic mulches can decrease growth and yield in some plants, including mint, thyme, potatoes, lavender, and various herbs. The other drawbacks are the capability of increasing pest infestation, causing microplastic contamination, soil puddling, soil structural loss and reducing activity of soil microorganisms (Getachew *et al.*, 2021). The use of plastic mulches in medicinal plants remains unknown, besides the obvious benefits of using plastic mulch in plants.

Moringa is recognized as a highly nutritious tree, rich in proteins, vitamins, and minerals (Sandeep *et al.*, 2019). The leaves of moringa contain essential minerals, including calcium, zinc, magnesium, potassium, and iron, which are vital for human growth (Islam *et al.*, 2021). The protein content in moringa leaves ranges from 20% to 35% of their dry weight (Mutar *et al.*, 2021). Research by Walia *et al.* (2022) indicates that Moringa leaves contain 21.8% crude protein (CP), 22.8% acid detergent fibre (ADF), and 30.8% neutral detergent fibre (NDF), along with 412.0 g/kg of crude fat, 211.2 g/kg of carbohydrates, and 44.3 g/kg of ash. These proximate nutrient compounds are beneficial for enhancing livestock production. Furthermore,

incorporating moringa leaves as a supplement can improve the quality of low-grade livestock fodder, leading to increased dry matter intake (DMI) and digestibility. A mixture of moringa leaves with 20% to 50% batiki grass has proven to improve DMI while providing a highly digestible source of dry matter (DM) and crude protein with a low NDF value (Abdel-Raheem and Hassan, 2021). Moreover, Moringa leaves serve as an excellent protein source and a convenient substitute for soybean and rapeseed meals in ruminant diets, promoting microbial protein synthesis in the rumen (Su and Chen, 2020).

The high protein content of moringa leaves is primarily composed of essential amino acids (Mutar *et al.*, 2021). These leaves can be consumed directly as food and can be preserved for months in their dried form with minimal loss of nutritional value (Grosshagauer *et al.*, 2021). Additionally, they possess a low caloric value, making them an excellent option for individuals seeking to manage their weight. Moringa leaves are a significant source of sulfur-containing amino acids, such as methionine and cysteine, which are often scarce in diets (Kamran *et al.*, 2021). Furthermore, dried moringa leaves can be ground into a powder and used as a nutritional additive in soups and meat dishes. Although some nutritional content may be lost during processing, the powder remains a valuable source of vitamin A (Olson *et al.*, 2016).

The evaluation and understanding of the potential application of coloured plastic mulches to enhance the crop growth, yield and nutritional quality of moringa remains largely unknown, despite the plant's high nutritional and economic attributes to society. This study seeks to examine how different mulching materials affect the nutrient composition and amino acid profile of moringa leaves, providing insights into how to maximize the nutritional benefits of this remarkable tree. By identifying effective mulching strategies, this research aims to contribute to the development of sustainable agricultural practices that not only encourage food security but also promote environmental resilience in the face of climate change.

5.2. Materials and Methods

5.2.1. Description of experimental site and design

The experiment in this section was conducted in the same field as described in Chapter 3 using the same design.

5.2.3. Leaf sample preparation

Chemical/nutritional analysis was done on oven-dried leaves collected at 90 days of planting. All the leaves in the plants were harvested by hand, sorted according to their treatment allocation and labelled per plot and treatment. The leaf samples were immersed into a large volume of potable water and shaken to remove impurities on the leaf surfaces. Thereafter, the leaves were taken out of water and oven dried at 40 °C prior to metabolites extraction and were considered dried when they were brittle, easy to crush and their moisture content dropped below 8% (Fahey, 2005). After drying, the samples were milled with the leaf grinder into a fine powder using an (SMC hammer mill; Cape Town) that could pass through 1 mm sieve and stored in zipper polythene plastic pockets until analysis.

5.3. Data collection

5.3.1. Nutrient composition of Moringa

Moisture, ash, and fat contents were determined using the standard procedures of Association of Official Analytical Chemistry (AOAC, 1990). Total nitrogen content was determined using the Kjeldahl method following Pearson (1976). Thereafter a factor of 6.25 was used to convert the nitrogen content into crude protein following Equation 1 (McDonald *et al.*, 2011).

$$\text{Crude protein (\%)} = \text{Total nitrogen (\%)} \times 6.25$$

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed by the methods described by Goering and Van Soest (1970). Micro and macro-elements were determined after digesting the leaf samples using aqua regia (3:1 v/v hydrochloric acid: nitric acid) in a MARS 5 microwave digester (CEM Corporation, Matthews, North Carolina, USA). Minerals were

thereafter determined using the ICP-OES method (Varian Inc., The Netherlands).

5.3.2 Determination of Amino acids

Amino acid sample preparation was carried out according to the manufacturer's instructions for the Ultra Derivatization Kit, as outlined by Mpai *et al.* (2018) in their studies. The analysis of individual amino acids—specifically Arginine (Arg), Serine (Ser), Glycine (Gly), Aspartate (Asp), Glutamate (Glu), Alanine (Ala), Proline (Pro), Histidine (His), Threonine (Thr), Methionine (Met), Lysine (Lys), Tyrosine (Tyr), Leucine (Leu), Phenylalanine (Phe), Asparagine (Asn), and Glutamine (Gln) was conducted using ultra-performance liquid chromatography, following the methods described by Mpai *et al.* (2018) without any modifications.

5.3.3 Statistical analysis

Data collected were subjected to statistical analyses using JMP 15.0 statistical software (SAS Institute, Inc., Cary, NC, USA). Means were separated using Turkey's test at $P < 0.05$ when ANOVA indicated a significant P-value. Significant differences were identified at three probability levels: $P < 0.05$, 0.01 and 0.001.

5.4. Results

The primary metabolic compound and nutrient analyses of moringa play a crucial role in providing enormous benefits for humans and animals through its nutritional significance. Moreover, despite the effectiveness of various mulching methods that have been employed on other plants, it is important to know their effects on the nutritional components of the moringa plant.

5.4.1. The influence of different mulching materials on the proximate nutrient composition of moringa

The results recorded from this current study indicated that moringa leaves contained appreciable amounts of crude protein (CP), ash, fat, acid detergent fibre (ADF), neutral detergent fibre (NDF) and moisture content (**Table 5.1**). The analysis of variance detected some significant differences ($p < 0.05$) between the variables being tested

as a response to different mulching methods.

Significant differences were detected in the moisture levels, where the plastic mulches were significantly higher than the organic and control mulches. The highest moisture content of 8.29% was obtained from Black plastic mulch, while the lowest moisture content of 7.28% was obtained from the control. The fat content in black plastic differed significantly ($p < 0.05$) from the ash content in red plastic, green plastic, organic mulch, and the control. However, the organic mulch was not significantly different ($p < 0.05$) from the control. Even though, the organic mulch was not significantly different ($p < 0.05$) from white, green, and red plastic mulch. Therefore, the highest ash content of 14.93% was obtained from black plastic mulch, and the lowest ash content of 12.84% was obtained from the control.

The fat content of black, white, and red plastic mulch was significantly different ($p < 0.05$) from green, organic mulch, and control. However, the effect of the red plastic mulch treatment on the fat content in the moringa leaves was not different from the green plastic mulch. Also, green plastic mulch, organic mulch and control were not significantly different ($p < 0.05$) from each other. The highest fat content of 4.92% was observed in black plastic mulched plots, while the lowest fat content of 3.95% was observed in the control.

The ADF content of moringa leaves obtained from plots that had black plastic mulch was significantly different ($p < 0.05$) from organic mulch and control. The highest ADF content of 29.87 was obtained from the black plastic mulch, whereas the lowest content of 22.99% was obtained from the control. The NDF of black plastic mulched plots' content differed significantly ($p < 0.05$) from the white, red, organic mulch and control. Also, the white, red, and green plastic mulch differed significantly ($p < 0.05$) from the control. As a result, the black plastic mulched plots exhibited the highest NDF content at 42.99%, while the control plots had the lowest content at 36.14%.

The crude protein of black plastic mulch was notably significantly different ($p < 0.05$) from white, green, organic material, and control plots. Moreover, the white and red plastic mulch differed significantly ($p < 0.05$) from the control. The highest crude protein percentage of 27.93 was obtained from black plastic mulched plots. In addition, the lowest crude protein percentage of 23.19 was observed from the control.

Table 5.1: Proximate nutrient analysis of dried moringa leaves as influenced by different mulching materials.

Treatment	Moisture	Ash	Fat	ADF	NDF	Crude Protein
Plastic black	8.29a	14.93a	4.92a	29.87a	42.99a	27.93a
Plastic white	7.84abc	14.20ab	4.78a	27.68ab	40.21b	25.53b
Plastic red	7.85abc	13.95b	4.64ab	26.97ab	40.11b	25.99ab
Plastic green	7.95ab	13.99b	4.22bc	26.61ab	40.74ab	25.00bc
Organic mulch	7.47bc	13.62bc	4.17c	25.27bc	38.25bc	24.34bc
No mulch	7.28c	12.84c	3.95c	22.99c	36.14c	23.19c
Mulch	***	***	***	**	***	***
CV (%)	4.40	4.22	9.55	9.53	5.21	5.85
LSD ($P < 0.05$)	0.51	0.88	0.64	3.82	3.12	2.23

Figures in the same column with the same letters are not significantly different from one another. CV= coefficient of variance, LSD= least significant difference

5.4.2. The effect of different mulching materials on the macronutrient composition of Moringa

The analysis of variance showed that macro-minerals were significantly affected by the different mulching materials. The phosphorus content in plots mulched with black plastic was significantly different ($p < 0.05$) from the rest of the treatments (**Figure 5.1a**). Notably, the phosphorus content in plots with black plastic mulch was 80% higher compared to the rest of the treatments. The plots with no mulch had 49% lower phosphorus content.

The nitrogen content in black plastic significantly increased and differed significantly ($p < 0.05$) from the control, even though the black plastic was not significantly different ($p < 0.05$) from other treatments used (**Figure 5.1b**). Moreover, the highest concentration of nitrogen (3.8%) was observed in plants grown under black plastic while the lowest concentration of nitrogen (3.4%) was observed in plants grown in plots that had no mulch.

Calcium content of the leaves in the current trial ranged between 2.73% and 3.32% (**Figure 5.1c**). The plants grown in black plastic mulch had the highest Ca content of 3.32% and were significantly different ($p < 0.05$) from the plants grown in control plots. The plants grown in control plots had the lowest Ca content of 2.73%.

The magnesium content of moringa leaves under the influence of the different mulching materials is presented in **Figure 5.1d**. The black plastic mulch was not significantly different ($p < 0.05$) from the red plastic mulch. However, it differed significantly ($p < 0.05$) from the green, white plastics, organic and the control. The highest Magnesium content of approximately 59% was recorded in plants that were grown in black plastic mulch. However, the lowest magnesium content of about 45% was observed in plants grown in the control.

Potassium content of moringa leaves grown under different mulching materials is presented in **Figure 5.1e**. The results of the analysis of variance indicated that organic mulch, green, and black plastic mulch were significantly different ($p < 0.05$) from the control. However, the white plastic mulch was not significantly different ($p < 0.05$) from the control. Moreover, the highest concentration of K (1.8%) was obtained from plants grown in black plastic mulch and the lowest concentration of K (1.3%) was observed from plants on the control plots.

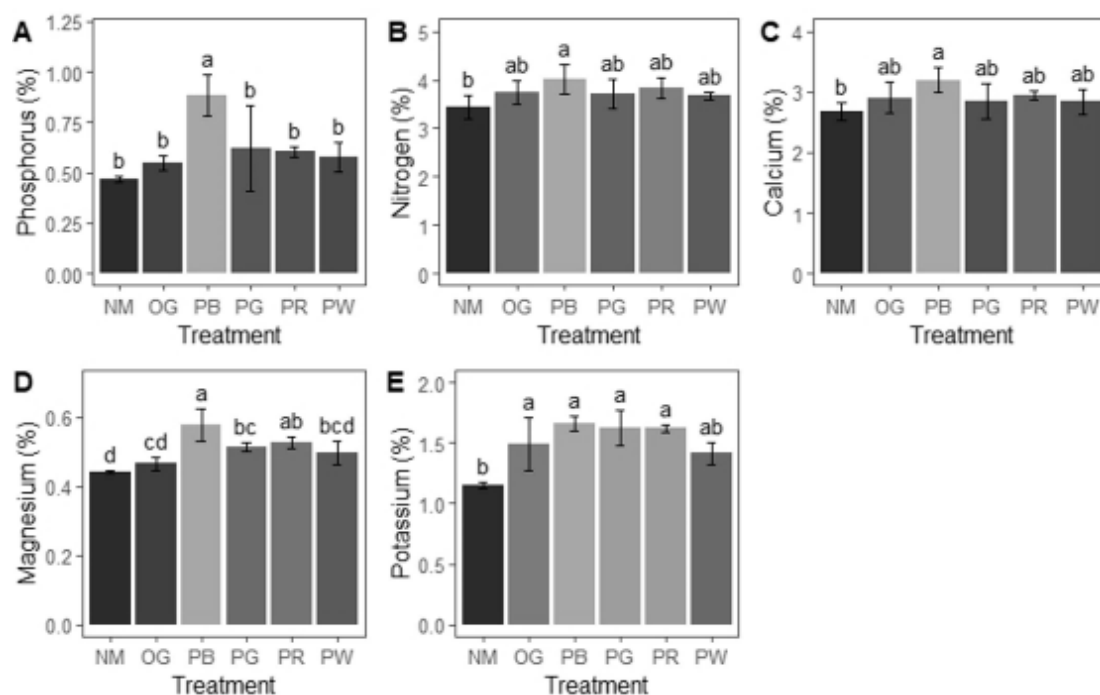


Figure 5.1: Macronutrient contents of moringa leaves grown in different mulching materials. Bars that share the same letters are not significantly different from one another. Error bars represent standard error.

5.4.3. The effect of different mulching materials on micronutrients composition of Moringa

The results of the study revealed that the analysis of variance showed that the determined micro-minerals were significantly affected by different mulching materials. In **Figure 5.2a**, the illustration shows that the application of different mulching materials significantly influenced the concentration of Na in moringa plants. The black plastic mulch and red plastic mulch treatment resulted in a significantly higher Na concentration in moringa leaves. The highest Mg concentration of 1387 mg/kg was observed from the red plastic mulch and the lowest Mg concentration of 672 mg/kg was observed from the control. This indicates that mulching was effective in the production of moringa as it reduces the accumulation of salts.

The results in **Figure 5.2b**, indicated that there was no significant difference between the mulching treatments applied regarding copper content in moringa leaf tissues. However, the treatments were not significant different ($p < 0.05$). The highest concentration of Cu was noted from plots mulched with black plastic and the lowest

concentration was noted in plots had no mulch and organic materials.

The manganese content indicated that the plants grown in black plastic mulch and red plastic mulch were significantly different from green, white, organic mulch and the control (**Figure 5.2c**). However, the red, green, and white plastic mulch were significantly different ($p < 0.05$) from the organic and the control plots. Moreover, the highest Mn content of 149 mg/kg was observed in plants grown in black plastic mulch and the lowest Mn content of 97 mg/kg was observed in plants in the control (**Figure 5.2c**).

The Iron content found in the black plastic mulch was significantly different ($p < 0.05$) from plants grown in white, green plastic mulch, organic mulch, and the control (**Figure 5.2d**). However, the black plastic mulch was not significantly different ($p < 0.05$) from the red plastic mulch. The highest Fe content of approximately 125 mg/kg was noted from plants mulched with black plastic, whereas the lowest Fe content of approximately 95 mg/kg was observed from plants grown in the control (**Figure 5.2d**).

The Aluminium content recorded in black plastic and red plastic mulch were significantly different from plants grown in green, and white plastic mulch (**Figure 5.2e**). Even though the red plastic mulch was not significantly different ($p < 0.05$) from the white plastic mulch. Moreover, the white and red plastic mulch were significantly different ($p < 0.05$) from the organic mulch and the control. Nonetheless, the highest Al content of approximately 71.25 mg/kg was noted from plants mulched with black plastic, whereas the lowest Al content of approximately 49.35 mg/kg was observed from plants grown in the control (**Figure 5.2e**).

The zinc content indicated that the plots mulched with plastics were significantly different from the organic mulch and the control (**Figure 5.2f**). However, a comparison within plastics indicated that the black plastic differed significantly ($p < 0.05$) from the green and white plastic mulch (**Figure 5.2f**). The highest Zn content of approximately 22.45 mg/kg was noted from plants mulched with black plastic, whereas the lowest Zn content of approximately 15.35 mg/kg was observed from plants grown in the control (**Figure 5.2f**).

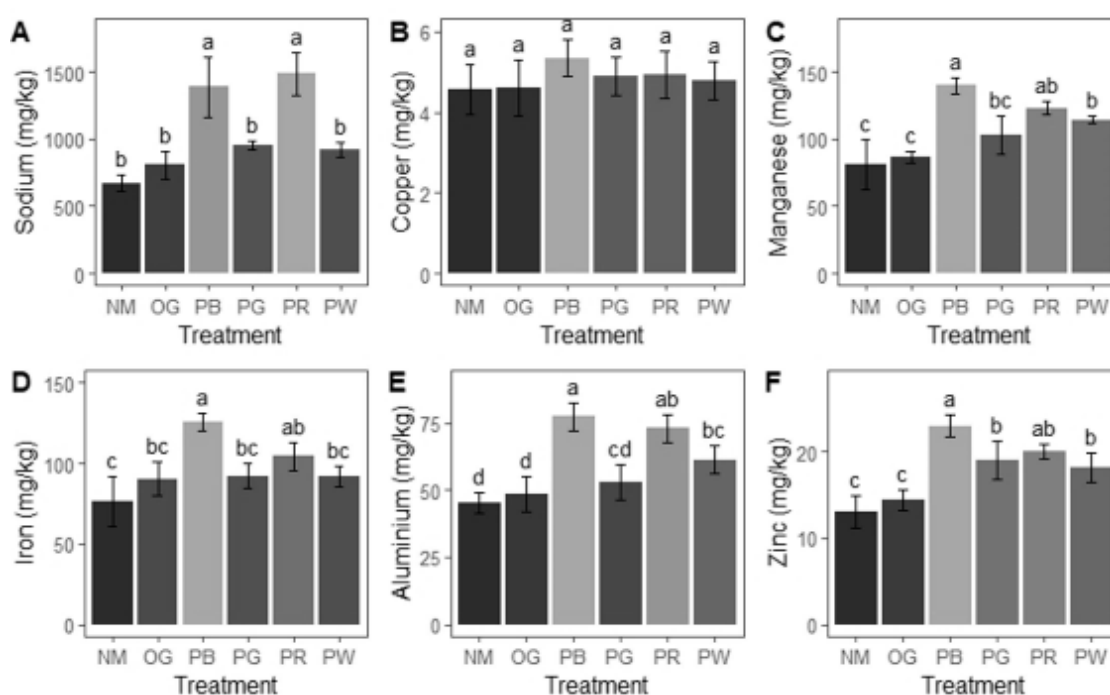


Figure 5.2: Micronutrient content of moringa leaves grown under different mulching materials. Bars that share the same letters are not significantly different ($p < 0.05$) from one another. Error bars represent standard error.

5.4.4. The influence of different mulching materials on Amino acids content of moringa

The results of the study reported sixteen (16) amino acids detected from dried moringa leaves (**Table 5.2**). Amongst the 16 amino acids detected, only two of the amino acids were significantly affected by the type of mulch used, namely, Aspartic acid and Glutamic Acid. However, the rest of the determined amino acids were Histidine, Arginine, Serine, Glycine, Threonine, Alanine, Proline, Lysine, Tyrosine, Methionine, Valine, Isoleucine, Leucine and Phenylalanine and no significant differences were detected amongst the treatment means. The aspartic acid and glutamic acid content of 2.30% and 3.56% were significantly higher in the control (NM), and the lowest content of 1.88% and 2.75% were obtained from the red plastic mulch (PR), respectively.

Table 5.2: The different classes of amino acids identified in moringa leaves as influenced by different mulching materials.

Treatment	Histidine	Arginine	Serine	Glycine	Aspartic	Glutamic	Threonine	Alanine	Proline	Lysine	Tyrosine	Methionine	Valine	Isoleucine	Leucine	Phenylalanine
Plastic black	0.59a	1.40a	1.06a	1.15a	2.21a	3.26ab	1.01a	1.47a	0.93a	1.38a	1.19a	0.29a	1.12a	0.88a	1.66a	1.13a
Plastic white	0.59a	1.38a	1.01a	1.02a	2.23ab	3.02ab	0.94a	1.40a	0.86a	1.16a	1.04a	0.20a	1.08a	0.84a	1.54a	1.01a
Plastic green	0.62a	1.38a	0.98a	0.98a	2.00ab	3.02ab	0.94a	1.30a	0.83a	1.03a	1.06a	0.23a	1.03a	0.78a	1.47a	0.98a
Plastic red	0.61a	1.18a	0.98a	0.99a	1.88b	1.75b	0.91a	1.31a	0.81a	1.05a	1.00a	0.22a	0.98a	0.76a	1.40a	0.92a
Organic mulch	0.64a	1.34a	1.02a	1.05a	2.13ab	3.14ab	0.96a	1.29a	0.88a	1.01a	1.13a	0.22a	1.04a	0.80a	1.48a	1.09a
No mulch	0.60a	1.32a	1.04a	0.99a	2.3a	1.56a	0.96a	1.30a	1.00a	1.20a	0.99a	0.25a	1.04a	0.83a	1.56a	1.02a
CV (%)	9.37	14.68	8.72	10.24	6.74	5.38	8.09	7.57	11.97	7.36	12.58	11.58	7.17	8.23	10.71	11.59
LSD (P< 0.05)	0.08	0.29	0.13	0.16	0.22	0.25	0.11	0.15	0.16	0.13	0.2	0.04	0.11	0.1	0.25	0.18

Figures in the same column with the same letters are not significantly different from one another. CV= coefficient of variance, LSD= least significant difference

5.5. Discussion

Plastic mulch enhances agricultural productivity, particularly in crops like moringa. Its ability to regulate soil temperature, retain moisture, and suppress weed growth significantly improves nutrient uptake and overall plant health (Chaudhary, 2022). In our study, black plastic mulch was shown to facilitate superior nutrient accumulation in moringa leaves compared to organic mulch and the control, highlighting its effectiveness in optimizing crop quality. While organic mulch is traditionally valued for its potential to enrich soil health through decomposition Betancur *et al.* (2023), using fresh organic material in our study did not yield the expected benefits. Fresh organic mulch takes time to decompose, potentially delaying nutrient release and leading to temporary nitrogen immobilization, hindering plant nutrient availability (El-Beltagi *et al.*, 2022). In contrast, plastic mulch provides immediate benefits by creating a stable environment for nutrient uptake.

The proximate nutrient analysis revealed that moringa leaves contained appreciable amounts of crude protein content that ranged between 23.19% and 27.93%. The highest crude protein content of 27.93% was obtained from black plastic mulched plots. The results of proximate nutrient analysis obtained from our study do not conform with most of the previous studies as documented in related literature except for a singular study by Osadebe *et al.* (2014) where a higher crude protein (23.94% to 42.12%) was recorded in fluted pumpkin in black plastic mulched plots. For instance, Nursandi *et al.* (2017) reported a significantly lower crude protein content that ranged from 13.60 to 14.43% and was obtained from plots that were mulched with wood shaving. Similarly, Sule *et al.* (2014) reported a lower crude protein content that ranges between 10.67 to 11.25% of maize grain in Nigeria.

The difference in protein content accumulation could be explained by the findings of Rafiq *et al.* (2010), who reported that mulches can preserve moisture content and increase soil fertility, thus increasing in crop protein content. In addition, Boomsma *et al.* (2009), indicated that plants that have access to adequate moisture and nitrogen in the soil can produce higher levels of chlorophyll and photosynthesis, which in turn can result in crops with higher protein content. A similar trend was obtained in Chapter 4 (**Table 4.4**) of the current study, where the plastic mulch plots had a significantly

increased chlorophyll content compared to organic mulch and the control. The protein value observed in this study was between 23.19% and 27.93% which is higher than the 3.3% recorded by the USDA nutrient database for standard reference (Gopalan *et al.*, 1989). This makes the plant advantageous as a rich source of vegetable protein.

The moisture content of 8.29% obtained with the black plastic mulched plots falls within the range of the standard moisture content recommendation by Fahey (2005). However, Osadebe *et al.* (2014) reported no significant difference in the values of the moisture content of the fluted pumpkin when subjected to different mulching materials. Kahraman *et al.* (2021) reported that high moisture content in vegetables is a quality attribute that indicates the extent of freshness in the vegetables. On the other hand, the high level of moisture content in the leaves of the plants often results in the growth of moulds; therefore, the level of moisture content obtained in the current study indicates that moringa leaves are protected from enzymatic decomposition.

An increase in ash content of moringa leaves indicates the amount of minerals available. The ash content obtained from the study was significantly higher in black plastic mulch although not significantly different from the red plastic mulches and differed significantly from the other mulching materials used. The ash content of 14.93% of the study obtained from black plastic mulch is relatively higher than the 13% ash content reported by Osadebe obtained from black polyethylene mulch and 8% of *Hibiscus esculentus* reported by (Akindahunsi and Salawu, 2005). However, Dairo and Adanlawo (2007), reported higher ash range of 16.30% to 17.31%, which was significantly higher than the ash content obtained from the current study. On the contrary, Adebimpe *et al.* (2024) reported that lower ash content of 5.45% stem to leaves of *E. purpurea* obtained from black plastic mulching materials.

The variation in the ash content may be attributed to different mulching materials used. In addition Adebimpe *et al.* (2024) opined that the variation observed in protein and ash content could be explained by many factors, involving the environment, abiotic stress, the genetic inheritance maturity of the plants and the type of mulching material applied during the cultivation of the plants. Therefore, the ash content observed in the current study irrespective of the treatment, indicates that dried leaves of moringa are a reliable source of mineral elements.

The ADF and NDF content accumulation was notably higher in plastic mulched plots in comparison with organic mulched plots and the control. No previous study in the literature have that reported the influence of plastic mulch on NDF and ADF. Therefore, the increased values of ADF and NDF content in moringa could be attributed to high temperatures and dry conditions in the summer season of the Eastern Cape Province. Extremely high temperature and moisture stress reduce forage quality by lowering the leaf/stem ratio, intensifying lignification, and increasing ADF and NDF contents (Kering *et al.*, 2011). The differences in NDF and ADF in this study are possibly due to differences in agro-climatic conditions, leaf ages, and harvest time. Even though mulching results in modified microclimatic conditions around the root zone of the plants, the black plastic traps more sun rays which results in increased ADF and NDF contents in black plastic mulched plots.

The likelihood of mulching to increase soil temperature may be linked to the increased levels of nitrogen, phosphorous, and potassium detected in leaves (Ghazal and Al-Nussairaw, 2022), which may improve root growth and their capacity to absorb water and nutrients from the soil. As a result, this is demonstrated by an increase in root and vegetative growth, which stimulates the growth of the tips of the plants and the synthesis of hormones like gibberellins and auxins (Ghazal, and Al-Nussairaw, 2022), due to the accumulation of nutrients in the leaves that enables vegetative growth characteristics to enhance the yield.

5.6. Conclusion

The study demonstrates that the use of black plastic mulch significantly enhances the nutritional composition of moringa leaves compared to other mulching materials and plastics evaluated. Black plastic mulch effectively regulates soil temperature, suppresses weeds, conserves soil moisture, improves plant productivity, and promotes early flowering, thereby facilitating early harvesting. Based on these findings, it is recommended to utilize black plastic mulch for moringa cultivation to optimize its nutritional quality. This approach can contribute to enhancing food security and nutritional outcomes in countries where moringa is cultivated, thereby supporting

efforts to combat malnutrition and improve sustainable agricultural productivity. As agricultural practices evolve towards more sustainable approaches, understanding the advantages of plastic mulch can inform better management strategies and support the growth of high-value crops, making it an essential consideration for modern cultivation practices.

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CHAPTER 6: THE IDENTIFICATION AND QUANTIFICATION OF SECONDARY METABOLITES FROM MORINGA LEAVES AS INFLUENCED BY DIFFERENT MULCH MATERIALS

ABSTRACT

Harvesting wild medicinal plants is crucial in rural economies as it alleviates poverty and enhances nutrition. Domestication of such plants is essential to ensure continued existence of such important plants among other factors. Moringa, valued for its significant economic and nutritional advantages, is commonly grown in rural areas to combat food insecurity and malnutrition. Despite its benefits, smallholder farmers encounter difficulties in moringa cultivation, especially with weed management, due to the plant's slow germination and growth. This current study evaluated the influence of various coloured plastic mulches on moringa's growth and secondary metabolites. The results of the analysis of variance revealed significant differences ($p < 0.05$) among the treatments. Various secondary metabolites were also affected. The total phenolic acid content was highest in organic mulch (4971.54 mg/kg), followed by black plastic mulch (4861.73 mg/kg), red plastic mulch (4725.26 mg/kg), white plastic mulch (4645.04 mg/kg), green plastic mulch (4565.92 mg/kg), and the control (4522.79 mg/kg). The total flavonoid content was increased in black plastic mulch (11735.50 mg/kg), followed by red plastic mulch (11233.75 mg/kg), organic mulch (11218.75 mg/kg), white plastic mulch (10460.25 mg/kg), green plastic mulch (10221 mg/kg), and the control (9892.50 mg/kg) respectively. Fatty acids were highest in black plastic mulch (2687.26 mg/kg), followed by green plastic mulch (2556.25 mg/kg), organic mulch (2445.52 mg/kg), red plastic mulch (2389.75 mg/kg), white plastic mulch (2336.50 mg/kg), and the control (2268.50 mg/kg). Organic compounds were most abundant in black plastic mulch (4562 mg/kg), followed by red plastic mulch (4256.25 mg/kg), organic mulch (4224.75 mg/kg), white plastic mulch (3942.73 mg/kg), green plastic mulch (3747 mg/kg), and the control (3550.50 mg/kg). Overall, black plastic mulch proved the most effective in improving Moringa's growth and metabolite composition, making it a viable strategy for enhancing productivity and managing weeds.

Key words: food security, growth, moringa production phytochemical composition and plastic mulch.

6.1. Introduction

Indigenous knowledge of medicinal plants has been utilized for centuries to address various health issues and enhance well-being (Roberson, 2008). The harvesting of wild medicinal plants is a significant economic activity in rural areas, contributing to poverty reduction and improved nutrition (Shackleton *et al.*, 2010). Moringa is cultivated in rural communities due to its substantial economic and nutritional benefits, which help mitigate food insecurity and malnutrition (Sokombela *et al.*, 2022). However, smallholder farmers face challenges in moringa production, particularly with weed management, due to the plant's slow germination and growth characteristics (Gadzirayi *et al.*, 2013). There are differences in the phytochemical composition of the medicinal plant's raw materials due to seasonal variation, cultivation practices, and post-harvest processing.

Mechanical weeding methods are time-consuming and costly, leading to reduced productivity (Bakare *et al.*, 2021). Mulching has been identified as a potential strategy to enhance crop performance and control weeds (Singh *et al.*, 2021). It influences soil moisture, temperature, fertility, and impacts plant growth and phytonutrient content (Lee and Thierfelder, 2017). Organic mulches such as plant residue, for example, maize stacks and sorghum straws are traditionally used for improving the plant's rhizosphere microclimate, and they often use plant-based material (Nyambo *et al.*, 2020). Plastic mulching has been extensively applied in horticultural crops, with different colours influencing crop yield and quality (Kasirajan and Ngouajio, 2012). Coloured plastic mulches affect light reflection and soil temperature, which can impact plant growth and yield (Kasperbauer and Loughrin, 2004; Manganelli, 2017). Although plastic mulching is established in other crops, its application in moringa production is relatively novel.

There has been a growing movement towards the domestication of wild medicinal plants to mitigate the pressures of over-harvesting, boost rural economy and malnutrition. One such species attracting attention is moringa, a versatile shrub. The tree's seeds, bark, roots, and leaves have valuable

uses, and its leaves are rich in vital nutrients and phytochemicals such as phenolic compounds, which exert antioxidant properties for improving human immunity (Anwar *et al.*, 2007; Verma *et al.*, 2009). These bioactive compounds play crucial roles in scavenging free radicals and protecting against oxidative stress, thereby potentially mitigating various health risks (Peng *et al.*, 2003; Dinda *et al.*, 2007). Research has indicated that phenolic compounds found in fruits and vegetables are essential for their antioxidant properties, prolonging shelf life and enhancing quality attributes by reducing oxidative degradation (Fan *et al.*, 2012; Khanizadeh *et al.*, 2009). Moringa leaves are recognized for their antioxidant content, including ascorbic acid, flavonoids, and phenolics, which protect cellular components from oxidative damage (Anwar *et al.*, 2007; Tshabalala *et al.*, 2020).

Researchers have reported the potential of moringa as a nutraceutical plant that improves both human and animal health (Sokombela *et al.*, 2022) and the application of its extracts to enhance the growth of other crops, such as tomatoes, wheat, and maize (Biswas *et al.*, 2016; Mashamaite *et al.*, 2022). Furthermore, critical information about agronomic practices, particularly weed management, is limited, although weed management significantly improves plant productivity. Literature reports evidence of investigation about the different phytochemicals in different parts of moringa (Biswas *et al.*, 2016, plant spacing and harvesting frequency that influences moringa growth and yield (Bopape-Mabapa 2019).

Production of moringa in South Africa is still at the developmental stage (Bopape-Mabapa, 2019). To foster sustainable production in South Africa, it is essential to establish effective agronomic practices such as weed management, water management, and fertilizer protocols to optimize yield and enhance nutritional quality. One effective and sustainable weed management practice is mulching, which involves applying a physical barrier on the soil surface (Nyambo *et al.*, 2020a; Zhang *et al.*, 2021).

Mulching not only suppresses weed growth, reducing associated costs, but also conserves soil moisture, moderates soil temperature, enhances microbial activity, improves soil fertility, and promotes overall plant growth and yield of high-quality produce (Lee and Thierfelder, 2017).

Literature on coloured plastic mulches in some horticultural crops was evidenced. For instance, blue plastic mulch has been associated with higher phytochemical concentrations in some plants (Taulavuori *et al.*, 2013; Taulavuori *et al.*, 2017) such as flavonoids (Ebisawa *et al.*, 2008), carotenoids and anthocyanins in lettuce (Li and Kubota, 2009) and anthocyanins in tomato (Giliberto *et al.*, 2005). However, the effects of light quality on flavonoid and phenolic acid biosynthesis in plants is species dependent (Taulavuori *et al.*, 2016), indicating that many plant traits are modified by the reflection of blue light from blue mulch, but many of them need to be explored regarding their agronomic importance. The yellow and green mulches have been attributed to increased levels of aromatic and phenolic compounds (Loughrin and Kasperbauer, 2001). These variations in response to different plastics were only observed in vegetable crops and, therefore, send a signal that it is worth evaluating the reaction of the other coloured plastic mulches in moringa plantation. The objective of this study was to evaluate the impact of different coloured plastic mulches on the composition of secondary metabolites in the moringa leaves.

6.2. Materials and methods

6.2.1. Description of the experimental site

The experiment in this chapter followed the experimental design and description in Chapter 3

6.3. Measurements

6.3.1. Sample preparation and chemicals for analyzing the untargeted metabolites in moringa leaves grown in different mulching materials

Sample extraction for untargeted metabolites was carried out following the protocol outlined by Mpai and Sivakumar (2018) with minor modifications, whereby 200 mg of the dried ground leaf sample was homogenized in 20 mL of an acidified methanol: HCL: distilled water (80:0.5:19.5 v/v/v). The homogenization was carried out in a thermostatic shaking water bath at 70°C for 15 minutes. Subsequently, the extracts were centrifuged for 15 minutes, dried under the N₂ gas, re-suspended, and filtered as described by Mpai and Sivakumar (2020) before biochemical analysis.

6.3.2. UPLC-MS untargeted metabolites analysis

The untargeted and targeted metabolites analysis was performed using a high-resolution Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA). This was further connected to a column eluate that first passed through a Photodiode Array (PDA) detector before going to the mass spectrometer, allowing simultaneous UV and MS spectra collection. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimized for best resolution and sensitivity. Data were acquired by scanning from m/z 150 to 1500 m/z in resolution and MSE modes. In MSE mode, two channels of MS data were acquired, one at a low collision energy (4 V) and the second using a collision energy ramp (40–100 V) to obtain fragmentation data. Leucine enkephalin was used as lock mass (reference mass) for accurate mass determination, and the instrument was calibrated with sodium formate. Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column. An injection volume of 2 µL was used, and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. The gradient started at 100% solvent A for 1 minutes and linearly changed to 28% B over 22 minutes It then went to 40% B over 50 s and a wash step of

1.5 minutes at 100% B, followed by re-equilibration to initial conditions for 4 minutes. The flow rate was 0.3 mL/min, and the column temperature was maintained at

55 °C.

Compounds were quantified relatively against a calibration curve established by injecting a range of quercetin standards from 0.5 to 100 mg/L chlorogenic acid.

6.4. Statistical analysis

Data obtained for the quantification of metabolites was run as one-way ANOVA and subjected to GraphPad statistical software version 15 and for the separation of means and standard deviation. The graphs and standard curve calculations were performed using the GraphPad Prism version 5. Unsupervised Principal Component Analysis (PCA) chemometric plots were generated using Metaboanalyst version 6 (as of July 22, 2024) to evaluate clustering patterns, identify differences, and determine the principal components contributing to the variation between sample groups treated with different mulching material and control (no mulch) groups. The data underwent normalization relative to the control feature, followed by base 10 logarithmic transformation. Additionally, the data was centred and Pareto scaled, which involves mean centering and dividing by the square root of the standard deviation for each variable. This approach was used to balance the features, preserve the data structure, reduce variable redundancy, and correct measurement errors (n=3).

6.4. Results

6.4.1: The identification and quantification of phenolic acids in moringa leaves as influenced by different mulching materials

The use of UPLC-QTOF-MS enabled the identification of 24 phenolic acid compounds, including: vanillobioside, gallic acid, 2-hydroxyphenylacetic acid O- β -D-glucoside, salicylic acid β -D-glucoside, canthoside A;(-)-canthoside A, gentesic acid 5-O-glucoside, 3,5-dihydroxybenzoic acid, racemosic acid, canthoside B;(-)-canthoside B, 3-[[1,1'-biphenyl]-4-yl]-9-methyl-5-phenyl-7H-furo[3,2-g]chromen-7-one, 4-Hydroxycinnamic acid, 6-benzyl-3-[[1,1'-biphenyl]-4-yl]-5-methyl-7H-furo[3,2-g]chromen-7-one, 4-hydroxycinnamic acid, 1-O-p-coumaroyl- β -D-glucose,

bungeiside D, dihydrocaffeic acid 3-O-glucuronide, dryopteriside;(+)-dryopteriside, trans-ferulic acid, caffeic acid, paeonolide, 2-hydroxybenzaldehyde O-[xylosyl-(1->6)-glucoside], 2-hydroxyphenylacetic acid O-b-D-glucoside, (??)-threo-1-(p-hydroxyphenyl) propylene glycol 4'-glucoside and vanillic acid. These compounds were derivatives of the phenolic glycosides, hydroxybenzoic, and hydroxycinnamic acid derivatives (**Table 6.1**). They were all detected in all moringa leaves grown under different mulching materials. The presence of caffeic acid, chlorogenic acid, and gallic acid has been authenticated by Vergara-Jimenez (2017), who describes their predominance in moringa leaves.

The quantification of these phenolic acids was influenced by the type of mulching material used. The application of different mulching materials significantly ($p < 0.05$) affected the phenolic acids contents in Moringa leaf. The total phenolic acid content showed variation within the mulching treatments. In descending order, organic mulch (4971.54 mg/kg), black mulch (4861.73 mg/kg), red mulch (4725.26), white mulch (4645.04 mg/kg), and green mulch (4565.92 mg/kg) performed better than the no-mulch (4522.79 mg/kg) material (**Table 6.1**). Black mulch showed enhanced accumulation of vanilloylside (83.00 mg/kg), gallic acid (131.00 mg/kg), canthoside A;(-)-canthoside A (57.75 mg/kg), vanillic acid (85.25 mg/kg), dihydrocaffeic acid 3-O-glucuronide (182.00 mg/kg), phenylacetic acid (75.75 mg/kg), 3-[[1,1'-biphenyl]-4-yl]-9-methyl-5-phenyl-7H-furo[3,2-g]chromen-7-one (246.75 mg/kg), chlorogenic acids (351.23 mg/kg), cinnamic acid (265.00 mg/kg) and 3-O-p-coumaroylquinic acid (268.25 mg/kg) than in other treatments. The highest caffeic acid was detected in Moringa leaves grown under the organic mulch, was one and a half times higher than the least-performing green mulch treatment (213.75 mg/kg).

Table 6.1: UPLC-QTOF-MS Quantification and identification of phenolic compounds detected in Moringa leaves grown under different mulching material.

COMPOUND	Black plastic (mg/kg)	White plastic (mg/kg)	Green plastic (mg/kg)	Red plastic (mg/kg)	Organic material (mg/kg)	No Mulch (mg/kg)
Vanilloside	83.00 ± 04.42a	71.75 ± 05.85b	83.00 ± 02.08a	82.25 ± 04.87a	75.75 ± 04.88b	72.25 ± 02.65b
Gallic acid	131.00 ± 02.82a	90.25 ± 01.60b	99.75 ± 03.79b	89.75 ± 04.12c	96.00 ± 03.48d	76.00 ± 03.82e
gentesic acid 5-O-glucoside	188.25 ± 02.74c	147.25 ± 01.16e	181.50 ± 04.2c	264.75 ± 01.23a	195.75 ± 02.85b	176.75 ± 04.76d
Canthoside A;(-)-Canthoside A	57.75 ± 01.31a	48.00 ± 02.94b	48.25 ± 03.88b	46.50 ± 03.13c	47.50 ± 04.51c	47.75 ± 02.92c
3,5-Dihydroxybenzoic acid	218.25 ± 02.43f	246.25 ± 04.59c	256.75 ± 02.56d	230.75 ± 04.28e	299.00 ± 03.73b	334.00 ± 02.67a
Racemosic acid	198.00 ± 02.75d	215.50 ± 03.70b	202.51 ± 03.34c	250.50 ± 01.06a	213.00 ± 02.50b	207.00 ± 03.74c
Vanillic acid	85.25 ± 05.25a	77.75 ± 02.09b	77.50 ± 04.05b	76.00 ± 04.02b	80.00 ± 04.79a	72.75 ± 02.49c
Canthoside B;(-)-Canthoside B	205.00 ± 02.24c	189.00 ± 02.46d	220.75 ± 03.95a	212.25 ± 03.65b	211.75 ± 02.64b	214.75 ± 04.54b
Dihydrocaffeic acid 3-O-glucuronide	182.00 ± 02.60a	152.75 ± 02.89d	171.00 ± 03.17b	120.25 ± 04.49f	166.25 ± 03.08c	139.00 ± 01.65e
Dryopteroid;(+)-Dryopteroid	189.50 ± 01.02c	180.75 ± 02.95b	236.25 ± 02.61a	201.75 ± 02.04b	176.50 ± 03.96d	178.50 ± 02.87d
trans-Ferulic acid	193.50 ± 01.28c	179.50 ± 04.65d	159.25 ± 01.51e	249.25 ± 01.87a	224.75 ± 04.12b	190.25 ± 03.01c
Caffeic acid	186.75 ± 03.23b	176.50 ± 03.04d	155.25 ± 01.46e	187.00 ± 03.71b	213.75 ± 02.37a	170.75 ± 03.62c
Salicylic acid beta-D-glucoside	175.75 ± 02.73c	164.00 ± 03.02d	160.25 ± 01.23d	144.25 ± 01.90e	223.00 ± 04.29a	208.25 ± 05.96b
Phenylacetic acid	75.75 ± 04.66a	70.50 ± 01.19b	72.25 ± 02.57b	68.50 ± 03.61c	70.75 ± 02.86b	68.00 ± 04.88c
2-Hydroxybenzaldehyde O-[xylosyl-(1->6)-glucoside]	196.50 ± 04.11c	196.00 ± 02.05c	222.50 ± 01.63b	229.25 ± 05.77b	269.25 ± 04.69a	172.75 ± 05.39d
3-[[1,1'-biphenyl]-4-yl]-9-methyl-5-phenyl-7H-furo[3,2-g]chromen-7-one	246.75 ± 0.83a	210.75 ± 0.21c	218.50 ± 03.25c	243.50 ± 04.28b	197.00 ± 02.16d	176.75 ± 02.91e
4-Hydroxycinnamic acid	208.50 ± 05.44d	230.00 ± 03.11c	176.00 ± 02.27e	261.75 ± 01.89a	251.75 ± 03.70b	257.00 ± 04.71b
1-O-p-Coumaroyl-beta-D-glucose	150.75 ± 01.50d	176.50 ± 01.14a	156.00 ± 05.70d	170.50 ± 01.44b	158.50 ± 03.21d	169.75 ± 03.01c
Bungeiside D	470.00 ± 0.33c	518.25 ± 03.68b	439.50 ± 04.79d	379.50 ± 03.24e	476.50 ± 01.42c	538.25 ± 02.43a
(-)-Gossypol	289.50 ± 03.17b	190.75 ± 04.45f	244.25 ± 04.97d	392.25 ± 03.54a	274.50 ± 05.65c	226.50 ± 02.25e
Chlorogenic acids	351.23 ± 01.21a	346.54 ± 01.15b	314.41 ± 02.41c	273.51 ± 03.52d	256.54 ± 04.21e	236.04 ± 02.21f
Cinnamic acid	265.00 ± 02.13a	156.25 ± 04.33d	172.50 ± 02.59c	187.75 ± 02.08b	146.00 ± 04.81e	112.50 ± 01.71f
3-O-p-Coumaroylquinic acid	268.25 ± 03.90a	230.00 ± 03.73b	146.75 ± 01.01d	114.50 ± 04.68f	169.50 ± 03.24c	122.50 ± 02.34e
3-O-Caffeoyl-4-O-methylquinic acid	245.50 ± 04.12d	353.25 ± 02.03b	351.25 ± 02.43b	249.00 ± 01.03c	478.25 ± 01.86a	354.75 ± 04.41b
Total phenolic acid	4861.73 mg/kg	4645.92 mg/kg	4525.26 mg/kg	4725.26 mg/kg	4971.54 mg/kg	4522.79 mg/kg

Figure 6.1 shows the principal component analysis model (PCA), and the sample variation based on the phenolic acid contents. There was a clear distinction between the two metabolome clusters based on the samples with higher phenolic content versus those with lower phenolic contents of phenolic compounds. Moringa leaves grown on the black mulch treatment exhibited the highest phenolic content compared to other treatment.

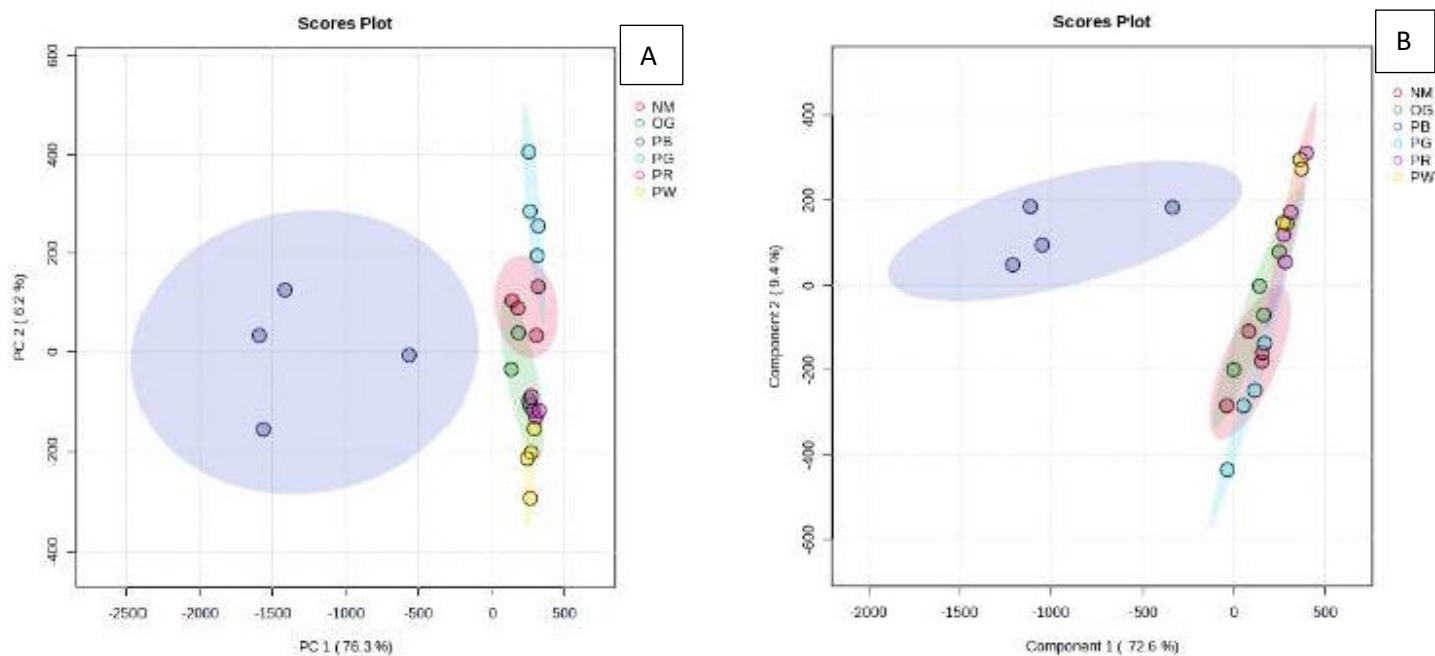


Figure 6.1: A: Principal component analysis and B: Orthogonal partial least of squares - Discriminant analysis showing clustering of phenolic compounds detected in Moringa leaves grown under different mulching material. NM: no mulch; OG: organic mulch; PB: black plastic; PG: green plastic; PR: red plastic, and PW: white plastic.

6.4.2: The identification and quantification of flavonoids compounds in moringa leaves as influenced by different mulching materials

Through the usage of UPLC-QTOF-MS different classes of flavonoids which contained 46 flavonoids compounds were identified (**Table 6.2**). Flavonoids detected in the moringa leaves grown under different mulching materials showed various flavonoids compounds falling within Flavonoid-3-O-glycosides including quercetin 3-(6"-malonylglucoside), Quercitrin, Kaempferol 3-(6-acetylgalactoside), 6"-Malonylastragalin, Rutin, Keioside, Quercitrin, 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl), Eujambolin, and Isorhamnetin 3-(6"-malonylglucoside). Moreover, other compounds from members of the Flavonoid-7-O-glycosides including Eriocitrin, Patuletin 3,7-bis(3-acetylramnoside), 6"-p-Coumaroylprunin, Patuletin 3-ramnoside-7-(3"',4"'-diacetylramnoside), 2-(3,4-dihydroxyphenyl)-5-hydroxy-3,6,7-trimethoxy-4H-chromen-4-one, Quercetin 3-O-(6"-malonylglucoside) 7-O-glucoside, Astragalin 7-ramnoside, Astragalin 7-ramnoside, Tricin 7-glucuronoside and Apigenin 7-[ramnosyl-(1->2)-galacturonide] were detected (**Table 6.2**).

Table 6.2 illustrates the quantification of the identified flavonoid compounds as affected by the different mulching materials. The application of different mulching materials significantly ($p < 0.05$) affected the flavonoid compounds observed in Moringa leaves. The total flavonoid content exhibited variation within the mulching treatments such that in descending order, black mulch (11735.50 mg/kg), red mulch (11233.75 mg/kg), organic mulch (11218.5 mg/kg), and white mulch (10460.25 mg/kg) performed

better than the green mulch (10221.00 mg/kg) and no-mulch (9892.50 mg/kg) material. The flavonoid content exhibited variation within the mulching treatments such that in descending order, black mulch, red mulch, organic mulch, and green mulch performed better than the white mulch and no-mulch material. Black mulch indicated an increased accumulation of Phaseollidin (85.75 mg/kg), Eriocitrin (96.75 mg/kg), Patuletin 3,7-bis(3-acetylramnoside) (385.75 mg/kg), Patuletin 3-ramnoside-7-(3'',4''-diacetylramnoside) (348.50 mg/kg), Quercetin 3-O-(6''-malonyl-glucoside) 7-O-glucoside (337.50 mg/kg), Quercetin 3-(6''-malonyl-glucoside) (337.50 mg/kg), Keioside (268.25 mg/kg), 5,7-dihydroxy-2-(4-hydroxy 3-methoxyphenyl)-3-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-4H-chromen-4 one (334.75), Eujambolin (617.50 mg/kg), 6-({3-[2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxo-7-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-1-benzopyran-8-yl]-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-1-benzopyran-7-yl]oxy)-3,4,5 trihydroxyoxane-2-carboxylic acid (363.50 mg/kg), Pierreione D (362.75 mg/kg), Ovalifolin;6-[(3-Methyl-2-butenyl)oxy]-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one, (566.00 mg/kg), and Embinin (267.25 mg/kg) which were significantly ($p < 0.05$) higher than the other treatments. Followed by the red plastic mulch, which resulted in increased concentrations of Glyceollidin I (268.50 mg/kg), Kanzonol P (265.00 mg/kg), 6''-p-Coumaroylprunin (85.00 mg/kg), Quercitrin (403.50 mg/kg), Kaempferol 3-(6-acetylgalactoside) (399.75), 5,7-Dihydroxyflavone 7-benzoate (293.25 mg/kg), 3-(2,4-diphenyl-4H-chromen-3-yl)-1-phenylprop-2-en-1-one (306.00 mg/kg), epicatechin-(4 β ->8)-4'-O-methylgallocatechin (369.25 mg/kg), Stephaflavone A;5,5'-Dihydroxy-7,7'-dimethoxy-2,2'-bis(4-methoxyphenyl)-[3,6'-bi-4H-1-benzopyran]-4,4'-dione (85.00 mg/kg), Epicatechin (369.25 mg/kg), Glyinflanin H (350.25 mg/kg), and Quercetin (686.50 mg/kg).

Table 6.2: UPLC-QTOF-MS Quantification and identification of flavonoids compounds detected in Moringa leaves grown under different mulching material.

Flavonoids	Treatments					
Tentative structural assignment	Black plastic (mg/kg)	White plastic (mg/kg)	Green plastic (mg/kg)	Red plastic (mg/kg)	Organic mulch (mg/kg)	No mulch (mg/kg)
Phaseollidin	85.75 ± 03.19a	82.50 ± 04.85b	82.50 ± 02.66b	80.25 ± 04.40c	66.25 ± 05.02d	62.50 ± 03.01d
Glyceollidin I	204.75 ± 03.44c	158.25 ± 02.43f	169.25 ± 06.87e	268.50 ± 01.90a	236.50 ± 02.52b	175.00 ± 03.80d
Kanzonol P	258.50 ± 05.41b	242.25 ± 05.59c	245.50 ± 04.52c	265.00 ± 02.72a	218.75 ± 01.04d	251.75 ± 02.04a
Eriocitrin	96.75 ± 02.23a	90.25 ± 02.78b	78.75 ± 03.59c	86.25 ± 03.06d	78.00 ± 03.93c	86.00 ± 03.18d
Patuletin 3,7-bis(3-acetylramnoside)	385.75 ± 04.45a	382.25 ± 02.85a	253.75 ± 05.88d	254.75 ± 01.59d	260.25 ± 01.55c	270.75 ± 05.43b
6"-p-Coumaroylprunin	76.00 ± 03.16c	86.25 ± 04.30a	81.50 ± 01.04b	85.00 ± 04.60a	81.75 ± 02.65b	68.00 ± 02.33d
Patuletin 3-rhamnoside-7-(3",4"-diacetylramnoside)	348.50 ± 01.15a	335.50 ± 03.12b	384.00 ± 03.39c	235.75 ± 02.01d	240.75 ± 10.18e	271.50 ± 04.43f
Quercetin 3-O-(6"-malonyl-glucoside) 7-O-glucoside	337.50 ± 03.62a	238.00 ± 04.63b	134.00 ± 03.58e	167.75 ± 01.42d	138.25 ± 03.87e	206.25 ± 02.62c
Astragalin 7-rhamnoside	258.00 ± 02.90d	247.75 ± 05.92e	279.50 ± 03.77c	210.75 ± 03.83f	372.25 ± 03.89a	353.00 ± 01.18b
Tricin 7-glucuronoside	284.00 ± 02.37d	272.50 ± 02.67e	367.00 ± 04.33b	348.75 ± 02.98c	426.75 ± 05.51a	285.00 ± 04.66d
Rutin	89.75 ± 02.16b	93.50 ± 02.21a	71.00 ± 05.18e	83.50 ± 02.87c	76.25 ± 04.40d	80.25 ± 01.54c

Flavonoids	Treatments					
Quercetin 3-galactoside	693.00 ± 03.89c	682.75 ± 03.05b	617.50 ± 02.97d	616.25 ± 03.90d	727.75 ± 03.14a	560.50 ± 02.62e
Quercetin 3-(6"-malonyl-glucoside)	337.50 ± 03.62a	238.00 ± 04.63b	134.00 ± 03.58e	167.75 ± 01.42d	138.25 ± 03.87e	206.25 ± 02.62c
Quercitrin	388.25 ± 01.86c	333.00 ± 03.49d	400.00 ± 05.06a	403.50 ± 02.40a	389.00 ± 03.80c	397.75 ± 03.11b
Kaempferol 3-(6-acetylgalactoside)	250.75 ± 04.67e	348.50 ± 02.69b	269.00 ± 03.43d	399.75 ± 04.65a	286.75 ± 02.25c	210.75 ± 02.26 f
6"-Malonylastragalin	72.75 ± 05.81e	82.50 ± 05.24c	87.25 ± 02.09a	85.25 ± 03.01b	79.00 ± 03.93d	74.75 ± 05.23e
Keioside	268.25 ± 02.45a	161.25 ± 03.73d	192.00 ± 03.91c	224.50 ± 02.29b	197.00 ± 01.47c	157.75 ± 03.31e
5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-4H-chromen-4-one	334.75 ± 02.06a	203.25 ± 01.19f	226.00 ± 04.72e	310.25 ± 03.50b	256.50 ± 02.38c	247.25 ± 02.11d
Eujambolin	617.50 ± 04.28a	476.00 ± 04.55e	404.25 ± 04.89f	611.25 ± 02.86b	555.75 ± 03.15c	453.50 ± 02.97d
Isorhamnetin 3-(6"-malonylglucoside)	250.50 ± 02.39d	252.00 ± 02.17d	320.75 ± 03.56c	341.25 ± 03.64b	382.00 ± 04.77a	218.75 ± 03.44e
5,7-bis(benzoyloxy)-2-phenyl-4H-chromen-4-one	418.75 ± 04.92d	479.50 ± 02.46b	403.25 ± 01.67e	368.50 ± 02.74e	512.75 ± 04.64a	446.25 ± 01.14c
5,7-Dihydroxyflavone 7-benzoate	218.25 ± 02.73b	146.50 ± 02.84d	135.50 ± 04.07e	293.25 ± 03.55a	208.50 ± 03.67c	293.75 ± 02.81a
3-(2,4-diphenyl-4H-chromen-3-yl)-1-phenylprop-2-en-1-one	230.50 ± 04.23c	282.25 ± 01.56b	198.50 ± 02.89e	306.00 ± 01.14a	234.50 ± 04.08c	216.00 ± 05.99d

Flavonoids	Treatments					
epicatechin-(4beta->8)- 4'-O-methylgallo catechin	281.50 ± 04.09c	254.25 ± 04.08d	288.75 ± 03.76b	369.25 ± 03.75a	208.00 ± 01.79e	281.75 ± 02.82c

Flavonoids	Treatments					
6-({3-[2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxo-7-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-1-benzopyran-8-yl]-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-1-benzopyran-7-yl]oxy)-3,4,5-trihydroxyoxane-2-carboxylic acid	363.50 ± 04.21a	238.25 ± 04.67e	358.00 ± 03.29b	299.50 ± 03.20c	227.75 ± 04.05f	269.25 ± 02.11d
Derriscandenoside C;8-Hydroxy-7,4'-dimethoxyisoflavone 8-O-(6"-rhamnosylglucoside)	178.75 ± 03.59b	176.25 ± 05.35b	213.75 ± 04.59a	117.25 ± 01.58c	160.50 ± 02.37d	153.25 ± 01.85e
Apigenin 7-[rhamnosyl-(1->2)-galacturonide]	693.75 ± 04.34d	721.25 ± 04.91c	808.75 ± 04.55a	717.00 ± 04.25c	791.25 ± 03.31b	582.00 ± 03.13e
Stephaflavone A;5,5'-Dihydroxy-7,7'-dimethoxy-2,2'-bis(4-methoxyphenyl)-[3,6'-bi-4H-1-benzopyran]-4,4'-dione	74.25 ± 02.01c	80.25 ± 04.76b	81.25 ± 02.89b	85.00 ± 02.06a	62.00 ± 04.67d	79.00 ± 01.47c
Euchrenone b11;5,5'-Dihydroxy-2'-methoxy-6-prenyl-(2",3":7,8),(2"',3"'':4',3')-bis(6,6-dimethylpyrano)isoflavone	301.50 ± 04.86e	322.50 ± 03.11c	314.00 ± 01.82d	332.25 ± 01.46b	361.25 ± 04.64a	307.00 ± 03.74e

Flavonoids	Treatments					
Pierreione D	362.75 ± 05.22a	326.50 ± 03.30c	346.75 ± 04.84b	262.75 ± 02.82f	318.00 ± 05.48d	295.75 ± 01.04e

Flavonoids	Treatments					
2',4-Dihydroxy-4',6'-dimethoxy-3'-prenylchalcone	260.00 ± 01.92d	306.75 ± 02.56a	260.75 ± 02.17d	302.00 ± 04.61b	213.75 ± 02.95e	273.00 ± 03.42c
Epicatechin	281.50 ± 04.09b	254.25 ± 04.08c	288.75 ± 03.76b	369.25 ± 03.75a	208.00 ± 01.79d	281.75 ± 02.82b
2-(3,4-dihydroxyphenyl)-5-hydroxy-3,6,7-trimethoxy-4H-chromen-4-one	79.00 ± 03.69c	83.00 ± 03.51a	64.75 ± 03.39e	81.50 ± 03.03b	79.25 ± 04.27c	75.00 ± 04.78d
(2S)-6-(gamma,gamma-dimethylallyl)-3',4'-dimethoxy-6'',6''-dimethylpyran[2'',3'':7,8]flavanone	205.00 ± 01.64e	242.25 ± 05.57d	202.25 ± 02.71e	315.75 ± 02.98b	324.25 ± 04.67a	307.25 ± 01.55c
Glyinflanin H	321.75 ± 04.45b	231.00 ± 01.73f	307.75 ± 02.54c	350.25 ± 03.68a	244.75 ± 02.05e	252.00 ± 04.46d
Ovalifolin;6-[(3-Methyl-2-butenyl)oxy]-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one	566.00 ± 04.45a	473.50 ± 02.73c	445.50 ± 05.74d	546.75 ± 03.43b	427.25 ± 41.03e	321.50 ± 03.56f
Embinin	267.25 ± 02.15a	171.00 ± 03.40c	251.75 ± 04.02b	185.00 ± 03.08d	232.75 ± 02.54c	176.25 ± 03.27e
Quercetin	633.00 ± 02.58d	664.75 ± 03.65b	453.50 ± 03.85f	686.50 ± 03.73a	598.25 ± 01.98e	644.50 ± 03.67c
Total flavonoids	11735.50 mg/kg	10460.25 mg/kg	10221 mg/kg	11233.75 mg/kg	11218.75 mg/kg	9892.50 mg/kg

Figure 6.2 indicates the principal component analysis model (PCA), and the sample variation based on the flavonoids compounds. There was a clear distinction between two metabolome clusters based on the samples with higher flavonoid content versus those with lower flavonoid contents of flavonoids compounds. Moringa leaves grown on the black mulch treatment revealed the highest flavonoid content in comparison to other treatments.

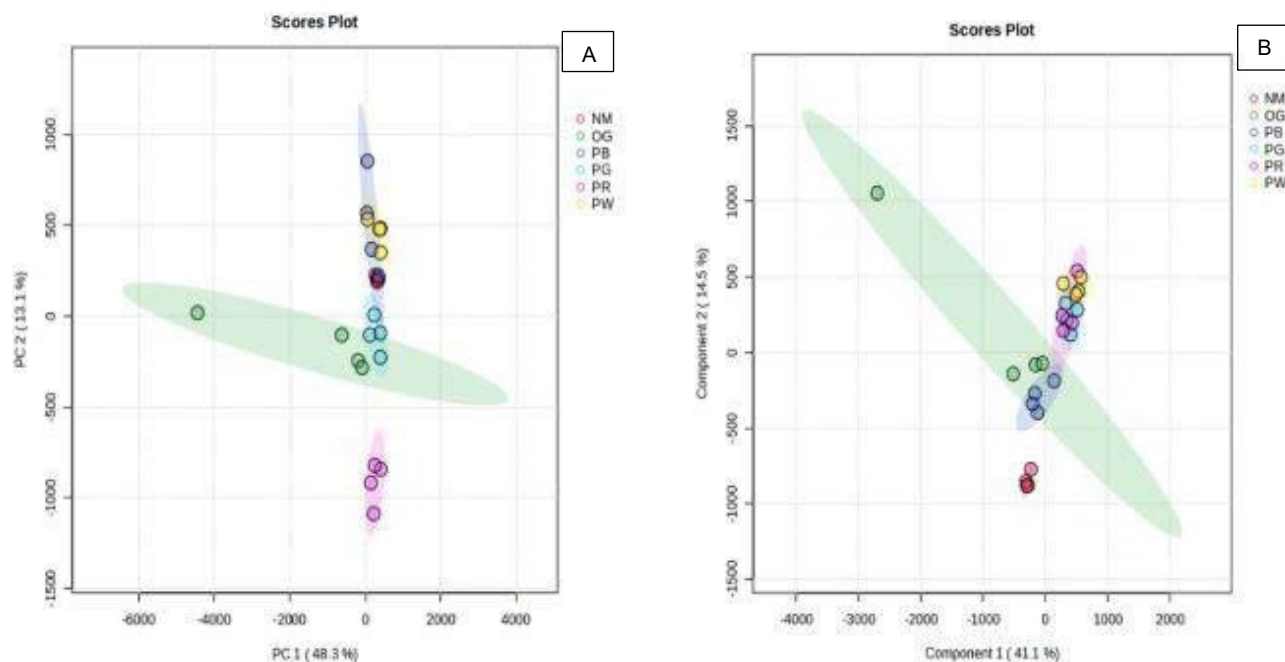


Figure 6.2: A: Principal component analysis and B: Orthogonal partial least of squares-Discriminant analysis showing clustering of flavonoids detected in moringa leaves grown under different mulching material. NM: no mulch; OG: organic mulch; PB: black plastic; PG: green plastic; PR: red plastic, and PW: white plastic.

6.4.3: The identification and quantification of fatty acids in moringa leaves as influenced by different mulching materials

The UPLC-QTOF-MS was used to determine fatty acid accumulation in moringa leaves grown under different mulching materials (**Table 6.3**). Different classes of fatty acids were obtained, such as long chain fatty acids, polyunsaturated fatty acids, fatty alcohols, and hydroxy fatty acids. The identified fatty acids were 12,13-EpOME, 12S-HHT, Gallicynoic acid F;(-)-Gallicynoic acid F, 13(S)-Hydroperoxylinolenic acid;(13S)-Hydroperoxy-cis-9,15-trans-11-octadecatrienoic acid;(13S)-HPLA, 13-L-Hydroperoxylinoleic acid, 3-Isopropylmalate, 3,7-Dimethyl-3-octene-1,2,6,7-tetrol, Ethyl (??)-3-hydroxybutyrate, and Corchorifatty acid F.

The total fatty acids content exhibited variation within the mulching treatments such that in descending order, black mulch (2687.26 mg/kg), green mulch (2556.25 mg/kg), organic mulch (2445.51 mg/kg), and red mulch (2389.75 mg/kg) performed better than the white mulch (2336.50 mg/kg) and no-mulch (control) (2268.50 mg/kg) material (**Table 6.3**). The black plastic mulch resulted to significantly higher concentrations of 12,13-EpOME, 12S-HHT, Gallicynoic acid F;(-)-Gallicynoic acid F, 13(S)-Hydroperoxylinolenic acid; (13S)-Hydroperoxy-cis-9,15-trans-11-octadecatrienoic acid;(13S)-HPLA, and 3,7-Dimethyl-3-octene-1,2,6,7-tetrol with fatty acid content of (80.25 mg/kg , 89.75 mg/kg, 799.75 mg/kg , 75.50 mg/kg and 326.25 mg/kg, respectively) when compared to the rest of the rest treatments. This was followed by the green mulch, which was significantly higher for 3-Isopropylmalate, Ethyl (??)-3-hydroxybutyrate, and Corchorifatty acid F with fatty acid content of (491.75 mg/kg, 779.0 mg/kg, and 82.25 mg/kg respectively).

Table 6.3.: UPLC-QTOF-MS Quantification of fatty acids found in moringa leaves as influenced by different mulching materials

Fatty acids	Treatments					
Tentative structural assignment	Black plastic (mg/kg)	White plastic (mg/kg)	Green plastic (mg/kg)	Red plastic (mg/kg)	Organic mulch (mg/kg)	No mulch (mg/kg)
12,13-EpOME	80.25 ± 05.02a	60.75 ± 03.11e	74.50 ± 05.38b	62.00 ± 04.54d	78.25 ± 02.39b	67.25 ± 03.81c
12S-HHT	89.75 ± 05.28a	50.25 ± 03.63e	58.75 ± 04.18d	80.75 ± 04.30b	88.25 ± 01.37a	72.25 ± 02.59c
Gallicynoic acid F;(-)-Gallicynoic acid F	799.75 ± 02.29a	625.50 ± 03.61d	763.00 ± 01.61b	763.00 ± 01.61b	616.50 ± 02.71e	643.00 ± 03.76
13(S)-Hydroperoxylinolenic acid;(13S)-Hydroperoxy-cis-9,15-trans-11-octadecatrienoic acid;(13S)-HPLA	75.50 ± 02.39a	71.75 ± 03.35b	52.25 ± 01.65e	59.00 ± 02.97d	72.00 ± 02.04b	61.00 ± 03.24c
13-L-Hydroperoxylinoleic acid	81.00 ± 04.08d	82.50 ± 02.10c	77.00 ± 01.15f	80.00 ± 04.88e	87.00 ± 04.14a	83.75 ± 01.52b
3-Isopropylmalate	413.50 ± 05.70b	323.50 ± 03.97f	491.75 ± 02.70a	389.00 ± 02.14c	358.50 ± 01.89e	375.50 ± 05.29d
3,7-Dimethyl-3-octene-1,2,6,7-tetrol	326.25 ± 03.17a	252.25 ± 05.50d	177.75 ± 02.47e	253.00 ± 01.55c	290.50 ± 05.67b	173.00 ± 01.10f
Ethyl (??)-3-hydroxybutyrate	755.75 ± 01.78b	727.75 ± 04.76c	779.00 ± 03.17a	626.25 ± 04.35e	778.00 ± 04.37a	714.00 ± 01.89d
Corchorifatty acid F	65.51 ± 04.59e	71.25 ± 04.95d	82.25 ± 01.70a	76.75 ± 03.56c	76.51 ± 05.02c	78.75 ± 08.48b
Total fatty acids	2687.26 mg/kg	2336.50 mg/kg	2556.25 mg/kg	2389.75 mg/kg	2445.51 mg/kg	2268.50 mg/kg

Figure 6.3 indicates the principal component analysis model (PCA), and the sample variation based on the fatty acids. There was a clear distinction between two metabolome clusters based on the samples with higher fatty acid contents versus those with lower fatty acid contents of fatty acid compounds. Moringa leaves grown on the black mulch treatment revealed the highest fatty acid content in comparison to other treatments.

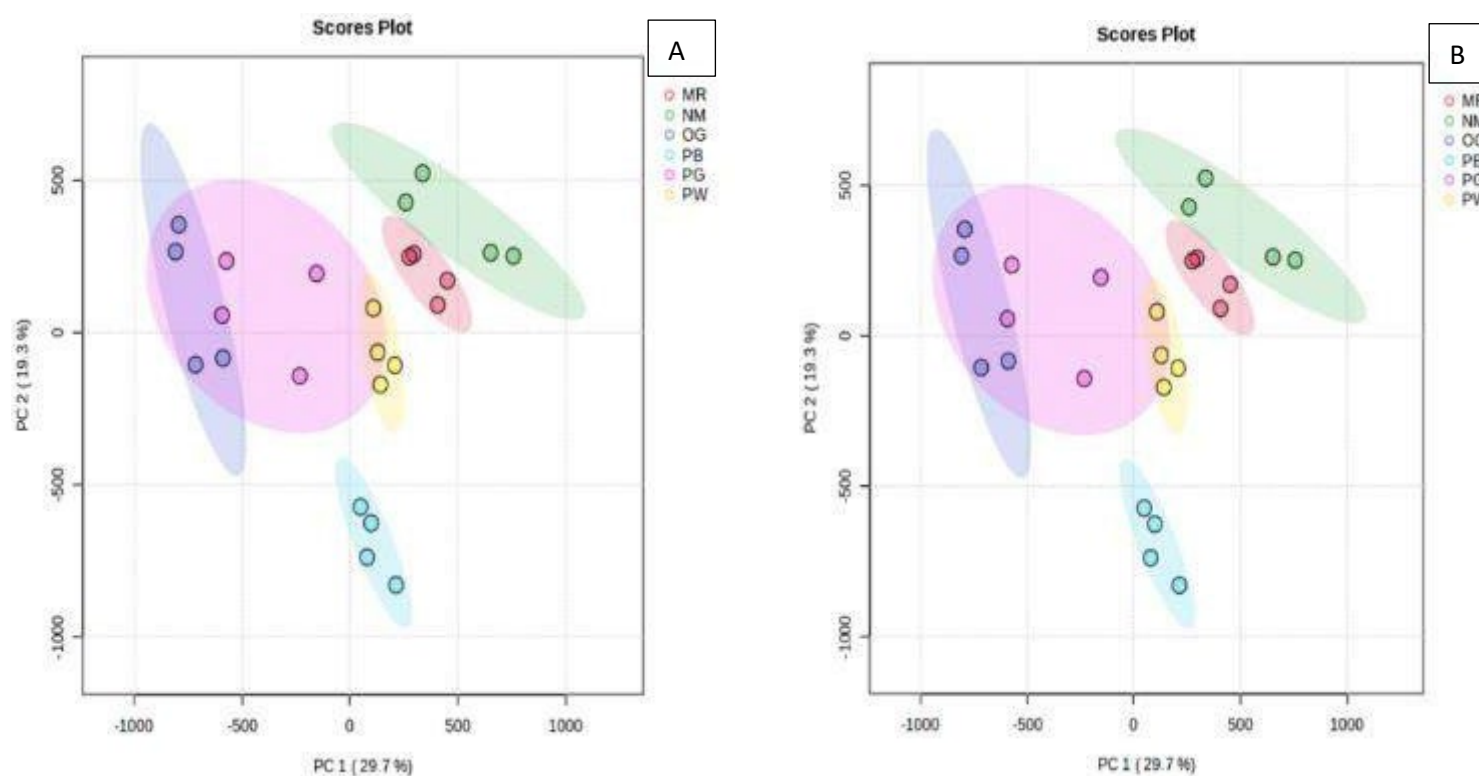


Figure 6.3: A Principal component analysis and B: Orthogonal partial least of squares-Discriminant analysis showing clustering of fatty acids detected in Moringa leaves grown under different mulching material. NM: no mulch; OG: organic mulch; PB: black plastic;

PG: green plastic; PR: red plastic, and PW: white plastic.

6.4.4: The identification and quantification of organic compounds in moringa leaves as influenced by different mulching materials

The UPLC-QTOF-MS was used to determine the organic compound accumulation in moringa leaves grown under different mulching materials (**Table 6.4**). Different classes of organic compounds were obtained from the moringa leaves for example: dicarboxylic acids, stilbenes, O-glycosyl compounds, benzoate ester, Saccharolipids, lignan glycosides and terpene glycosides. The identified organic compounds were Adipic acid, Azelaic acid, Suberic acid, 3,3',4'5-Tetrahydroxystilbene, 6-Hydroxy-4-methoxy-3-(3-methyl-2-butenyl)-2-(2-phenylethenyl)benzoic acid, Lysidiside E;(+)-Lysidiside E, Globosumone C;(-)-Globosumone C, Licoagroside B, b-D-Xylopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-D-fucose, Lariciresinol-9-O-beta-D-glucoside, 3-Hydroxydodecanedioic acid, 2-Hydroxydecanedioic acid, Pisumionoside, 2,3-Butanediol glucoside, 3R-(3R-hydroxybutyryloxy)butyric acid, and Quinic acid across all treatments tested.

The total organic compounds exhibited variation within the mulching treatments such that in descending order, black mulch (4562.00 mg/kg), red mulch (4256.25 mg/kg), organic mulch (4224.75 mg/kg), and white mulch (3942.75 mg/kg) performed better than the green mulch (3747.00 mg/kg) and no-mulch (3550.50 mg/kg) material (**Table 6.4**). The black mulch was significantly higher in the concentration of Adipic acid, Azelaic acid, 6-Hydroxy-4-methoxy-3-(3-methyl-2-butenyl)-2-(2-phenylethenyl)benzoic acid, b-D-Xylopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-D-fucose, 3-Hydroxydodecanedioic acid, 2-Hydroxydecanedioic acid, Pisumionoside, 2,3-Butanediol glucoside, 3R-(3R-hydroxybutyryloxy) butyric acid, and Quinic acid with concentrations of 191.50 mg/kg, 253.7 mg/kg, 326.50 mg/kg, 425.75 mg/kg, 335.00 mg/kg, 192.50 mg/kg, 359.25 mg/kg, 396.25 mg/kg, 315.50 mg/kg, and 373.75 mg/kg, respectively, compared to the rest of the treatments in the study.

Table 6.4: UPLC-QTOF-MS Quantification of Organic compounds detected in Moringa leaves grown under different mulching material

Organic compounds	Treatments					
Tentative structural assignment	Black plastic (mg/kg)	White plastic (mg/kg)	Green plastic (mg/kg)	Red plastic (mg/kg)	Organic mulch (mg/kg)	No mulch (mg/kg)
Adipic acid	191.50 ± 04.98a	180.50 ± 05.56b	145.25 ± 04.57d	174.75 ± 03.53b	153.50 ± 01.93c	137.00 ± 02.81e
Azelaic acid	253.75 ± 03.19a	205.00 ± 03.23 b	175.00 ± 03.01d	173.25 ± 04.01d	195.75 ± 01.97b	180.00 ± 01.98c
Suberic acid	235.50 ± 03.45f	412.00 ± 04.61a	248.50 ± 02.69e	345.00 ± 05.23b	274.25 ± 02.76d	307.00 ± 03.88c
3,3',4'5-Tetrahydroxystilbene	344.25 ± 01.79c	418.50 ± 01.96b	340.25 ± 01.78c	315.50 ± 01.58d	450.00 ± 02.51a	315.75 ± 02.87d
6-Hydroxy-4-methoxy-3-(3-methyl-2-butenyl)-2-(2-phenylethenyl)benzoic acid	326.50 ± 04.46a	272.75 ± 03.30b	246.25 ± 05.41c	272.75 ± 02.03b	229.25 ± 01.63d	226.75 ± 04.57d
Lysidiside E;(+)-Lysidiside E	301.75 ± 05.35b	215.75 ± 04.65d	207.25 ± 03.59e	341.50 ± 04.79a	207.75 ± 01.08e	266.50 ± 02.34c
Globosumone C;(-)-Globosumone C	63.50 ± 01.94b	55.50 ± 02.10e	57.00 ± 01.70d	68.75 ± 02.28a	60.25 ± 02.21c	63.75 ± 01.75b
Licoagroside B	63.50 ± 01.29b	63.00 ± 01.30b	60.50 ± 04.5c	57.00 ± 03.90d	76.00 ± 04.35a	57.25 ± 03.72c
b-D-Xylopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-D-fucose	425.75 ± 05.50a	271.00 ± 05.22d	328.00 ± 03.78c	327.50 ± 01.20c	379.75 ± 3.24b	225.75 ± 02.11e
Lariciresinol-9-O-beta-D-glucoside	383.75 ± 04.61d	327.25 ± 05.35f	417.75 ± 03.02c	442.75 ± 02.46b	459.75 ± 03.90a	374.75 ± 2.02e
3-Hydroxydodecanedioic acid	335.00 ± 04.24a	208.75 ± 01.41f	275.00 ± 01.90e	320.50 ± 01.15b	285.75 ± 02.60d	306.25 ± 04.72c
2-Hydroxydecanedioic acid	192.50 ± 02.04a	166.00 ± 01.60d	172.00 ± 01.12c	177.75 ± 02.83c	180.75 ± 02.41b	129.25 ± 03.97e
Pisumionoside	359.25 ± 04.21a	294.75 ± 03.28d	297.25 ± 02.92c	314.25 ± 03.20a	313.50 ± 03.81b	209.75 ± 04.03e

Organic compounds	Treatments					
2,3-Butanediol glucoside	396.25 ± 03.55a	264.25 ± 03.57d	245.00 ± 02.31f	392.25 ± 04.04b	296.75 ± 03.63c	262.00 ± 08.37e
3R-(3R-hydroxybutyryloxy)butyric acid	315.50 ± 02.51a	304.75 ± 03.13b	206.50 ± 02.83e	266.50 ± 04.15e	294.00 ± 02.64c	271.50 ± 05.51d
Quinic acid	373.75 ± 01.22a	283.00 ± 01.58d	325.50 ± 01.68c	266.25 ± 01.50e	367.75 ± 01.42b	217.25 ± 02.51f
Total organic compounds	4562 mg/kg	3942.75 mg/kg	3747 mg/kg	4256.25 mg/kg	4224.75 mg/kg	3550.50 mg/kg

Figure 6.2 indicates the principal component analysis model (PCA), and the sample variation based on the organic compounds. There was a clear distinction between two metabolome clusters based on the samples with higher organic content versus those with lower organic contents of the organic compounds. Moringa leaves grown on the black mulch treatment revealed the highest organic content in comparison to other treatments.

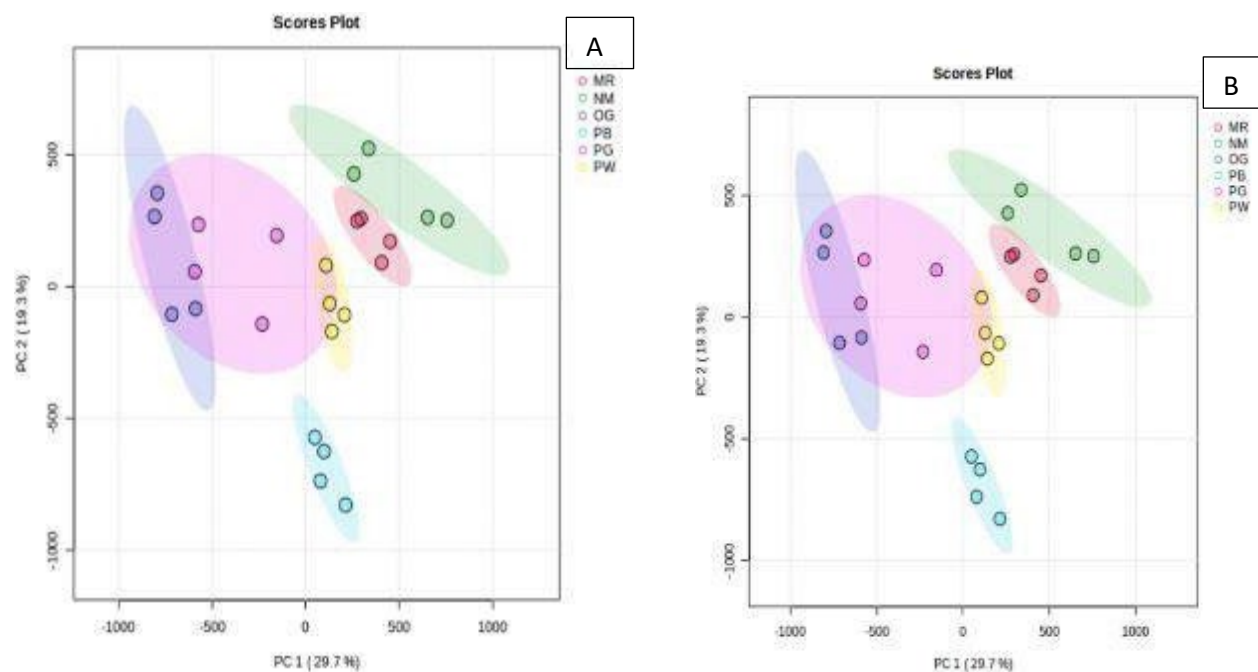


Figure 6.4: A Principal component analysis and B: Orthogonal partial least of squares-Discriminant analysis showing clustering of organic compounds detected in Moringa leaves grown under different mulching material. NM: no mulch; OG: organic mulch; PB: black plastic; PG: green plastic; PR: red plastic, and PW: white plastic.

6.5. Discussion

Farmers main focus is to produce healthy food containing appreciable amounts of antioxidant compounds, mainly phenolics and flavonoids, which can potentially serve human and animal health. The beneficial effects of plant products can be associated with the biological activities of their phytochemical and antioxidant constituents (Dinda *et al.*, 2007). Studies indicated that phenolics observed in fruits and vegetables are essential compounds responsible for antioxidant capacity (Fan *et al.*, 2012). According to Khanizadeh *et al.* (2009), phenolic compounds can extend shelf life and enhance the quality attributes of fresh fruits, thus delaying senescence induced by oxidative degradation. Anwar *et al.* (2007) reported that moringa leaves are a good source of antioxidants such as ascorbic acid, flavonoids, and phenolics.

These bioactive molecules protect radical and reactive oxygen cells and decrease oxidative damage of molecular compounds such as lipids, proteins, and nucleic acids (Tshabalala *et al.*, 2020). Literature indicates that the most common flavonoids found in moringa include myricetin, quercetin, kaempferol, isorhamnetin, and rutin (Leone *et al.*, 2015). In addition, Vergara-Jimenez (2017) reported that caffeic acid, chlorogenic acid and gallic acid, which are phenolic acids, are prevalent in moringa leaves. Similar polyphenol compounds were identified in significant quantities in the current study.

The results of the study indicated that the phytochemicals of moringa leaves in black and red plastic mulches were significantly higher than those of control, organic mulch, white, and green plastic mulches. Coloured plastic mulches are widely used for the production of horticultural crops. The extensive usage of these mulches is mostly because of plastic thermal and light properties. Jahan *et al.* (2018) report that the use of coloured plastic treatments had a significant effect on the phytochemical concentrations and nutritional value of lettuce. Particularly, lettuce grown in soil mulched with black polyethylene had greater concentrations of chlorophyll, anthocyanin, phenols, and carotenoid contents.

According to Shiukhy *et al.* (2015), strawberry plants grown under red plastic mulch had far higher levels of anthocyanin and flavonoid content than strawberry plants grown in black plastic mulch or in plots without mulch. Moreover, the increase in flavonoid and anthocyanin contents in red plastic mulch could be associated with the reflected light quality (Shiukhy *et al.*, 2015). According to studies by Sarıdaş *et al.* (2021) and Zhang *et al.* (2021) coloured plastic mulches, especially those that are red, are particularly effective in increasing fruit antioxidant levels and improving fruit quality. In contrast, the current study's flavonoids, such as Phaseollidin, Kanzonol P, Eriocitrin, and Keioside, were identified in significantly greater quantities in the mulch composed of black plastic. Peng *et al.* (2003) observed that flavonoids are among the chemicals with greater chemical stability and hydrophilicity; they are typically found in plants as their glycosides. This suggests that flavonoids or other stable antioxidants may be linked to the antioxidative activity of these plants. Leone *et al.* (2015), reported that moringa has predominant accumulation of Myricetin, quercetin, kaempferol, isorhamnetin, and rutin flavonoids compounds. This validates the flavonoids compounds identified in the current study. There is assumption that the enhanced flavonoid concentration in black plastic mulch may be associated with radiation and thermal characteristics that alter the microclimates in the root zone of the plant. Diffuse light enhances light scattering into the plant canopy, although spectral radiation modulation may stimulate physiological responses, according to Shahak *et al.* (2014). Thus, crop physiological responses are generally affected by changes in agricultural microenvironment.

Kasperbauer and Loughrin (2004) reported that basil leaves planted in green and yellow plastic mulches had considerably greater concentrations of phenolics than those grown over the other colours. In addition, similar results were obtained for some phenolic compounds such as Canthoside B; (-)-Canthoside B, and Dryopteriside; (+)-Dryopteriside were in greater concentrations in green plastic mulch. In contrast Shahak *et al.* (2014) reported no significant difference in the phenolic content of strawberries planted in different coloured plastic mulch. Research has demonstrated that some agricultural practices, like date planting and mulching systems, can positively increase the yield and phytochemical composition of strawberries (Fan *et al.* 2012). A similar trend

was observed in the current study where mulching significantly increased the yield and metabolomic composition of moringa.

The research demonstrated that moringa phytochemicals have a significant antioxidant capacity against free radicals, suggesting the plant's high nutritional value. A combination of factors, including the high concentration of cinnamic acid generated by the leaves, moringa is believed to possess medicinal properties that may aid in combating cancer (Hassan *et al.*, 2021). It additionally has the potential in the production of pharmaceutical products, like face creams, considering the leaves secrete azelaic acid and salicylic acid beta-D-glucoside. Additionally, Coventry *et al.* (2003) reported plastic mulch colour results in a considerable increase in total phenolics, flavonols, and anthocyanins in plants. While Asami *et al.* (2003) demonstrated substantial variations in total phenolics between conventional, organic, and sustainably cultivated marionberries and strawberries, Pliakoni *et al.* (2010) obtained a greater concentration of phenolic compounds in nectarines under mulching.

Conclusions

The objective of the study was to determine whether different coloured plastic mulch and organic mulch influence the biological and phytochemical composition of moringa leaves. The results of the study proved that different colours have a significant influence on the phytochemical composition of moringa leaves particularly due to variation on the concentrations of different phytochemicals detected in the current study. The black plastic mulch for most of the detected phytochemicals showed significantly higher concentration in comparison with other treatments, followed by red plastic mulch. This could be attributed to the potential of black plastic mulch that tends to warm soil and consequently improve the soil microclimate. The color of the mulches affects the light spectrum, particularly the ratio of red to far-red light (R/FR). Red plastic mulch reflects light with a lower R/FR ratio than natural sunlight, potentially influencing plant growth in a specific way. However, black plastic mulch does not alter the R/FR ratio, meaning it has no impact on the light spectrum that reaches the plants in terms of this specific ratio. Essentially, the black plastic mulch does not influence the light conditions in the same way the red plastic mulch does. Therefore, we reject the null hypothesis which stated that the use of mulching materials will not increase the biological activity and phytochemical compounds of moringa

leaves.

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CHAPTER 7: GENERAL DISCUSSION

Moringa recognized for its therapeutic and nutritional properties, has gained global attention due to its extensive utility across various cultures and climates. The versatility of moringa as a food source and medicinal plant underscores its potential to alleviate nutritional deficits in regions where balanced diets are scarce (Anwen and Bhanger, 2003). The plant's role in addressing malnutrition, particularly among children and households with health challenges, emphasizes its local importance.

In intensive vegetable crop cultivation, managing environmental stresses like biotic and abiotic factors is essential. Plastic mulches have emerged as effective tools for manipulating soil temperature, moisture retention, and plant photobiology, thereby enhancing agricultural productivity (Sharmila-Bharathi *et al.*, 2018). These mulches modify the microclimate by influencing the radiation balance at the soil surface, which in turn affects nutrient availability and metabolic processes critical for plant growth (Sharmila-Bharathi *et al.*, 2018). The adoption of plastic mulches has significantly improved production efficiency, particularly in regions with limited rainfall where fruit and vegetable farming is prevalent. However, the effectiveness of plastic mulches depends on various factors, with mulch colour being particularly crucial. Different coloured plastic mulches, such as black, white, and red, induce distinct effects on soil temperature and light penetration, which have direct implications for plant physiology and developmental processes (El-Beltagi *et al.*, 2022). These effects on the microclimate underline the importance of selecting appropriate mulching materials based on environmental conditions and crop requirements.

The results of this study revealed unexpected findings where the plastic mulch (PM) treatments, except for sodium and potassium, and the interaction between season and temperature did not exhibit significant differences (**Table 3.2**). Sarkar *et al.* (2019) investigated the effects of black, white, blue, and silver PM compared

to no mulch on soil properties and reported significant differences in all measured soil properties (N, P, K, S, and pH). In contrast, Subrahmaniyan *et al.* (2008) found significant effects on nitrogen (N) but no significant effects on available phosphorus (P) and available potassium (K) when comparing black and white PM to a control.

The study by MingFu *et al.* (2022) shares similarities with the current study, as they examined the impact of mulching on soil properties during potato growth over two seasons, collecting data at multiple time points each season. They reported no significant differences in soil carbon (C) levels between seasons yet observed varying significant levels in pH, available potassium (K), available phosphorus (P), and nitrogen (N) across different sampling dates. While some soil properties in this study did not show significant differences under different PM treatments and seasonal variations, these findings align with or deviate from previous studies' observations, highlighting the complexity and variability in the effects of plastic mulching on soil parameters in agricultural settings.

Plastic mulch can significantly influence soil enzyme activity compared to organic mulch and bare soil, particularly in the context of growing moringa. It typically retains more heat and moisture, which can enhance the activity of certain enzymes involved in nutrient cycling due to increased microbial activity. However, excessively high temperatures may inhibit some enzyme functions. In contrast, while organic mulch contributes organic matter that supports diverse microbial communities and enzyme production, fresh organic mulch may not enhance enzyme activity as effectively as well-decomposed material. Additionally, plastic mulch alters microbial community dynamics, potentially favouring specific microorganisms. Ultimately, while plastic mulch offers benefits like moisture retention and temperature regulation for immediate plant growth, organic mulch may provide long-term advantages through enhanced organic matter and microbial activity, making the choice of mulching strategy crucial for optimizing moringa growth and soil health.

Research comparisons have shown that the choice of mulch colour significantly affects plant performance. For instance, Shah *et al.* (2018) demonstrated that black polyethylene mulch increased leaf number, length, and plant width in lettuce, while other studies, like Samaila *et al.* (2011), found red plastic mulch to perform better in different crops. Consistently, black plastic mulch has been found to promote taller plants compared to other mulch types, as observed in potatoes and cucumbers (Karki *et al.*, 2020). Our study on moringa demonstrated that mulching enhances plant growth, as evidenced by increasing stem diameter throughout the growing season, irrespective of the mulch type used. Numerous studies have reported that black plastic mulching enhances various growth parameters such as plant height, leaf area, flowering, fruiting, and overall yield compared to no mulch or other types of mulch (Awasthi *et al.*, 2022; Bhatt *et al.*, 2011; Parmar *et al.*, 2013). Amare and Desta (2021) highlighted that the effectiveness of plastic mulches in mitigating environmental stresses on crops varies depending on the mulch colour. Conversely, Taylor *et al.* (2008) found that black plastic mulch reduced thyme yield compared to unmulched plots. However, our findings showed higher yields in moringa plots mulched with black plastic compared to those without mulch.

Similar trends have been observed in other crops such as chilli plants (Ashrafuzzaman *et al.*, 2011) and tomatoes (Miles *et al.*, 2019), where black plastic mulch consistently resulted in maximum yields, while white plastic mulch and no mulch conditions yielded the lowest. The variations in plant biomass observed in our study can be attributed to factors like improved moisture conservation, nutrient availability, and uptake facilitated by plastic mulching. Overall, the use of plastic mulch benefits moringa growth by creating favourable microclimatic conditions, enhancing nutrient availability, and optimizing plant physiological processes, thereby contributing to increased plant growth and biomass compared to no mulch conditions.

The use of different coloured plastic mulch has been shown to significantly enhance the growth of moringa compared to organic mulch and bare soil. Each colour of plastic mulch affects light reflection and soil temperature differently, which can influence plant growth.

For instance, black mulch retains heat and can create a warmer microenvironment that promotes faster germination and root development. In contrast, reflective mulches, such as silver or white, can help reduce soil temperature and repel pests, benefiting plant health. In our study, moringa plants grown under coloured plastic mulch exhibited greater biomass and improved overall quality than those grown with organic mulch or in bare soil. This suggests that coloured plastic mulches not only provide a barrier against weeds but also optimize the growing conditions for moringa, highlighting their potential as a practical tool for smallholder farmers aiming to maximize yields and improve crop quality.

The moisture content of moringa leaves under black plastic mulch, measured at 8.29%, aligns with the standard recommended range Fahey (2005), suggesting effective protection against enzymatic decomposition associated with high moisture levels that promote mould growth. Regarding crude protein content, the study recorded a higher CP under black plastic mulch, contrasting with lower values reported by Nursandi *et al.* (2017) using wood shaving mulch. This discrepancy may be attributed to mulch effects on moisture retention and soil fertility, which enhance nitrogen availability and chlorophyll production, thereby boosting protein synthesis in plants like moringa. The ash content observed at 14.93% under black plastic mulch exceeds values reported for other mulching materials such as black polyethylene and *Hibiscus esculentus* L. This variation underscores the influence of mulching materials on mineral accumulation in moringa leaves. Additionally, the higher ADF (acid detergent fibre) and NDF (neutral detergent fibre) contents in plastic mulched plots, compared to organic mulched plots and the control, suggest that environmental factors like high temperatures and moisture stress in the Eastern Cape Province during summer can intensify lignification and reduce forage quality by increasing fibre contents.

Using decomposed organic material as mulch offers numerous benefits for smallholder farmers, including the enhancement of soil nutrients and improved plant growth and quality. Decomposed mulch enriches the soil with essential nutrients, promotes moisture retention, and fosters a healthy microbial environment, all of which contribute to robust

plant development. However, in our current study, we utilized fresh organic material instead, which did not undergo decomposition. As a result, the plants grown with this organic mulch did not demonstrate significant growth compared to the control group. In contrast, the plants cultivated under plastic mulch showed marked improvements in growth and quality, particularly in moringa. This highlights the importance of using well-decomposed organic materials to maximize their benefits and suggests that while organic mulches can be advantageous, their effectiveness is contingent upon proper decomposition.

Using different coloured plastic mulch, particularly black plastic mulch, significantly enhances the phytochemical composition of moringa compared to organic mulch and bare soil. The black mulch promotes higher soil temperatures and moisture retention, which can stimulate increased photosynthetic activity and the production of bioactive compounds such as flavonoids and phenolic acids. This leads to healthier plants that produce higher levels of beneficial phytochemicals. In contrast, organic mulch, while beneficial for soil health, may not provide the same immediate effects on phytochemical content due to variations in decomposition rates and nutrient release.

Conclusion

In conclusion, this study highlights the significant role of different mulching materials, particularly black plastic mulch, in enhancing the growth and phytochemical composition of *Moringa oleifera*. Our findings demonstrate that black plastic mulch improves soil moisture retention and temperature regulation and positively influences the phytochemical profile of moringa leaves, leading to higher levels of beneficial compounds. While the effects of plastic mulch colour on soil properties were generally limited, with notable exceptions for sodium and potassium, this research demonstrates the need for further exploration into the interactions between mulching techniques and soil health.

Moringa is renowned as a miracle tree, providing a rich source of highly digestible proteins, calcium, iron, and vitamin C. The presence of these essential nutrients positions moringa leaves as a valuable ingredient for both animal and human consumption, potentially addressing malnutrition-related diseases. Given the importance of moisture retention, nutrient availability, and fibre composition in determining the nutritional quality of moringa, our results affirm that integrating plastic mulches into moringa cultivation can optimize growth conditions and enhance productivity.

Future research should focus on refining mulching techniques tailored to local climates and production objectives. Investigating the long-term effects of various plastic mulch colours on soil health and nutrient dynamics will be crucial for maximizing moringa cultivation's nutritional and economic benefits. Additionally, studies exploring the interactions between plastic mulching and other sustainable agricultural practices could further enhance the role of moringa in promoting food security and supporting resilient agricultural systems.

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APPENDICES I: ANOVA TABLES FOR CHAPTER 3

EXCHANGEABLE ACIDITY

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
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Season	1	0.03891	0.03891	13.201	0.000866 ***
Treatment	5	0.01779	0.00356	1.207	0.325586
Season: Treatment	5	0.00200	0.00040	0.136	0.982949
Residuals	36	0.10612	0.00295		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

PHOSPHORUS (P)

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	797	796.9	3.227	0.0808
Treatment	5	1945	389.1	1.575	0.1919
Season: Treatment	5	1060	212.1	0.859	0.5181
Residuals	36	8891	247.0		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

SODIUM (Na)

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	14913	14913	93.032	1.62e-11 ***
Treatment	5	5160	1032	6.438	0.000228 ***
Season: Treatment	5	495	99	0.617	0.687361
Residuals	36	5771	160		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

POTASSIUM (K)

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	6557	6557	6.383	0.0161 *
Treatment	5	14716	2943	2.865	0.0281 *
Season: Treatment	5	5239	1048	1.020	0.4204
Residuals	36	36979	1027		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

CALCIUM (Ca)

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	13002	13002	1.893	0.177
Treatment	5	13637	2727	0.397	0.847

Season: Treatment	5	19655	3931	0.572	0.721
Residuals	36	247209	6867		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

MAGNESIUM (Mg)

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	4351	4351	6.76	0.0134 *
Treatment	5	2248	450	0.699	0.6278
Season: Treatment	5	1745	349	0.542	0.7429
Residuals	36	23162	643		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

pH

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	0.021	0.02083	0.218	0.643
Treatment	5	0.313	0.06253	0.654	0.660
Season: Treatment	5	0.402	0.08039	0.841	0.529
Residuals	36	3.440	0.09556		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TOTAL CARBON

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	0.06977	0.06977	19.278	9.5e-05 ***
Treatment	5	0.02241	0.00448	1.239	0.3115
Season: Treatment	5	0.04212	0.00842	2.328	0.0625
Residuals	36	0.13029	0.00362		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TOTAL NITROGEN

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	0.000574	0.0005741	6.057	0.0188 *
Treatment	5	0.000492	0.0000984	1.038	0.4104
Season: Treatment	5	0.000158	0.0000316	0.333	0.8895
Residuals	36	0.003412	0.0000948		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

β-GLUCOSIDASE ENZYME

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	0.905	0.9046	1.922	0.174
Treatment	5	3.461	0.6923	1.471	0.224
Season: Treatment	5	1.726	0.3452	0.733	0.603
Residuals	36	16.945	0.4707		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

PHOSPHATASE ENZYME

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Season	1	311.1	311.07	25.192	1.42e-05 ***
Treatment	5	17.2	3.44	0.279	0.922
Season: Treatment	5	55.6	11.12	0.901	0.491
Residuals	36	444.5	12.35		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

APPENDECES II: ANOVA TABLES FOR CHAPTER 4

Plant height

Week 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	133.79	44.597	0.52	0.6780
Treat	5	8648.71	1729.742	19.98	0.0000
Error	15	1298.46	86.564		
Residual	14	1007.27	71.948		
Total	23	10080.96			

Week 2

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	10950.83	3650.278	10.16	0.0007
Treat	5	56251.33	11250.267	31.30	0.0000
Error	15	5391.67	359.444		
Residual	14	5127.87	366.276		
Total	23	72593.83			

Week 3

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	7605.46	2535.153	8.05	0.0020
Treat	5	56425.71	11285.142	35.85	0.0000
Error	15	4721.79	314.786		
Residual	14	3807.92	271.994		
Total	23	68752.96			

Week 4

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	6386.17	2128.722	6.29	0.0056
Treat	5	67702.83	13540.567	40.04	0.0000
Error	15	5072.83	338.189		
Residual	14	4424.42	316.030		
Total	23	79161.83			

Week 5

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1705.00	568.333	2.14	0.1379

Treat	5	89418.83	17883.767	67.34	0.0000
Error	15	3983.50	265.567		
Residual	14	3779.56	269.969		

Total	23	95107.33			
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Week 6

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1593.00	531.000	2.28	0.1209
Treat	5	86318.00	17263.600	74.18	0.0000
Error	15	3491.00	232.733		
Residual	14	3231.08	230.791		

Total	23	91402.00			
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Week 7

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1315.50	438.500	2.10	0.1432
Treat	5	84594.33	16918.867	81.03	0.0000
Error	15	3132.00	208.800		
Residual	14	2944.18	210.298		

Total	23	89041.83			
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Week 8

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1479.17	493.056	1.97	0.1625
Treat	5	114123.83	22824.767	90.99	0.0000
Error	15	3762.83	250.856		
Residual	14	3559.40	254.243		

Total	23	119365.83			
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STEM DIAMTER

Week 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	97.32	32.442	1.01	0.4162
Treat	5	2632.15	526.431	16.37	0.0000
Error	15	482.43	32.162		
Residual	14	404.33	28.881		

Total	23	3211.91			
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Week 2

Degrees of Freedom	Sum of Squares
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Source	Freedom	Squares	Mean Square	F-value	Prob
Block	3	200.93	66.977	0.73	0.5480
Treat	5	3670.59	734.118	8.04	0.0007
Error	15	1369.54	91.303		
Residual	14	1351.91	96.565		
Total	23	5241.06			

Week 3

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	546.72	182.238	2.97	0.0655
Treat	5	1030.28	206.056	3.36	0.0312
Error	15	920.87	61.391		
Residual	14	357.18	25.513		
Total	23	2497.87			

Week 4

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	874.35	291.452	3.16	0.0554
Treat	5	1467.56	293.512	3.19	0.0370
Error	15	1381.30	92.087		
Residual	14	788.07	56.291		
Total	23	3723.22			

Week 5

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	369.05	123.016	1.61	0.2292
Treat	5	6571.43	1314.287	17.19	0.0000
Error	15	1146.96	76.464		
Residual	14	1127.55	80.540		
Total	23	8087.44			

Week 6

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	304.46	101.486	1.38	0.2885
Treat	5	6527.38	1305.475	17.69	0.0000
Error	15	1106.79	73.786		
Residual	14	1103.13	78.795		
Total	23	7938.63			

Week 7

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	282.46	94.153	1.26	0.3234
Treat	5	6511.21	1302.242	17.44	0.0000
Error	15	1120.29	74.686		
Residual	14	1119.60	79.972		
Total	23	7913.96			

Week 8

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	266.83	88.944	1.17	0.3547
Treat	5	6293.50	1258.700	16.53	0.0000
Error	15	1142.17	76.144		
Residual	14	1141.89	81.564		
Total	23	7702.50			

Chlorophyll Content

Week 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	88.60	29.534	1.11	0.3746
Treat	5	891.10	178.220	6.72	0.0018
Error	15	397.83	26.522		
Residual	14	381.23	27.231		
Total	23	1377.53			

Week 2

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	374.14	124.714	8.66	0.0014
Treat	5	834.74	166.948	11.59	0.0001
Error	15	216.05	14.403		
Residual	14	214.74	15.339		
Total	23	1424.93			

Week 3

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	279.17	93.056	6.59	0.0047
Treat	5	789.50	157.900	11.18	0.0001
Error	15	211.83	14.122		
Residual	14	204.93	14.638		
Total	23	1280.50			

Week 4

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	523.50	174.500	3.80	0.0329
Treat	5	1022.50	204.500	4.46	0.0109
Error	15	688.50	45.900		
Residual	14	600.24	42.875		
Total	23	2234.50			

Week 5

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	460.00	153.333	3.52	0.0414
Treat	5	1009.33	201.867	4.63	0.0094
Error	15	654.00	43.600		
Residual	14	575.29	41.092		
Total	23	2123.33			

Week 6

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	485.33	161.778	3.93	0.0297
Treat	5	847.00	169.400	4.11	0.0149
Error	15	617.67	41.178		
Residual	14	518.72	37.051		
Total	23	1950.00			

Week 7

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	392.79	130.931	3.39	0.0458
Treat	5	755.21	151.042	3.91	0.0180
Error	15	578.96	38.597		
Residual	14	432.62	30.901		
Total	23	1726.96			

Week 8

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	392.79	130.931	3.39	0.0458
Treat	5	755.21	151.042	3.91	0.0180

Error	15	578.96	38.597
Residual	14	432.62	30.901
Total	23	1726.96	

Leaf length
Week 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	89.46	29.819	0.30	0.8247
Treat	5	5030.71	1006.142	10.13	0.0002
Error	15	1489.79	99.319		
Residual	14	1438.86	102.776		
Total	23	6609.96			

Week 2

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	172.46	57.486	0.80	0.5145
Treat	5	7093.71	1418.742	19.67	0.0000
Error	15	1081.79	72.119		
Residual	14	1024.69	73.192		
Total	23	8347.96			

Week 3

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	166.79	55.597	0.92	0.4545
Treat	5	7569.71	1513.942	25.08	0.0000
Error	15	905.46	60.364		
Residual	14	881.17	62.941		
Total	23	8641.96			

Week 4

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	146.12	48.708	1.01	0.4161
Treat	5	6818.71	1363.742	28.25	0.0000
Error	15	724.13	48.275		
Residual	14	711.41	50.815		
Total	23	7688.96			

Week 5

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
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Block	3	380.12	126.708	1.56	0.2401
Treat	5	7785.71	1557.142	19.19	0.0000
Error	15	1217.13	81.142		
Residual	14	1158.92	82.780		

Total	23	9382.96			
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Week 6

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	882.00	294.000	2.74	0.0802
Treat	5	9626.50	1925.300	17.92	0.0000
Error	15	1611.50	107.433		
Residual	14	1430.42	102.173		

Total	23	12120.00			
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Week 7

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1139.50	379.833	2.77	0.0782
Treat	5	9902.50	1980.500	14.42	0.0000
Error	15	2060.50	137.367		
Residual	14	1893.97	135.284		

Total	23	13102.50			
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Week 8

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	984.50	328.167	2.67	0.0853
Treat	5	9264.50	1852.900	15.06	0.0000
Error	15	1845.50	123.033		
Residual	14	1650.97	117.926		

Total	23	12094.50			
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Leaf width

Week 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	354.83	118.278	1.41	0.2778
Treat	5	7793.83	1558.767	18.63	0.0000
Error	15	1255.17	83.678		
Residual	14	1253.16	89.512		

Total	23	9403.83			
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Week 2

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	2296.83	765.611	3.60	0.0386
Treat	5	9882.50	1976.500	9.30	0.0003
Error	15	3189.17	212.611		
Residual	14	2392.02	170.859		
Total	23	15368.50			

Week 3

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	812.79	270.931	2.73	0.0804
Treat	5	7779.71	1555.942	15.70	0.0000
Error	15	1486.46	99.097		
Residual	14	1080.46	77.175		
Total	23	10078.96			

Week 4

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	445.13	148.375	1.62	0.2269
Treat	5	6837.88	1367.575	14.92	0.0000
Error	15	1374.63	91.642		
Residual	14	914.79	65.342		
Total	23	8657.63			

Week 5

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	559.67	186.556	2.49	0.1001
Treat	5	6562.00	1312.400	17.51	0.0000
Error	15	1124.33	74.95		
Residual	14	800.82	57.201		
Total	23	8246.00			

Week 6

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	473.79	157.931	2.08	0.1460
Treat	5	6658.71	1331.742	17.53	0.0000
Error	15	1139.46	75.964		

Residual	14	807.31	57.665
Total	23	8271.96	

Week 7

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	513.50	171.167	1.87	0.1781
Treat	5	7246.83	1449.367	15.83	0.0000
Error	15	1373.50	91.567		
Residual	14	809.16	57.797		
Total	23	9133.83			

Week 8

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	513.50	171.167	1.87	0.1781
Treat	5	7246.83	1449.367	15.83	0.0000
Error	15	1373.50	91.56		
Residual	14	809.16	57.797		
Total	23	9133.83			

Petiole Diameter

Week 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	24.40	8.132	3.78	0.0334
Treat	5	172.31	34.463	16.03	0.0000
Error	15	32.24	2.150		
Residual	14	32.20	2.300		
Total	23	228.95			

Week 2

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1.05	0.351	0.04	0.9901
Treat	5	355.37	71.074	7.49	0.0011
Error	15	142.33	9.489		
Residual	14	140.00	10.000		
Total	23	498.75			

Week 3

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	0.20	0.068	0.81	0.5100

Treat	5	2.93	0.587	6.98	0.0015
Error	15	1.26	0.08		
Residual	14	1.10	0.079		

Total	23	4.40			
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Week 4

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1.33	0.445	2.35	0.1134
Treat	5	5.38	1.075	5.68	0.0039
Error	15	2.84	0.189		
Residual	14	1.81	0.129		

Total	23	9.55			
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Week 5

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	9.08	3.027	4.18	0.0246
Treat	5	12.09	2.418	3.34	0.0318
Error	15	10.87	0.72		
Residual	14	8.08	0.577		

Total	23	32.04			
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Week 6

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	50.80	16.934	13.42	0.0002
Treat	5	16.29	3.257	2.58	0.0707
Error	15	18.92	1.261		
Residual	14	12.99	0.928		

Total	23	86.01			
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Week 7

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	67.46	22.486	14.48	0.0001
Treat	5	16.21	3.242	2.09	0.1237
Error	15	23.29	1.553		
Residual	14	21.89	1.563		

Total	23	106.96			
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Week 8

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
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Block	3	116.46	38.819	25.00	0.0000
Treat	5	14.21	2.842	1.83	0.1673
Error	15	23.29	1.553		
Residual	14	21.47	1.533		

Total	23	153.96			
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Petiole length

Week 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	49.13	16.375	6.12	0.0063
Treat	5	297.38	59.475	22.23	0.0000
Error	15	40.13	2.67		
Residual	14	32.87	2.348		

Total	23	386.63			
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Week 2

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	97.12	32.375	12.10	0.0003
Treat	5	265.71	53.142	19.87	0.0000
Error	15	40.13	2.675		
Residual	14	30.04	2.145		

Total	23	402.96			
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Week 3

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	129.46	43.153	10.83	0.0005
Treat	5	266.38	53.275	13.37	0.0000
Error	15	59.79	3.98		
Residual	14	42.32	3.023		

Total	23	455.63			
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Week 4

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	178.13	59.375	10.34	0.0006
Treat	5	254.38	50.875	8.86	0.0004
Error	15	86.13	5.742		
Residual	14	74.21	5.301		

Total	23	518.63			
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Week 5

Degrees of Sum of

Source	Freedom	Squares	Mean Square	F-value	Prob
Block	3	178.13	59.375	10.16	0.0007
Treat	5	275.88	55.175	9.45	0.0003
Error	15	87.63	5.842		
Residual	14	75.87	5.419		
Total	23	541.63			

Week 6

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	200.79	66.931	11.48	0.0004
Treat	5	214.38	42.875	7.35	0.0012
Error	15	87.46	5.83		
Residual	14	83.05	5.932		
Total	23	502.63			

Week 7

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	156.46	52.153	10.06	0.0007
Treat	5	242.71	48.542	9.36	0.0003
Error	15	77.79	5.186		
Residual	14	77.01	5.501		
Total	23	476.96			

Week 8

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	194.83	64.944	17.50	0.0000
Treat	5	224.00	44.800	12.07	0.0001
Error	15	55.67	3.711		
Residual	14	55.61	3.972		
Total	23	474.50			

Leaf biomass

Fresh mass

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	2.34	0.781	2.11	0.1415
Treat	5	266.76	53.353	144.28	0.0000

Error	15	5.55	0.37		
Residual	14	3.17	0.226		
Total	23	274.65			
Dry mass					
Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	0.03	0.010	3.00	0.0638
Treat	5	5.70	1.140	342.00	0.0000
Error	15	0.05	0.003		
Residual	14	0.03	0.002		
Total	23	5.78			
Dry Matter Content %					
Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1.19	0.397	0.95	0.4406
Treat	5	8.58	1.716	4.12	0.0149
Error	15	6.26	0.417		
Residual	14	5.30	0.379		
Total	23	16.03			

APPENDICES III: ANOVA TABLES FOR CHAPTER 5

Moisture content

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1.42	0.473	4.09	0.0263
Treat	5	1.68	0.336	2.90	0.0500
Error	15	1.74	0.11		
Residual	14	1.74	0.124		
Total	23	4.84			

Ash content

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1.64	0.546	1.58	0.2356
Treat	5	7.36	1.472	4.26	0.0130
Error	15	5.18	0.345		
Residual	14	5.18	0.370		
Total	23	14.18			

Fat

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	1.31	0.437	2.42	0.1068
Treat	5	0.63	0.126	0.70	0.6350
Error	15	2.71	0.181		
Residual	14	2.65	0.189		
Total	23	4.65			

ACID DETERGENT FIBRE (ADF)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	21.10	7.035	1.09	0.3820
Treat	5	36.56	7.312	1.14	0.3833
Error	15	96.43	6.428		
Residual	14	89.52	6.394		
Total	23	154.09			

NEATRAL DETERGENT FIBRE (NDF)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
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Block	3	11.71	3.905	0.91	0.4590
Treat	5	64.04	12.808	2.99	0.0455
Error	15	64.27	4.285		
Residual	14	63.55	4.540		
Total	23	140.03			

Protein content

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Block	3	14.33	4.778	2.18	0.1333
Treat	5	30.95	6.189	2.82	0.0546
Error	15	32.93	2.195		
Residual	14	32.19	2.299		
Total	23	78.21			

Macro-nutrients Phosphorus

SV	DF	Sum Sq	Mean Sq	F Value	Pr(>F)
Treatment	5	0.3076	0.06152	4.217	0.0103 *
Residuals	18	0.2626	0.01459		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Nitrogen

SV	DF	Sum Sq	Mean Sq	F-value	Pr(>F)
Treatment	5	0.6956	0.13911	2.499	0.0691
Residuals	18	1.0018	0.05566		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Calcium

SV	DF	Sum Sq	Mean Sq	F-value	Pr(>F)
Treatment	5	0.5649	0.11298	2.208	0.0986
Residuals	18	0.9213	0.0511		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Magnesium

SV	DF	Sum Sq	Mean Sq	F-value	Pr(>F)
Treatment	5	0.04524	0.009047	13.21	1.69e-05 ***
Residuals	18	0.01232	0.000685		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Potassium

SV	DF	Sum Sq	Mean Sq	F-value	Pr(>F)
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Treatment	5	0.4610	0.09221	2.654	0.0575
Residuals	18	0.6253	0.03474		
Significance codes: 0 '***' 0.001 '**' 0.01 '.' 0.1 ' ' 1					

Sodium

SV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	1612201	322440	7.037	0.000834 ***
Residuals	18	824755	45820		
Significance codes: 0 '***' 0.001 '**' 0.01 '.' 0.1 ' ' 1					

Copper

SV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	1.593	0.3187	1.02	0.435
Residuals	18	5.625	0.3125		
Significance codes: 0 '***' 0.001 '**' 0.01 '.' 0.1 ' ' 1					

Manganese

SV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	3601	720.3	1.267	0.321
Residuals	18	10236	568.7		
Significance codes: 0 '***' 0.001 '**' 0.01 '.' 0.1 ' ' 1					

Iron

SV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	2136	427.2	1.386	0.276
Residuals	18	5550	308.3		
Significance codes: 0 '***' 0.001 '**' 0.01 '.' 0.1 ' ' 1					

Aluminium

SV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	1336	267.2	2.151	0.106
Residuals	18	2236	124.2		
Significance codes: 0 '***' 0.001 '**' 0.01 '.' 0.1 ' ' 1					

Zinc

SV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	115.4	23.07	1.194	0.351
Residuals	18	347.7	19.32		
Significance codes: 0 '***' 0.001 '**' 0.01 '.' 0.1 ' ' 1					