INVESTIGATING THE USE OF NETTING STRUCTURES AS CONDITIONS SUITABLE FOR OFF-SEASON PRODUCTION OF *MORINGA OLEIFERA* IN SOUTH AFRICA

by

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[redacted]

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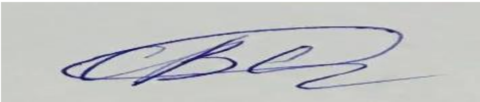
DECLARATION

I, Setlale Happiness declare that the full dissertation hereby submitted to the University of Limpopo, for the degree Master of Agricultural Management (plant production) has not been submitted previously by me or nobody for a degree at this or any other university. This is my work in design and in execution, and related materials contained herein has been duly acknowledged.



29 March 2025

Setlale H **Date**

DEDICATION

I dedicate this dissertation to my parents, Mr Allen Setlale and Mrs Thandy Setlale in appreciation of their unwavering support and inspiration during my academic journey.

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List of abbreviations

°C= Degrees Celsius

CAF= Central Analytical Facility

CCM- Chlorophyll Content Meter

Cm= Centimeters

CS= Closed Sides

CTS= Closed Top and Sides

E= East (geographical direction)

G= Grams

Ha= Hectares

HSD= Honestly Significant Difference

HSS= High Strength Steel (context-specific; could also refer to High-Resolution Sampling System)

Kg= Kilograms

Mm= Millimeters

MS= Mass Spectrometry

RCBD= Randomized Complete Block Design

S= South (geographical direction)

SWATH-MS= Sequential Window Acquisition of All Theoretical Mass Spectra-Mass Spectrometry

TADB= Technical Advanced Digital Balance

TC= Top Cover

UHPLC-MS-QTOF= Ultra-High Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry

UK: United Kingdom

UPLC-MS= Ultra Performance Liquid Chromatography-Mass Spectrometry

VSN= VSN International (software developers)

µL= Microliter

USA= United States of America

Abstract

Moringa oleifera, part of the Moringaceae family, is a subtropical plant renowned for its medicinal properties. Its leaves are often used as leafy vegetables or as primary ingredients in green smoothies. However, *Moringa oleifera* is highly sensitive to cold temperatures, which can severely hinder its growth and yield. The enhancement and assessment of growth, yield, and phytochemical composition of *Moringa oleifera* using Coolaroo frost cloth and a 40% shade net has not been previously documented. The aim of this study was to generate scientific information regarding the effects of the application of a white Coolaroo frost cloth and a white 40% shade nets with closed sides, top cover, closed sides and top, to protect *Moringa oleifera* during the winter season. The objectives of this study were, (1) to investigate the effect of a 40% shade net and Coolaroo Frost Cloth, with closed sides, top cover, closed sides and top, on the growth and yield attributes, and (2) phytochemical profiling of *Moringa oleifera* during winter. Following a randomized complete block design (RCBD), *Moringa oleifera* seedlings were transplanted onto plots covered with 40% shade net and established Coolaroo frost cloth with closed sides, top cover, and closed top sides. This process was carried out for 120 days, with three replications and six plants per replicate. Data were subjected to statistical analysis using GenStat 18th version statistical package (VSN International, Hempstead, UK). Mean separation for significant ($p \leq 0.05$) treatments were achieved using Tukey's Honestly Significant Difference (HSD). The growth parameters, including plant height, stem diameter, and root length, were measured weekly until the experiment was completed. Yield parameters including plant biomass and number of leaves were recorded at harvest. After collection, the leaves, roots, and twigs were analyzed for their phytochemical composition. The growth and yield parameters of *Moringa oleifera* were significantly influenced ($p \leq 0.05$) by the Coolaroo frost cloth and the 40% shade net. At termination (120 days after transplanting), the Coolaroo frost cloth with closed sides and top had significantly increased the stem diameter, plant height, biomass production, root length and number of leaves. Untargeted metabolite profiling of the leaves, twigs, and roots was conducted using UPLC-MS to evaluate the effects of shading on secondary metabolite production, including phenolic compounds, flavonoids, and glycosides. Chemometric analysis of the UPLC-MS data revealed distinct effects of shading on different plant tissues. While the root metabolites showed no significant variation

across shading treatments, the twigs and leaves exhibited pronounced differences. Twigs grown under 40% shade net and Coolaroo frost cloth with closed sides retained higher concentrations of key flavonoids such as quercetin 3-galactoside, rutin, and astragalin 7-rhamnoside. The leaves formed distinct clusters based on metabolite composition, with shading structures, especially the 40% shade net with closed top and sides, enhancing the accumulation of important phytochemicals like quecetrin and kaempferol derivatives. Overall, the study concludes that the use of Coolaroo frost cloth and shade nets provides a practical and cost-effective method for protecting *Moringa oleifera* from cold stress. These shading techniques not only improve plant growth and biomass yield but also enhance the phytochemical content, making the plant more valuable nutritionally and medicinally. The findings offer a sustainable solution for year-round Moringa cultivation in cold-prone regions, contributing to food security and agricultural sustainability.

Chapter 1: General introduction

1.1 Background

Native to northern India's sub-Himalayan regions, Moringa (*Moringa oleifera* Lam.) is a tree that grows quickly and can withstand drought. It belongs to the Moringaceae family, which comprises 13 distinct species (Murro *et al.,* 2003). Over time, *Moringa oleifera* has become increasingly popular and has spread to many tropical and subtropical regions of Africa, Southeast Asia, and South America, mostly as a result of its ability to adapt to a variety of weather conditions (Jahn, 1988). Its agricultural and economic importance is increasingly acknowledged, especially in regions where it is cultivated for both subsistence and commercial purposes. The tree's capacity to thrive on marginal lands, combined with its rapid growth and high productivity, positions it as a valuable asset for enhancing food security, generating income, and supporting sustainable development efforts in many communities (Habtemariam, 2016). *Moringa oleifera* was likely introduced to South Africa during the 1800s via ports in KwaZulu-Natal. This introduction coincided with the expansion of sugarcane plantations in the province's subtropical coastal lowlands (Naidoo and Coopoosamy, 2011).

All parts of the *Moringa oleifera* plant, including its leaves, pods, seeds, flowers, and roots, exhibit diverse biological activities and are widely recognized for their medicinal properties in addressing and preventing numerous health conditions. Studies show that *Moringa* leaves are a rich source of essential vitamins, minerals, fatty acids, and amino acids. (Teixeira *et al.,* 2014; Razis *et al.,* 2014). Additionally, these leaves are recognized for their antioxidant components, including ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani *et al.,* 2013; Vongsak *et al.,* 2014). Additionally, they serve as a valuable source of saponins, which have demonstrated anticancer properties (Ekor, 2014). Moringa stems are traditionally utilized to treat various ailments, including toothaches, colds, epilepsy, and bites from scorpions and snakes (Fahey, 2005; Stevens *et al.,* 2013). Due to its extensive beneficial properties, research from multiple disciplines suggests that *Moringa oleifera* has significant potential as a preventative medicinal plant (Pandey *et al*., 2019). Furthermore, its high nutritional content may contribute to food security through the availability of its tender green leaves and pods (Mabapa *et al*., 2017).

1.1.1 Description of the research problem

Due to the tree's great susceptibility to frost and freezing weather, the *Moringa oleifera* crop performs best throughout the summer season and less so during the winter (Price, 2007). This implies that the tree's winter cultivation may not be feasible in all the regions of South Africa, particularly in areas known for their chilly and sometimes rainy winters, such as the Western Cape, the Eastern Cape, and parts of the Highveld (Daron, 2014). For smallholder farmers relying on Moringa cultivation as a source of income, the seasonal variation in biomass and yield can impact profitability. During the winter season, when the plant's growth is hampered, farmers may experience lower yields and reduced income from selling Moringa leaves. However, the demand for leaf products often remains steady or even increases during the winter, leading to higher prices in the market. The geographic expansion of *Moringa oleifera* may be restricted as a result of yield loss in the winter season. There is limited documentation available about the impact of netting structures on the growth, yield, and quality of *Moringa oleifera* during winter. Studies on other high-value plants, such avocados, have shown that covering young "Reed" avocado trees with shade nets throughout the winter period improved physiological parameters including leaf chlorophyll content and trunk diameter, as well as vegetative development (Chernoivanov *et al.,* 2022). Similarly, the growth of lettuce under 40% shade has been shown to decrease the yield of fresh leaves and the proportion of marketable produce at harvest (Ntsoane *et al.,* 2016). Consequently, the use of shade nets has gained popularity as a means of mitigating the adverse effects of climate change, offering a potential solution to enhance crop resilience in challenging conditions.

1.1.2 Impact of the research problem

Moringa oleifera holds significant potential to benefit humanity, especially for populations in developing countries where challenges such as unemployment, poverty, and inadequate healthcare are prevalent (Kou *et al.,* 2018; Singh *et al.,* 2020). Governments in sub-Saharan Africa recognize *Moringa oleifera* as a vital resource, often referring to it as the "developmental tree" of choice. This designation reflects the plant's perceived ability to address a range of socioeconomic challenges prevalent in the region, including food insecurity, malnutrition, and poverty. Studies by Hassan and Ibrahim (2013) and Leone *et al*. (2015) support this perspective, further emphasizing the significant role *Moringa oleifera* can play in tackling these pressing issues. By harnessing the potential of *Moringa oleifera,* governments and communities can work towards improving food security, addressing malnutrition, and alleviating poverty. Its versatility as a nutritional supplement, income source, and sustainable resource underscores its importance as a tool for socioeconomic development and empowerment in resource-constrained regions.

1.1.3 Possible causes of the research problem

According to García Milla *et al.* (2021), *Moringa oleifera* flourishes in conditions characterized by direct sunlight temperatures between 25°C and 35°C. However, the plant cannot withstand colder climates, implying that the tree is quite susceptible to freezing conditions. This susceptibility to cold conditions poses challenges for cultivation during winter months, potentially leading to yield loss and constraining the geographical expansion of *Moringa oleifera* cultivation. There is limited information regarding how various netting structures impact the growth, yield, and quality of *Moringa oleifera* during the winter months.

1.1.4 Possible solutions

The off-season production of *Moringa oleifera* can benefit from alternative production system including shading. In addition to protecting plants from sunburn, frost, and hail, shade nets also influence soil conditions, temperature, humidity, radiation levels, and the diversity of microorganisms (Guastella *et al.,* 2020). Although the use of shade nets of different colors (red, pearl grey and white) has effectively enhanced quality attributes by altering the phytonutrients to improve crop adaptive mechanisms in commercial crops such as tomatoes and sweet pepper (Bertin and Génard, 2018; Ilić *et al*., 2012; Mashabela *et al*., 2015), such information is currently limited in the production of *Moringa oleifera.*

1.1.5 General focus of the study

The study is focused on generating information on conditions suitable for off-season production of *Moringa oleifera* in South Africa by the use of a 40% shade net structure and a standard Coolaroo frost cloth (Coolaroo®, South Africa).

1.2 Rationale of the study

Moringa oleifera is well-known around the world for its nutritionally dense components, as well as economic and health benefits (Foidl *et al*., 2001). Given that the plant prefers tropical and subtropical climates and does not produce blossoms in winter, growing *Moringa oleifera* in winter becomes difficult posing a threat to food security. It is therefore, suggested to plant *Moringa oleifera* under difficult conditions due to the tree's strong adaptation characteristics to various environment and viewed as a potential plant to improve food quality. The significant economic value of *Moringa oleifera* products makes its production a viable source of income that could help South African smallholder farmers improve their standard of living (Maponya and Mpandeli, 2012). Due to its extensive cultivation, Moringa is susceptible to various diseases and pests; however, its resilience demonstrates that Moringa is vital in adapting to climate change (Mridha *et al.,* 2017). It was therefore regarded as a great way to fight malnutrition because of how easily phytochemicals could be produced and dispersed throughout the plant, including the leaves, flowers, pods, and seeds (Mabapa *et al.,* 2017). Over the past decade, producers in various countries have adopted cost-effective techniques to enhance food nutrition and improve agricultural outcomes (Ilic *et al.,* 2017). Among these techniques, shade nets have been extensively utilized to provide multiple benefits, such as protecting plants from birds and insects, as well as shielding them from environmental stresses like frost, hail, drought, and extreme heat (Kittas *et al.,* 2014). Basil cultivated under red netting exhibited the highest levels of total phenols, flavonoids, and antioxidant activity, demonstrating how shading can affect a plant's biochemical characteristics (Milenković *et al.,* 2014). The findings by Elad *et al.* (2007) and Shahak (2008) regarding the impact of various shade nets on *Capsicum annuum* yields align with the broader understanding of how shade nets can influence plant growth, which is also relevant to *Moringa oleifera*. Both studies highlight that different shade net colors and percentages can have varying effects on plant performance, with the effects sometimes differing by cultivar. For example, Elad *et al.* (2007) observed increased yields with 40% shade nets for certain Capsicum cultivars, particularly when using silver netting, though the results varied with different net colors. Similarly, Shahak (2008) reported increased yields for bell peppers under pearl and red nets compared to black netting, indicating that colored nets may enhance plant production more effectively than black ones. These studies reflect the variability in plant responses to different shade factors, suggesting that the effects of shade nets on Moringa may also depend on factors like net color, shade percentage, and environmental conditions.

Coolaroo frost cloth, a widely used protective material in horticulture and agriculture, is a lightweight and breathable fabric specifically designed to safeguard plants from frost damage. By trapping heat, it creates a slightly warmer microenvironment around plants, reducing the risk of frost injury. This fabric also serves as a barrier against strong winds and pests while maintaining its permeability to essential elements such as sunlight, air, and moisture. As a result, Coolaroo frost cloth promotes healthy plant growth even under challenging weather conditions (Coolaroo, n.d.). Despite its widespread application in mitigating cold weather impacts, there is currently limited information available on the specific effects of Coolaroo frost cloth, particularly regarding its influence on plant growth, yield, and phytochemical properties. Further research is necessary to fully understand its potential and optimize its usage in diverse agricultural contexts. Apart from this, the Department of Science and Innovation (DSI) has identified *Moringa oleifera* as an alternative crop for food and nutritional security in South Africa. Thus, this project is envisaged to promote *Moringa oleifera* for both commercialisation, food and nutritional security and domestic use.

1.3 Purpose of the study

1.3.1 Aim

Establishment of information on conditions suitable for off-season production of *Moringa oleifera* in South Africa by the use of a 40% shade net structure and a standard frost net (Coolaroo Frost Cloth, Coolaroo®, South Africa).

1.3.2 Objectives

To investigate whether a white 40% shade net and Coolaroo Frost Cloth, with open sides, open top, closed sides and top, will have an effect on the growth and yield parameters of Moringa tree.

To investigate whether a white 40% shade net and Coolaroo Frost Cloth, with open sides, open top, closed sides and top, will have an effect on the phytochemical profiling of Moringa tree.

1.3.3 Hypotheses

Coolaroo Frost Cloth and 40% shade net, with open sides, open top, closed sides and top, will significantly affect the growth and biomass yield parameters of Moringa tree.

Coolaroo Frost Cloth and 40% shade net, with open sides, open top, closed sides and top, will significantly affect the phytochemical profiling of Moringa tree.

1.4 Reliability, validity and objectivity

Reliability was ensured by performing suitable statistical analyses using GenStat 18th version (VSN International, Hempstead, UK) at a significance level of ($p \leq 0.05$) and GraphPad Prism Software V5.0 (California, USA). Validity was established through replication of both treatment and control, as well as repeating experiments over time. Objectivity was maintained by discussing findings grounded in empirical evidence to eliminate any potential subjectivity (Leedy and Ormrod, 2005).

1.5 Bias

Bias was minimized by reducing experimental error through the use of replications, assigning treatments randomly within a suitable research design, and accounting for the variability in environmental conditions. Specifically, a randomized complete block design (RCBD) was employed to address environmental variability (Leedy and Ormrod, 2005).

1.6 Scientific significance of the study

This study is valuable as it will provide essential insights for farmers interested in cultivating *Moringa oleifera* during winter, focusing on using netting structures to manage plant growth and development. With climate change expected to negatively affect agriculture, it is important to understand how shade netting influences plant growth, yield, and nutritional properties, especially given the growing demand for Moringa in South Africa. Moreover, this cost-effective protected cultivation technique can mitigate environmental challenges (both biotic and abiotic), potentially enhancing plant growth and productivity (Kittas *et al*., 2012). Supporting Moringa production could also benefit the health and well-being of people in marginalized communities (Mashamaite, 2020).

1.7 Structure of the dissertation

Chapter 1 provided a detailed outline of the research problem. Chapter 2 examined the work completed on the problem and outlined the work that was not completed. After that, Chapters 3 and 4 addressed objectives 1 and 2, respectively. The data from each chapter were compiled and explained in the final chapter (Chapter 5), which concluded by synthesizing the entire study and providing recommendations for further research. This full-dissertation followed the Harvard style using author-alphabets as approved by University of Limpopo Senate.

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Chapter 2: Literature review

There is insufficient data to fairly discuss the literature review on how shading nets affect the growth and yield of *Moringa oleifera* during off-season production.

2.1 Work done on *Moringa oleifera*

2.1.1 Morphology and physical characteristics of *Moringa oleifera.*

Moringa is a medium-sized tree with widely spreading branches. Its trunk is covered with thick, corky, whitish-brown bark that features vertical fissures (Yadav and Sardesai, 2002). According to Gupta (2018), the leaves are longitudinally fractured, with a main axis ranging from 30 to 75 cm in length. The branches are joined at glandular points, and the leaflets are solid and smooth on both sides, with either blunt or mucronate tips. The petiole at the base is sheathed. The tree blooms in large clusters, either at the branch tips or in the axils of the leaves. The calyx is cup-shaped with five unequal lobes resembling petals, linear-lanceolate in shape, measuring 10–12 mm long, and bent backward. It has five spatula-shaped petals of varying lengths (10–15 mm) that are creamy white in color. The tree also has five stamens, alternating with 5 to 7 sterile staminodes. The reproductive system consists of three fused carpels, an oblong ovary, and a cylindrical style. The pods are long and pendulous, cylindrical to triangular in cross-section, with nine ridges, growing up to 50 cm in length (Naik, 1998). Moringa seeds measure roughly 12.9 mm in length, 11.4 mm in width, and have an average weight of approximately 268.9 mg. They typically contain around 34% oil, with the potential to reach up to 39%, underscoring their commercial significance, especially for the generation of premium biofuels (Rashid *et al.,* 2008). The seeds are oil-rich and do not undergo a dormancy phase, allowing them to be sown immediately after maturation (Valdés-Rodríguez *et al.,* 2018).

2.1.2 Uses and medicinal benefits of *Moringa oleifera*

Moringa oleifera is widely acknowledged for its numerous medicinal applications and nutritional advantages across the globe. Every part of this versatile plant is utilized for a variety of domestic functions, such as purifying water, enhancing agricultural practices through alley cropping and as green manure, producing biogas, serving as animal fodder and natural fertilizer, and extracting gum from its trunk. In addition to these uses, *Moringa* is employed in various industries, including sugar cane clarification, hide tanning, bio-pesticide manufacturing, pulp processing, oil extraction for lubrication, rope production, and perfumery due to its aromatic compounds, as well as in hair care products (Brilhante *et al.,* 2017). Studies have indicated that every part of the *Moringa oleifera* plant, including flowers, seeds, leaves, fruits, and young pods, is traditionally eaten as food, highlighting their significant nutritional benefits (Siddhuraju and Becker, 2003). The plant is especially esteemed in the fields of medicine and health for its antibacterial properties. Its rich fiber and protein content position it as a valuable dietary supplement, while extracts from *Moringa* have been utilized to treat bacterial and fungal skin infections (Fuglie, 1999). Studies have explored the antibacterial effectiveness of various extracts derived from *Moringa* against common human enteric bacteria, including *E. coli*, *S. aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae* (Zaffer, 2014). Table 2.1 outlines the various medicinal applications and advantages of Moringa.

In agricultural contexts, *Moringa* has been investigated for its potential to boost crop yields. Components extracted from *Moringa* have shown promise in enhancing soil fertility and promoting overall plant health (Francis and Liogier, 1999). When included in livestock feed, *Moringa* leaves have been found to increase the net mass gain of animals by as much as 35%. Additionally, supplementing dry animal feed with *Moringa* has been associated with improved milk production, highlighting its dual role in enhancing both animal nutrition and productivity (Foidl *et al.,* 2001).

Table 2.1: Medicinal uses and benefits of different parts of the Moringa tree.

Plant parts	Uses	Methods and Solvents Used for Extraction	References
Seed	The seed oil is employed in the treatment of various conditions such as gout, Crohn's disease, herpes simplex virus, arthritis, and epilepsy.	Cold-pressed extraction using mechanical methods or solvent extraction with hexane.	Thurber and Fahey; 2010; Kasolo *et al.,* 2010; Anwar *et al*., 2007.
Leaves	The leaves are employed to treat a wide range of ailments including scurvy, headaches, skin conditions, ear and eye infections, bronchitis, asthma, influenza, heartburn, malaria, diarrhea, pneumonia, and hyperglycemia. They have the ability to help reduce cholesterol levels and regulate blood pressure in humans.	Aqueous extraction (hot water) or ethanol/methanol extraction using Soxhlet or maceration techniques.	Mbikay, 2012; Rockwood *et al*., 2013; Ijarotimi *et al*., 2013; Sengupta and Gupta, 1970; Amaglo *et al.,* 2010.
Flower	It is utilized as a hypocholesterolemic and anti-arthritic agent and is also effective in treating urinary issues and colds.	Ethanolic or aqueous extraction using maceration	Sutalangka *et al*, 2013; Amaglo *et al.,* 2010.

Root	The root bark exhibit strong antiulcer and anti-inflammatory properties, along with potential as a heart stimulant.	Hydroalcoholic extraction (ethanol and water) or methanolic extraction.	Choudhary *et al.,* 2013; Sengupta and Gupta, 1970.
Gum	It is used to treat dental cavities and serves as both an astringent and a rubefacient. When mixed with sesame oil, the gum is employed to alleviate headaches, fevers, digestive problems, dysentery, and asthma. Additionally, it is sometimes utilized as an abortifacient and in the management of syphilis and rheumatism.	Aqueous extraction through dissolution or alcohol-based extraction.	Fuglie, 2001; Anwar *et al.,* 2007; Siddhuraju and Becker 2003.
Pod	It has shown potential in treating diarrhea, liver and spleen disorders, as well as joint pain.	Ethanolic extraction using Soxhlet.	Fugile, 2005; Anwar *et al.,* 2007.

2.1.3 Optimal climatic conditions for *Moringa oleifera.*

Moringa oleifera is a fast-growing plant capable of flourishing in challenging climatic and environmental conditions that could negatively affect many other agricultural crops (Tshabalala *et al.,* 2020; Korsor *et al.,* 2019). This species is mainly located in tropical and subtropical areas, thriving best at temperatures between 25 and 35 °C. Nevertheless, it can tolerate short bursts of heat reaching up to 48 °C, particularly when it has some shade (Palada *et al.,* 2019). While Moringa generally favors tropical climates, it possesses physiological adaptations that enable it to thrive even in less-than-ideal conditions, allowing it to tolerate lower temperatures (Mabapa *et al.,* 2017). The plant can tolerate temperatures ranging from -1 to 3 °C during the coldest months and can survive short, mild frosts. However, prolonged exposure to such cold conditions can be harmful (Trigo *et al.,* 2021). Regarding rainfall, *Moringa* can withstand annual precipitation between 250 mm and 3000 mm (Mahmood *et al.,* 2010). This remarkable resilience is mainly attributed to its long, tuber-like taproot, which penetrates deeply into the soil to access water reserves. This feature enables the tree to endure moderate droughts when many other crops may fail (Ndubuaku *et al.,* 2014). As a result, *Moringa* stores water in its roots and fleshy tissues, allowing it to cope with dry periods rather than depending entirely on rainfall.

2.1.4 Phytoconstituents

Moringa oleifera is recognized as a rich source of tannins, phenolics, steroids, flavonoids, and terpenes. It also offers a range of nutraceuticals, including dietary fiber, carbohydrates, proteins, fats, and numerous vitamins such as riboflavin, thiamine, pantothenic acid, niacin, folate, vitamin C, provitamin A, and vitamin K. Additionally, it is rich in essential minerals, including iron, calcium, manganese, magnesium, phosphorus, sodium, potassium, and zinc (Gopalakrishnan *et al.,* 2016). Various phytoconstituents in *Moringa oleifera* have been identified and studied, as outlined in Table 2.2.

Table 2:2 Phytoconstituents found in various parts of *Moringa oleifera*.

Parts of the Plant	Phytoconstituents	Health benefits
Leaves	Isothiocyanates, total polyphenols, flavonoids, omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), and E-lutein.	➢ Antioxidant: Reduces oxidative stress. ➢ Anti-inflammatory: Alleviates inflammation-related conditions. ➢ Eye health: E-lutein supports retinal health. ➢ Heart health: PUFAs help in reducing cholesterol and improving cardiovascular health.
Stem	Contains β-sitosterone, vanillin, octacosanoic acid, 4-hydroxymellein, β-sitosterol, and 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate.	➢ Anti-cancer: β-sitosterol and vanillin may inhibit tumor growth. ➢ Anti-inflammatory: Reduces inflammation. ➢ Wound healing: Promotes tissue repair.
Root	Contains benzylglucosinolate and 4-(α-L-rhamnopyranosyloxy) benzylglucosinolate.	➢ Antimicrobial: Effective against bacteria and fungi. ➢ Detoxification: Glucosinolates promote liver detox pathways. ➢ Pain relief: Traditionally used to alleviate joint pain.

Gum	Includes sugars such as leucoanthocyanin, L-arabinose, L-rhamnose, D-galactose, D-xylose, D-mannose, and D-glucuronic acid.	➢ Anti-inflammatory: Leucoanthocyanin reduces swelling. ➢ Gut health: Polysaccharides act as prebiotics, supporting gut microbiota. ➢ Immune support: Enhances immune response.
Mature flower	Comprised of protein, D-glucose, ascorbic acid, D-mannose, and polysaccharides.	➢ Antioxidant: Ascorbic acid neutralizes free radicals. ➢ Immune booster: Polysaccharides stimulate immunity. ➢ Nutritional benefits: Rich source of essential amino acids.
Pod	Includes compounds such as isothiocyanate, thiocarbamates, o-ethyl-4-[(α-1-rhamnosyloxy)-benzyl] carbamate, o-[2'-hydroxy-3'-(2''-heptenyloxy)] propylundecanoate, nitriles, β-sitosterol, and methyl-p-hydroxybenzoate.	➢ Antimicrobial: Inhibits bacterial and fungal growth. ➢ Cancer prevention
Seed	Seed contains phytosterols, the most abundant of which are β-sitosterol, stigmasterol and campesterol. Alkaloids, saponins, phytates, tannin	➢ Cholesterol-lowering: Phytosterols reduce LDL cholesterol. ➢ Antioxidant: Phenolic compounds combat oxidative stress.

	and phenolic compounds (quercetin and p-hydroxybenzoic acid) can also be found.	➢ Anti-diabetic: Quercetin helps regulate blood sugar levels.

Source: Mishra *et al.,* 2011; Gopalakrishnan *et al.,* 2016; Anwar *et al.,* 2007; Oliveira *et al.,* 1999, Asiedu-Gyekye *et al.,* 2014; Karthivashan *et al.,* 2016; Caceres *et al,* 1991; Vergara-Jimenez *et al.,* 2017, Mbikay, 2012, Tiloke *et al.,* 2013 and Amaglo *et al.,* 2010.

2.2 Cultivation methods used to improve the growth and yield of *Moringa oleifera*

2.2.1 Mineral nutrition

Understanding soil composition is vital for evaluating the nutritional quality of plants. The application of agrochemicals, including insecticides and fertilizers, has led to increased levels of nitrogen, phosphorus, and potassium in the soil, improving the nutritional value of specific plant species (Dania *et al.,* 2014). Research has shown that the use of inorganic fertilizers can significantly enhance the vegetative growth of *Moringa oleifera*. Studies indicate that fertilizers high in phosphorus and nitrogen lead to notable improvements in growth, particularly by stimulating the development of the root system and promoting leaf growth (Fuglie, 1999). These findings are supported by research from Sarwar (2020), which demonstrated that the application of a combined NPK (nitrogen, phosphorus, potassium) fertilizer and compost resulted in a substantial increase in carbohydrate levels within *Moringa* plants. Additionally, Haukioja *et al.* (1998) introduced the C/N (carbon to nitrogen) balance hypothesis, suggesting that the presence of readily available nitrogen encourages plants to produce nitrogen-rich compounds, such as proteins, which in turn promotes overall growth.

Further investigations into the nutrient concentrations in *Moringa* have focused on organic fertilizers, particularly poultry manure. Research has indicated that poultry manure yields especially positive results when compared to other sources of nutrients like phosphorus, sodium, potassium, nitrogen, and manganese, underscoring its potential to enhance the nutrient profile of *Moringa* on a global scale (Mbikay, 2012). Furthermore, nitrogen fertilization has been found to impact the chemical makeup of *Moringa oleifera*, considerably increasing its protein levels (Mouchili *et al.,* 2019). Foliar application of selenium has also been explored as a method to elevate the selenium content in *Moringa oleifera* (Tang *et al.,* 2020). Overall, these studies indicate that increased fertilizer application can significantly enhance the nutritional properties of *Moringa oleifera*, making it a more valuable crop in terms of its health benefits.

2.2.2 Pruning

Pruning is a crucial horticultural technique that supports continuous leaf production in *Moringa oleifera,* thereby helping to fulfill the nutritional requirements of families in developing countries. In dry-tropical areas, *Moringa oleifera* naturally drops its leaves during the dry season and regrows them when the rains arrive. Pruning can encourage the growth of additional leaves and lateral buds, which can boost overall productivity. Proper pruning helps prevent *Moringa oleifera* from developing into a tall, straight tree with sparse foliage and fruit primarily on the main stem, a condition often seen without pruning intervention (Palada *et al*., 2003). Research shows that pruning enhances both the yield and quality of Moringa plants (E.S. du Toit *et al*., 2020). Vijayakumar (2000) discovered that pruning early, specifically 60 days after planting, is more advantageous than waiting until 90 days, as it promotes faster growth rates and results in higher forage yields. Root pruning, a common practice in horticulture, involves managing both the above-ground and below-ground plant components to control vegetative growth. Research has demonstrated several advantages of root pruning, including enhanced water-use efficiency (Ma *et al*., 2009; Wang *et al.,* 2014), enhanced fruit characteristics (Yang *et al.,* 2011), enhanced nodule development (Cazenave *et al*., 2014), and elevated leaf nutrient concentrations alongside increased photosynthetic activity (Jing *et al.,* 2017). By adopting horticultural techniques like pruning, the sustainable cultivation of *Moringa oleifera* leaves can be promoted, which is vital for meeting nutritional requirements in developing areas. However, there is a notable scarcity of research focused on pruning strategies specifically tailored for *Moringa oleifera*, with existing literature offering varying recommendations (Crosby and Craker 2007; Patricio *et al.,* 2015).

2.2.3 Planting density

Various studies have highlighted the impact of planting density on the growth and biomass production of *Moringa oleifera*. Higher planting densities generally result in increased competition for resources such as light, water, and nutrients, leading to reduced plant size and slower growth rates. For instance, studies by Foidl *et al*. (2001) showed that higher densities lead to stunted growth and reduced leaf area, affecting the overall biomass yield per plant. However, when biomass yield per unit area is considered, higher densities can result in higher total yield due to the increased number of plants. Planting density has been recognized as an essential management practice affecting biomass production and leaf quality (Sánchez *et al.,* 2006; Gadzirayi *et al.,* 2013). Mabapa *et al*. (2017) found that planting *Moringa oleifera* at a higher density significantly boosted biomass production, with a planting density of 435,000 plants per hectare leading to increased biomass accumulation across all cutting intervals. Research has found that denser planting conditions may reduce the nutrient content in the leaves. For example, Sanchez *et al.* (2006) observed that lower planting densities resulted in higher concentrations of essential nutrients. This is likely because reduced competition allows plants to accumulate more resources. Planting density also affects the leaf-to-stem ratio, which is crucial for harvesting leaves for human consumption. Foidl *et al.* (2001) found that lower planting densities favored higher leaf yields and a better leaf-to-stem ratio, improving the economic value of *Moringa* leaves. On the contrary, high-density planting leads to an increase in stem biomass at the expense of leaf production.

2.3 Work done on the problem statement

Sharma Bharathi (2019) observed that adjusting *Moringa oleifera's* flowering cycle through management practices can significantly enhance its off-season yield. Plastic mulches are widely utilized in horticulture to control soil temperature and moisture, prevent rainwater disruption, and potentially impact plant photobiology. These mulches primarily alter the field's microclimate by modifying the surface radiation balance, influencing plant growth and overall yield (Liakatas *et al.,* 1986). Pruning at the optimal time in the growing season can further increase Moringa's yield potential, with effective pruning supporting early flowering and fruit development by improving light exposure, nutrient availability, and moisture conditions.

Growth retardants like Uniconazole also contribute by slowing vegetative growth and encouraging flowering (Sharma Bharathi *et al.,* 2019). In Sharma Bharathi (2019) study, the combination of plastic mulch (50-micron polyethylene), July pruning, and post-pruning Uniconazole spray at 50 ppm (applied one to two months afterward) led to early off-season flowering and higher fruit set. This specific treatment resulted in increased flower and panicle production per plant, along with improved fruit yield during the off-season, compared to other treatments. The study suggested that *Moringa oleifera* performs well in dry conditions with limited water, which promotes flowering and fruiting outside the typical season. However, there is a notable lack of comprehensive research focused on *Moringa oleifera*'s response to off-season environmental conditions.

2.4 Work done on other crops regarding the problem statement

The effect of shade nets on plant growth and development of *Moringa oleifera.*

Shade nets are highly effective for moderating temperature fluctuations, especially during winter when nighttime temperatures can drop significantly. Rajasekar *et al*. (2013) found that mean weekly temperatures in both summer and winter were generally higher in open fields than under shade net conditions. The cooler temperatures within shade net houses promoted greater plant height, increased branch numbers, longer internodal spacing, higher average fruit weight, and improved yield per plant, outperforming open-field conditions. These results are consistent with those of Kumar *et al.* (2000) in polyhouse environments. Further studies by Chernoivanov *et al.* (2022) and Zait *et al.* (2020) emphasize the effectiveness of shading nets in shielding agricultural crops from harsh weather and cold-related damage.

Research in Florida also demonstrates the substantial benefits of shade netting on plant health. In a study on avocado trees, shaded leaves had notably higher chlorophyll content compared to those exposed directly to sunlight (Reed *et al.,* 2012). Leaves grown in shade absorb more light and consequently produce more chlorophyll, optimizing their capacity to absorb diffuse radiation and transform it into carbohydrates necessary for plant development. Findings by Beneragama and Goto (2010) also support this, indicating that levels of chlorophyll a and b increase under lower light conditions. Beyond temperature and light regulation, shade nets help to retain soil moisture and manage humidity. Maintaining adequate humidity during winter is crucial, as it prevents excessive water loss due to cold winds and low relative humidity. Shade nets reduce wind speed and retain moisture, fostering a more humid environment around plants that lowers evapotranspiration and conserves water (Ilić *et al.,* 2012). This is particularly valuable in regions with limited water resources or for crops vulnerable to drought stress during winter.

The effect of shade nets on phytochemical profiles

Studies have also highlighted the effects of shade nets on the phytochemical profiles of crops, with findings showing increased levels of secondary metabolites such as phenolic compounds, flavonoids, and chlorogenic acids in crops grown under shade nets during winter (Ramphinwa *et al.,* 2022). The cooler temperatures and adjusted light levels under shade nets can induce stress responses in plants, stimulating higher production of these compounds, which are associated with improved antioxidant activity and enhanced nutritional quality. For example, a study on tea plants found that those cultivated under an 80% white shade net in winter accumulated considerably higher levels of chlorogenic acids compared to plants grown under different types of shade nets or exposed to direct sunlight (Ramphinwa *et al.,* 2022). This suggests that shade nets provide both winter protection and an environment conducive to increased phytochemical content, potentially boosting the nutritional and medicinal value of crops.

According to Ramphinwa *et al*. (2022), shading offers an effective means of shielding plants from extreme temperatures, whether hot or cold, helping prevent cold-related damage and leaf injury. Zaman *et al*. (2022) found that in tea plants, shade improved physiochemical properties and antioxidant activity. Additionally, a study by Ju *et al.* (2023)

demonstrated that light-selective sunshade nets resulted in increased levels of total phenols, tannins, and flavonols in grapes, although there was a reduction in total flavonoid and anthocyanin levels. In winemaking, most phenolic compounds showed elevated levels, and the study concluded that light-selective sunshade nets effectively enhanced the formation of various phenolic compounds while improving the aroma profile in grapes.

Other research has shown that colored shading nets can further encourage plant growth (Strydom *et al.,* 2018), boost antioxidant levels (Zhang *et al.,* 2015), produce more nutrient-dense fruits (Ilic *et al*., 2018), and promote the accumulation of metabolites useful in pharmaceuticals (Ribeiro *et al*., 2018). Together, these findings indicate that shade nets not only shield crops in winter but can also elevate phytochemical content, contributing to improved nutritional and medicinal value.

2.4 Addressing the identified gaps.

There is a significant lack of comprehensive research on developing *Moringa oleifera* varieties or cultivars that can thrive in colder temperatures. Investigating the genetic factors responsible for cold sensitivity and breeding for cold tolerance could provide valuable insights. Additionally, there is limited research exploring the effectiveness of environmental control methods, such as greenhouse or polytunnel cultivation, to extend the growing season of *Moringa oleifera* into colder months. Understanding the optimal conditions for off-season production could enhance both yield and quality. Furthermore, there is a scarcity of information on the effects of different protective measures, such as netting structures, on *Moringa oleifera* growth, yield, and quality during the winter season. Research in this area could offer practical solutions for growers in colder climates. Cultural practices that can mitigate the impact of cold stress on *Moringa oleifera* such as mulching, irrigation management, and tailored nutrient supplementation for off-season production, also require further investigation. Moreover, understanding the post-harvest physiology of *Moringa oleifera* during off-season production is crucial for maintaining quality and extending shelf life. Research could focus on determining optimal harvesting times, storage conditions, and processing techniques specific to off-season harvests.

Addressing these research gaps could lead to the development of sustainable practices for off-season production of *Moringa oleifera*, potentially expanding its cultivation into regions with colder climates.

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Chapter 3: The effect of 40% shade net and coolaroo frost cloth on the growth and biomass yield of *Moringa oleifera*

Abstract

Moringa oleifera Lam is a fast-growing species known for its drought resilience, yet its sensitivity to frost and freezing restricts its growth in colder regions. This study aimed to assess the effect of using Coolaroo frost cloth and 40% shade nets to protect *Moringa oleifera* during winter. Seven treatments were arranged in a Randomized Complete Block Design replicated three times: 40% shade net with top cover (TC), Coolaroo frost cloth with top cover (TC), 40% shade net with closed sides (CS), Coolaroo frost cloth with closed sides (CS), 40% shade net with closed top and sides (CTS), Coolaroo frost cloth with closed top and sides (CTS), and a control with no netting. Weekly measurements of growth parameters (plant height, stem diameter, and root length) were recorded, while yield (number of leaves and biomass) and physiological (chlorophyll content) parameters were assessed at harvest. Data were subjected to statistical analysis using GenStat 18th version statistical package (VSN International, Hempstead, UK). Mean separation for significant (P=0.00) treatments were achieved using Tukey's Honestly Significant Difference (HSD). Both Coolaroo frost cloth and 40% shade net with closed top and sides (CTS) significantly improved plant growth and development, with the Coolaroo frost cloth with closed top and sides providing the highest protection against freezing temperatures. Shading nets offer a viable strategy for reducing cold stress in *Moringa oleifera*, enabling its cultivation in cooler climates and potentially minimizing grower losses.

Keywords: *Moringa oleifera*, frost protection, Coolaroo frost cloth, 40% shade net, cold stress, plant growth, biomass yield.

3.1. Introduction

Moringa oleifera Lam., also referred to as the "drumstick tree" or "miracle tree," is renowned for its fast growth, growing to heights of 10 to 12 meters within a few years under optimal conditions (Foidl *et al.,* 2001). With an ideal pH range of 4.5 to 8, this versatile plant thrives in a variety of soil types, with the exception of hard clay and wet soils. It can be cultivated at elevations of up to 2000 meters. The most suitable regions for cultivating *Moringa oleifera* are those with tropical and subtropical climates, featuring dry to moist conditions, annual rainfall between 760 to 2500 mm (requiring less than 800 mm with irrigation) and temperatures between 18°C and 28°C (Nouman *et al*., 2014). Propagation methods for *Moringa oleifera* include directly sowing seeds into the soil or utilizing seedling trays in nurseries (Price, 2007). The tree holds considerable significance in traditional medicinal systems across many developing nations, including South Africa, where herbal plants are vital to healthcare practices (Soni *et al.,* 2015). Research conducted by Saini *et al*. (2021) emphasizes the diverse utility of *Moringa oleifera*, as nearly every part of the tree possesses medicinal and nutraceutical properties, producing valuable compounds for various health-related applications.

Temperature is a critical factor influencing the respiration rates of plants, which in turn affects photosynthesis and overall growth and development (Munir, 2004). Research conducted by Manduwa *et al*. (2018) indicated that temperature plays a crucial role in the reproductive phase of *Moringa oleifera*. In particular, low temperatures (≤15 °C) can negatively affect fruit set during the flowering stage, whereas temperatures ranging from 30 °C to 35 °C promote better fruit set. Expanding *Moringa oleifera* cultivation into cooler climates may broaden its production range (Muhl *et al.,* 2011[a]). Although the plant flourishes in warmer conditions, it can still produce growth and yields in less-than-ideal climates (Manduwa *et al.,* 2018). The increasing frequency of extreme cold events due to climate change presents a challenge to *Moringa oleifera*, especially regarding its vulnerability to frost damage (Zhang *et al.,* 2012). Therefore, it is crucial to develop strategies to mitigate these risks during the winter months to maintain production levels and ensure the resilience of *Moringa oleifera* cultivation amidst changing climatic conditions.

Shading nets are commonly used to shield crops from sun, wind, hail, and other extreme weather conditions (Shahak *et al.,* 2004, 2008), but their ability to mitigate the damage caused by cold temperatures is not well-known. High-density shade nets have been demonstrated in previous studies to be effective in shielding banana plants from frost damage (Zait *et al*., 2020). Similarly, it has been demonstrated that during cold winters, high-density shade nets increased the fruit production and chlorophyll fluorescence of mature avocado Pinkerton trees (Rubinovich *et al.,* 2023). Despite the fact shading nets have been demonstrated to be effective in mitigating frost damage in crops like bananas and avocados, there is limited information available on their impact on *Moringa oleifera* growth and yield during the winter season. This study is the first to investigate the effects of a particular type of shading net, specifically a white 40% shade net and a standard Coolaroo frost cloth on the growth, yield, and quality of *Moringa oleifera* trees during the winter months. Therefore, the objective of this chapter was to investigate the effect of a white 40% shade net and a standard Coolaroo frost cloth with different structures (closed top, closed sides and closed top and sides), on the productivity of *Moringa oleifera* in South Africa.

3.2 Materials and methods

3.2.1 Description of the study site

The field experiment was carried during winter season (May-August 2023) at the Green Biotechnology Research Center of Excellence, University of Limpopo, South Africa, in an open field condition (23°50'S; 29°0'E). The area was characterized by chilly weather, with day/night temperatures ranging from 4 to 20 °C and relative humidity levels ranging from 30% to 60%.

3.2.2 Treatments and research design

Seven treatments were arranged in a Randomized Complete Block Design (RCBD), replicated three times (7 treatments x 3 replication), the treatments were as follows:

- 40% shade covering netting structure with open sides
- Coolaroo Frost Cloth covering netting structure with open sides
- 40% shade net boxed netting structure with open top
- Coolaroo Frost Cloth boxed netting structure with open top
- 40% shade net boxed netting structure with closed top and sides
- Coolaroo Frost Cloth boxed netting structure with closed top and sides
- control with no netting structure

Each plot consisted of six plants.

3.2.3 Procedures

Moringa olefera Lam. seeds (Cultivar PKM1) procured from the World Vegetable Centre (Taiwan) were planted and grown in 200 cavity polystyrene trays filled with Hygromix® (Hygrotech seed company, South Africa). The *Moringa oleifera* seedlings were transplanted directly into the soil at four leaf stage. Prior transplanting, seedlings were hardened off for a week outside the greenhouse. The size of the individual plot was 1m x 1 m, with 0.4 m intra-row spacing and 0.45 m inter-row spacing as depicted in Figures 3.1 and 3.2 below. The plots were spaced 0.5 m apart. The white 40% shade net and Coolaroo frost cloth were installed at 35 days after transplanting in the field. Termination was done at 120 days after transplanting. The plants were watered with drip irrigation system with recommended amounts which cumulated with plants growth stage following the methods described by (Chitiyo *et al*., 2021). Weeding was performed manually throughout the cropping season to maintain a weed-free experimental plot, following the methods established in earlier studies (Mudau *et al.,* 2007). The NPK fertilizer was applied two weeks post-transplanting at doses of 300 kg N ha^{-1}, 300 kg P ha^{-1}, and 200 kg K ha^{-1}.

Figure 3.1: Treatments used in the study. A - Coolaroo Frost Cloth side cover with top open; B – Coolaroo® Frost Cloth side open with top cover; C - Coolaroo Frost Cloth side cover with top cover; D – White 40% Shade net side cover with top open; E - White 40% Shade net side open with top cover; F - White 40% Shade net side cover with top cover.

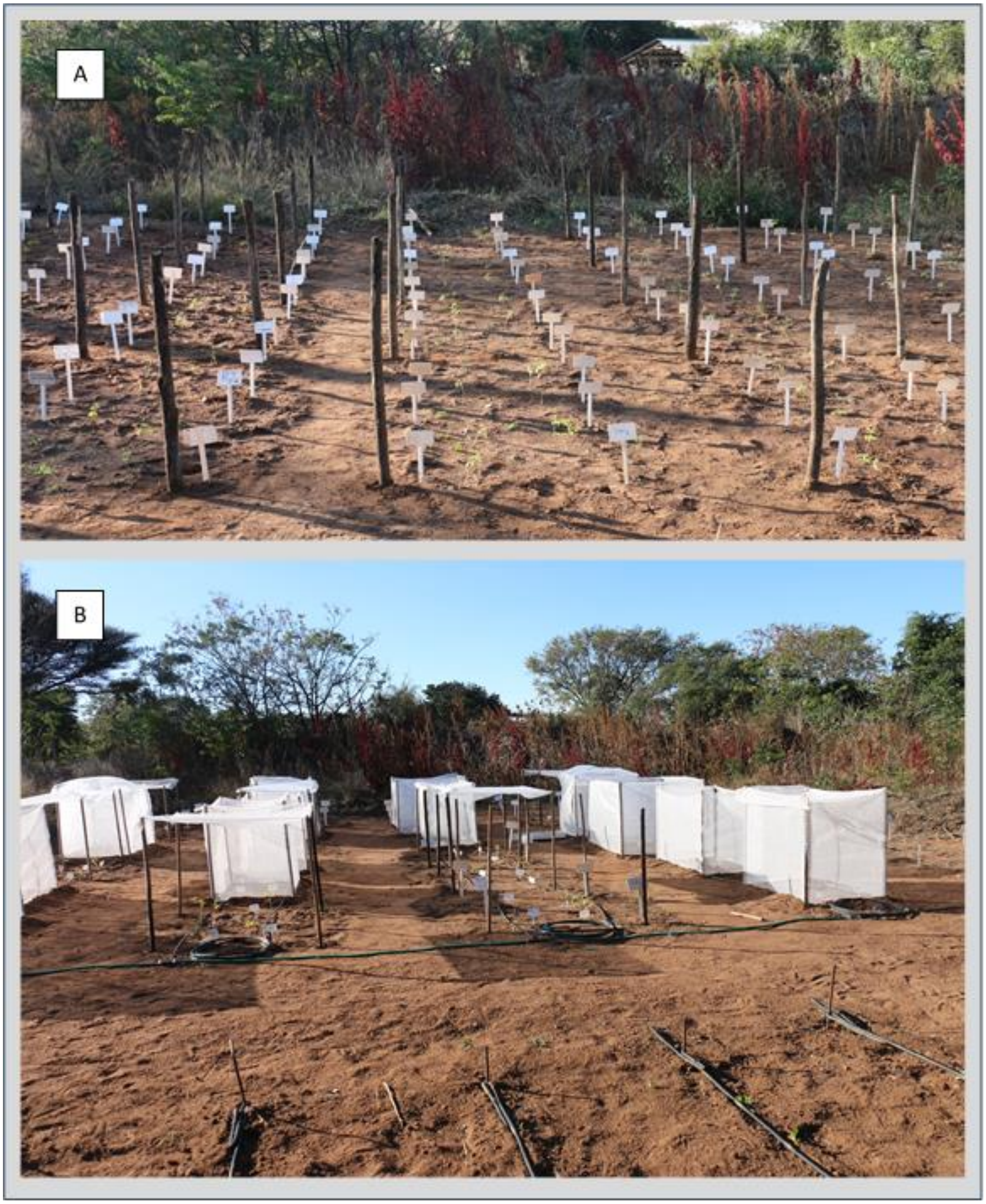

Figure 3.2: Experimental setup. A – Control without netting structures and B – Netting structures setup.

3.2.4 Data collection

Four plants from each replicate were randomly selected, tagged, and their growth and yield parameters were recorded. Throughout the experiment, growth parameters such as plant height, stem diameter, and root length were measured until the study was completed. Plant height was recorded with a measuring tape, measuring from the base of the stem to the tip of the highest leaf. Stem diameter was recorded 30 mm above the soil surface using a digital vernier caliper, with the measurements expressed in millimeters (mm). At termination, 120 days after transplanting, yield parameters including biomass and leaf count were assessed. The total chlorophyll content was measured using a CCM-200 plus chlorophyll content meter between 06:00 and 07:00 on the last day of the trial, from four randomly selected plants in each plot. The number of leaves on the selected plants was counted manually. Root length was measured with a meter scale, and results were reported in centimeters (cm). The entire plant (including stem, leaves, and roots) was uprooted and weighed for fresh weight (biomass) using a TADB 220-4-B balance (Germany), with results expressed in grams per plant.

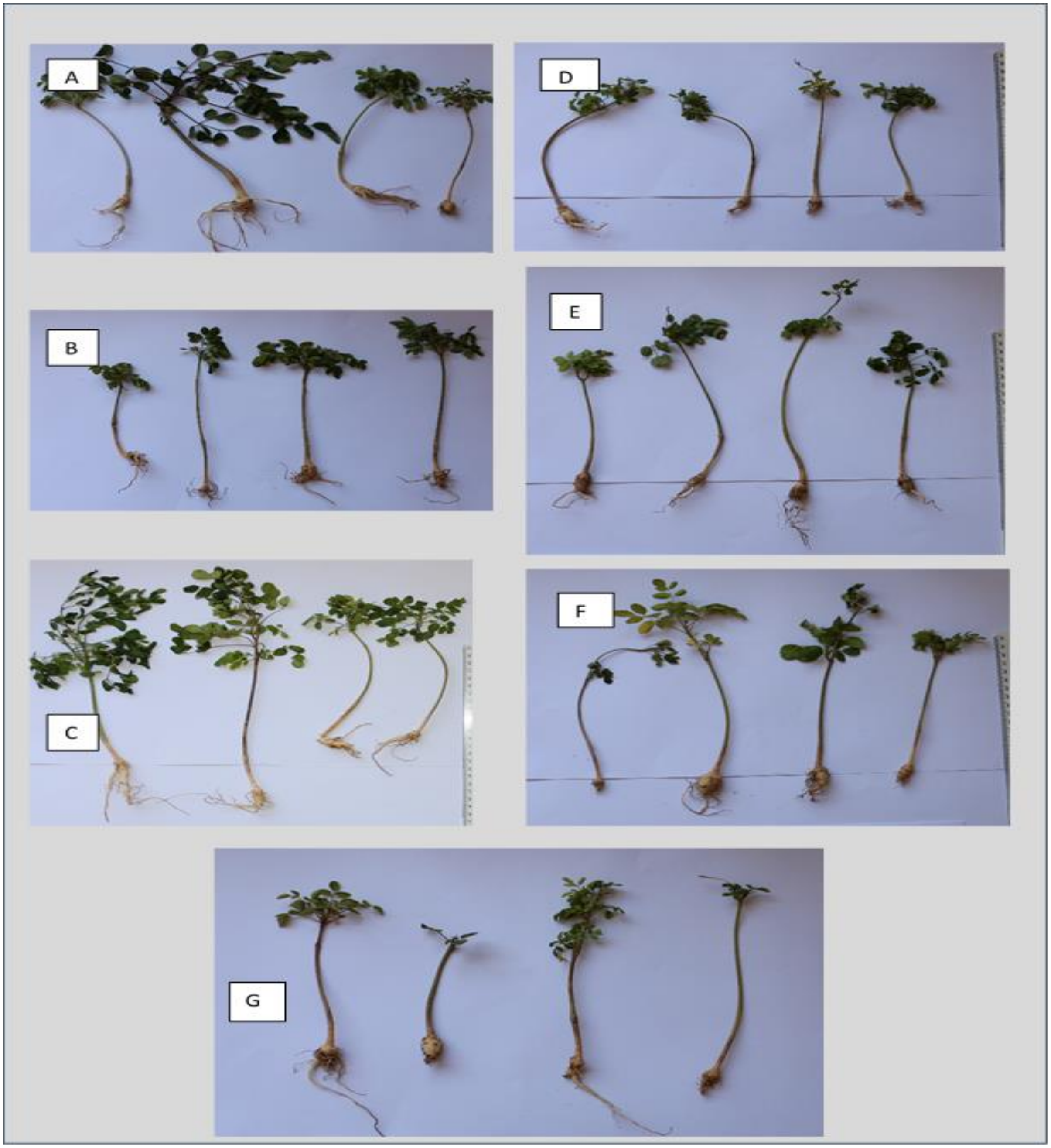

Figure 3.3: Freshly harvested *Moringa oleifera* plants. A- Coolaroo frost cloth with closed sides; B- Coolaroo frost cloth with top cover; C- Coolaroo frost cloth with closed top and sides; D- White 40% shade net with closed sides; E- White 40% shade net with top cover; F- White 40% shade net with closed top and sides; G- Control.

3.2.5 Data analysis

Data were subjected to statistical analysis using GenStat 18th version statistical package (VSN International, Hempstead, UK). Mean separation for significant ($p \leq 0.05$) treatments were achieved using Tukey's Honestly Significant Difference (HSD).

3.3 Results and discussion

3.3.1. The effect of White 40% shade net and Coolaroo frost cloth on the growth of *Moringa oleifera.*

Figures 3.4, 3.5 and 3.6 illustrate a significant difference (p=0.00) in plant growth parameters, such as plant height and stem diameter, for *Moringa oleifera* cultivated under the White 40% shade net and Coolaroo frost cloth with top cover, closed sides, and closed top and sides.

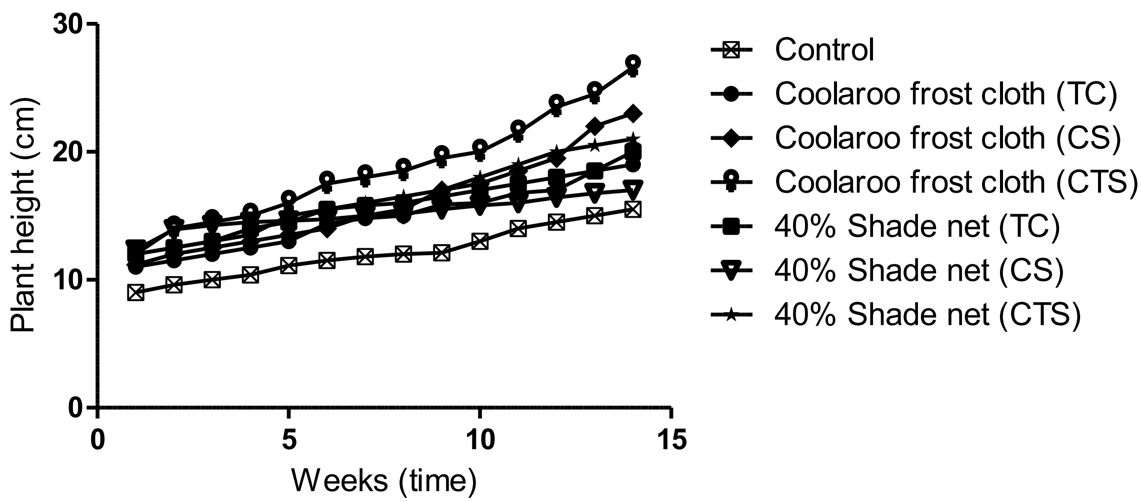

Figure 3.4: The effect of a white 40% Shade net and Coolaroo frost cloth on plant height (cm) of *Moringa oleifera* cultivate during winter. Note: CS (closed sides), TC (top cover), CTS (closed top and sides).

Plant height was significantly (p=0.00) influenced by shade nets, according to means comparison over control (figure 3.3). Among the shade nets treatments, Coolaroo frost cloth with closed top and sides (CTS) resulted in tallest plants, followed by Coolaroo frost cloth with closed sides (CS), while the control recorded the lowest plant height throughout the growing period. At harvest, plants cultivated under Coolaroo frost cloth with closed sides (CS) were taller than plants grown under White 40% shade net with closed sides (CS). However, there was no significant difference between White 40% shade net with top cover (TC) and Coolaroo frost cloth with top cover (TC). The unshaded plants (Control) consistently showed lower plant height, followed by plants cultivated under White 40% shade net with closed sides (CS). Overall, the findings suggest that Coolaroo frost cloth with closed top and sides (CTS) outperformed all the studied treatments over the growing period. Furthermore, both Coolaroo frost cloth and 40% shade net contributed to improved plant height compared to unshaded plants (Control). García-Sánchez *et al*. (2015) demonstrated that shade netting has an impact on plant height, which is another aspect of vegetative growth.

The results of this study are consistent with those of Gaurav *et al*. (2015), who found that *Cordyline* plants grown in shaded conditions were taller and had more leaves than those grown in direct sunlight. Similarly, Gaurav *et al*. (2016) found that due of the varied light spectra, plants grown under colored shade nets showed distinct growth patterns, with plants produced under white and red nets growing taller than those in the unshaded control group. However, the findings of this study differ from previous research, this suggested that changes in the microclimate, due to varying spacing and shading levels, led to considerable differences in growth and development. According to that study, plants in full sun grew more quickly than those in shade (Kumar *et al.,* 2013). It has also been demonstrated that shading promotes the buildup of carbohydrates in leaves (Raveh *et al.,* 2003), which would account for the faster vegetative growth seen in shaded plants as opposed to those in direct sunlight.

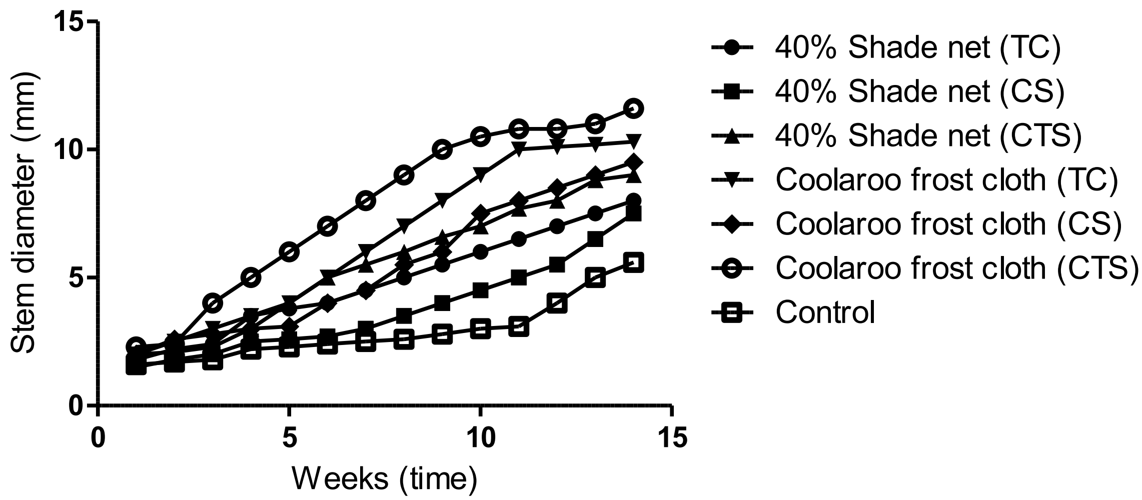

Figure 3.5: The effect of white 40% shade net and Coolaroo frost cloth on stem diameter (mm) of *Moringa oleifera* cultivated during winter. Note: TC (top cover), CS (closed sides) and closed top and sides (CTS).

Stem diameter was significantly influenced by shade nets, according to means comparison over control (figure 3.4). The highest stem diameter was obtained under Coolaroo frost cloth with closed top and sides (CTS) as compared to White 40% shade net with closed top and sides (CTS) with the lowest obtained from the control over the growing period. At termination, both the shading nets had a stem diameter that was higher than the control. The control resulted in a gradual increase in stem diameter from week 11 until the end of the experiment. The Coolaroo frost cloth with top cover (TC) plants were taller than White 40% shade net with top cover (TC). Similarly, Coolaroo frost cloth with closed sides (CS) were taller than White 40% shade net with closed sides (CS). However there was no significant difference in white 40% shade net with closed top and sides (CTS) and Coolaroo frost cloth with closed sides (CS). Consistently, the unshaded plants (control) recorded the lowest plant height, followed by those under White 40% shade net with closed sides (CS). These findings suggest that Coolaroo frost cloth with closed top and sides (CTS) outperformed all other treatments studied over the growing period. Furthermore, both Coolaroo frost cloth and 40% shade net contributed to improved stem diameter compared to unshaded plants, emphasizing the positive impact of these shading methods on plant growth.

The importance of stem diameter in Moringa cultivation is highlighted by its role in enhancing yield potential. Interestingly, our results do not align with those of Gilbert *et al.* (2013), who documented that Eucalyptus hybrid plants cultivated in open environments had the largest stem diameter. Similarly, Ocimum selloi plants in open settings had larger stem diameters, according to Costa *et al.* (2010), but colored shade nets had no discernible impact on stem diameter. Stem diameter is important because it is linked to the vascular tissues, which directly affect the ability to transport carbohydrates and water, as well as indirectly influence the storage capacity of these metabolites (Stuefer and Huber, 1998). As such, variations in environmental conditions, including sunlight exposure and shading, can influence stem diameter and, consequently, plant physiology and productivity. In the context of *Moringa oleifera* cultivation, our results emphasize the importance of understanding the specific requirements of this plant species and the potential impact of different environmental factors, such as shading, on stem diameter and overall yield potential.

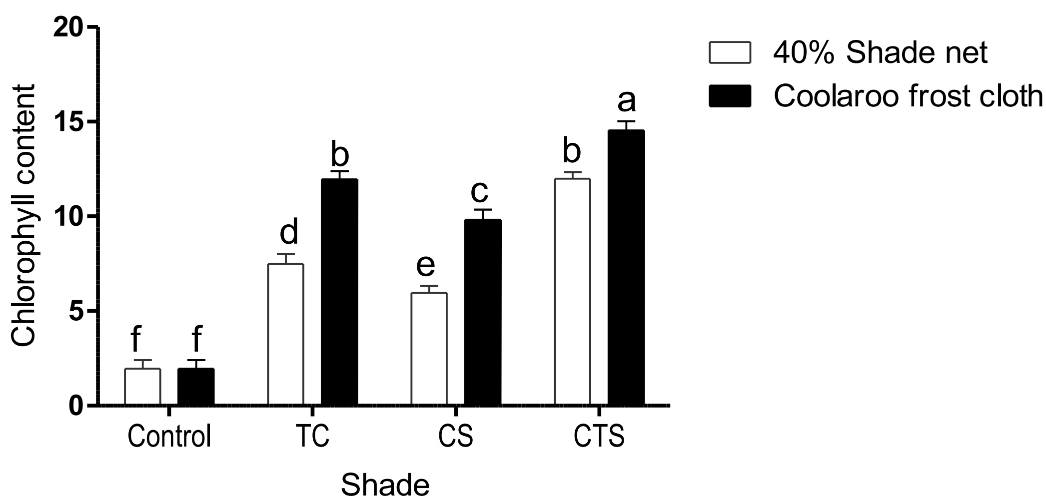

Figure 3.6: The effect of Coolaroo frost cloth and white 40% Shade net on chlorophyll content of *Moringa oleifera*. Note: TC (top cover), CS (closed sides) and (CTS).closed top and sides

All shade nets significantly increased the chlorophyll content, compared to the chlorophyll content values obtained from control plants (figure 3.5). Coolaroo frost cloth with closed top and sides (CTS) recorded the highest chlorophyll content, in comparison to the white 40% shade net with closed top and sides (CTS), recording 14.5 and 11.9, respectively. Leaves of *Moringa oleifera* plants cultivated under Coolaroo frost cloth with top cover (TC) recorded the highest chlorophyll content, in comparison to white 40% shade net with top cover (TC), recording 11.9 and 5.9, respectively. However, there was no significant different between plants cultivated under Coolaroo frost cloth with top cover (TC) and white 40% shade net with closed top and sides (CTS) with a chlorophyll content of 11.9 and 11.9, respectively. Unshaded plants (control) recorded the lowest chlorophyll content of 1.96. These findings suggest that shade nets, particularly Coolaroo frost cloth and White 40% shade net with closed sides and top, positively impact chlorophyll content in *Moringa oleifera* plants, likely due to their ability to moderate sunlight exposure and create a more favorable growing environment. The higher chlorophyll content indicates enhanced photosynthetic activity, which is important for plant growth and productivity.

Manja and Aoun (2019) noted that the use of shading nets fosters an environment conducive to the production of photosynthetic enzymes, resulting in an increase in chlorophyll content per unit leaf area. Shading materials such as Coolaroo frost cloth and a 40% white shade net may enhance the chlorophyll levels in the leaves. Shade-grown leaves, compared to those exposed to direct sunlight, are better at absorbing light and consequently exhibit higher chlorophyll content. Although they do not receive direct sunlight, these leaves produce more chlorophyll to capture diffuse radiation, which is essential for synthesizing the carbohydrates necessary for plant growth (Ilić *et al.,* 2017). The findings of this study align with research by Reed *et al*. (2012) conducted in Florida, which indicated that avocado leaves grown in shaded conditions had higher chlorophyll concentrations than those exposed to sunlight. Similarly, research conducted by Liu *et al.* (2020) discovered that apple trees cultivated in shaded conditions exhibited higher chlorophyll levels than those exposed to full sunlight. Since the concentration of chlorophyll in leaves is indicative of a plant's physiological condition and its ability to photosynthesize and generate biomass, the notable increase in chlorophyll content in shaded plants likely contributed positively to their vegetative growth (Croft *et al*., 2020).

Chilling stress may be the cause of the significant decrease in chlorophyll concentration in the unshaded plants (control). Chilling stress may be the cause of the significant decrease in chlorophyll content in the unshaded plants (control) (Chernoivanov *et al.,* 2022). Growing plants under chilling stress have substantial physiological and morphological alterations, such as decreased appearance of seedlings, commencement of leaves, disorganized division of root cells, and elongation (Farooq *et al.,* 2009; Mulchupadhyay and Roychaudhury 2018). In addition to reducing water and nutrient uptake, the decrease in root architectural growth also reduces nutrient usage efficiency (Grossnickle, 2005). For example, after being subjected to cold stress, chlorophyll content in the leaves of Quercus ilex, an evergreen tree, dropped by 30%, potentially as an adaptive reaction to regulate excessive light absorption in chilly environments (Oliveira and Peñuelas, 2000). Another potential explanation is that in leaves exposed to high light intensities, the rate of chlorophyll breakdown exceeds the rate of its production, which impedes chloroplast formation and results in a decline in chlorophyll content. As a result, studies have observed that leaves grown in shaded environments generally possess greater levels of chlorophyll per unit of leaf weight than those cultivated in direct sunlight (Fu *et al*., 2012; Kosma *et al.,* 2013).

3.3.3 The effect of white 40% shade net and Coolaroo frost cloth on the yield of *Moringa oleifera.*

As shown in Figures 3.7, 3.8, and 3.9, plants cultivated under both white 40% shade net and Coolaroo frost cloth with top cover, closed sides and closed top and sides, significantly (p=0.00) showed greater biomass, number of leaves and root length (cm) than plants grown in unshaded (control).

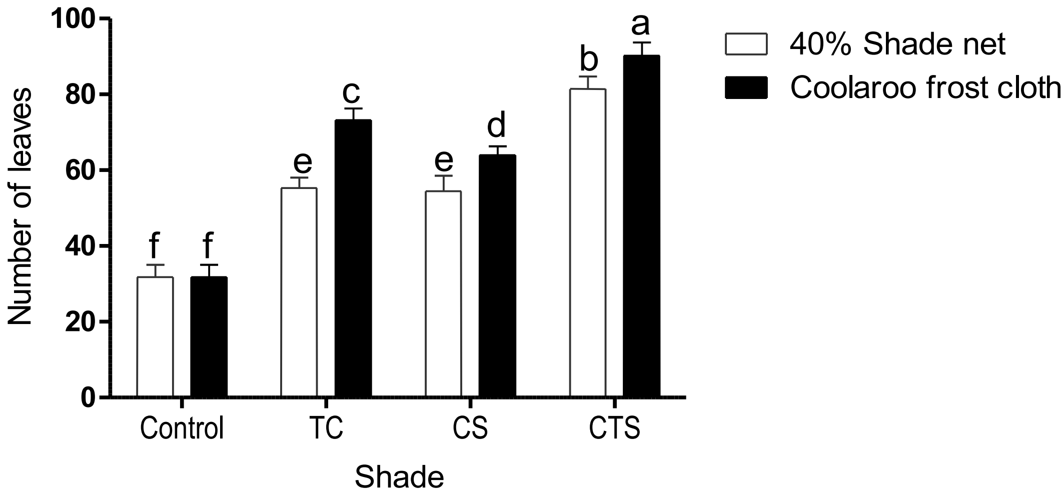

Figure 3.7: The effect of white 40% shade net and Coolaroo frost cloth on leaf number of *Moringa oleifera.* Note: TC (top cover), CS (closed sides) and CTS (closed top and sides).

Plants grown under Coolaroo frost cloth with closed sides and top (CTS) recorded the highest number of leaves, followed by White 40% shade net with closed top and sides (CTS), while the lowest was recorded in control, recording 90, 81 and 31 number of leaves, respectively. Contrary, to the white 40% shade net with top cover (TC), which recorded 55 leaves and all the treatments studied, the Coolaroo frost cloth with top cover (TC) recorded the highest number of leaves (73). Similarly, the Coolaroo frost cloth with closed sides (CS) with 63 number of leaves, recorded the highest in comparison to white 40% shade net with closed sides (CS) with 54 number of leaves. There was no significant difference in White 40% shade with top cover (TC) and Coolaroo frost cloth with closed sides (CS), recording 55 and 54, respectively. The unshaded plants (control) recorded the lowest number of leaves (31). The present study revealed that netting structures had a positive effect on leaf number on *Moringa oleifera.*

The results of this study are in line with those of (Incesu *et al*. (2016), who found that trees cultivated under aluminet and black shade nets, providing shading intensities of 50% and 75% respectively, had a greater number of leaves compared to the control group. This suggests that higher shading intensity, as provided by certain shade nets, can positively influence leaf number, likely by mitigating stress from excessive sunlight. However, our results differ from those reported for *Spinacea oleracea* by Meena *et al.* (2015), where, in contrast to the various colored shade nets, the highest number of leaves was seen in open conditions. This change may be attributed to differences in plant species and their responses to shading, highlighting the importance of considering species-specific requirements when implementing shading techniques. The loss of *Moringa oleifera* leaves during winter could potentially be the cause for the significant drop in the number of leaves under control. It goes dormant and loses its leaves when the temperature drops (Muhl *et al.,* 2011[b]). In summary, while our findings align with certain studies regarding the positive impact of shade nets on leaf number, differences in species responses and environmental conditions must be considered. Furthermore, the observed drop in leaf number under control conditions during winter highlights the importance of managing environmental factors, such as temperature, for optimal plant growth and productivity.

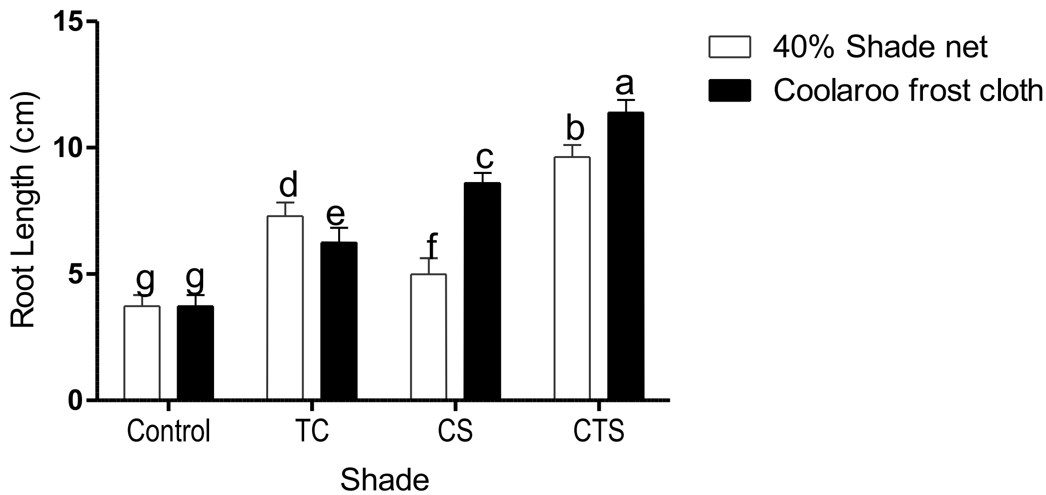

Figure 3.8: The effect of white 40% shade net and Coolaroo frost cloth on the root length (cm) of *Moringa oleifera*. Note: TC (top cover), CS (closed sides) and CTS (closed top and sides).

The study reveals that at harvest, root length (cm) was significantly influenced by shade nets, with both Coolaroo frost cloth and 40% shade net showing increased root length compared to the control (figure 3.7). Among the shade net treatments, Coolaroo frost cloth with closed top and sides (CTS) resulted in the highest root length, recording 11.4 cm, followed by 40% shade net with closed top and sides (CTS) with 9.6 cm, and the control group with 3.7 cm (as shown in Figure 3.7). Interestingly, White 40% shade net with top cover (TC) recorded the highest root length compared to Coolaroo frost cloth with top cover (TC), measuring 7.3 cm and 6.2 cm, respectively. Similarly, Coolaroo frost cloth with closed sides (CS) resulted in higher root length compared to White 40% shade net with closed sides (CS), with measurements of 8.6 cm and 4.9 cm, respectively. These findings indicate that both Coolaroo frost cloth and 40% shade net have a positive impact on root development in *Moringa oleifera* plants, leading to increased root length compared to unshaded plants (control). The specific effects may vary depending on the type of shade net used, highlighting the importance of selecting the appropriate shading method for optimizing root growth and overall plant health.

The observed maximum of root length in plants grown under Coolaroo frost cloth, followed by those under white 40% shade net, and the minimum in unshaded plants (control) aligns with the interpretation that shaded environments provide more favorable growing conditions, as suggested by Gaurav *et al*. (2015). A number of variables, including temperature, light intensity, water availability, and nutrient levels, affect root growth and development; these are all often enhanced in shaded conditions. The development of longer roots in shaded plants may enhance water and nutrient uptake, leading to a better source-sink relationship and increased photosynthesis with assimilate accumulation. Additionally, shade netting has been linked to enhanced nutrient absorption, including nitrogen and potassium, likely because it encourages the development of longer root systems under specific shade netting conditions, as observed by Al-Helal and Abdel-Ghany (2010) and Zhou *et al.* (2018). The findings of Desai *et al*. (2016), which observed longer root length in plants grown under red shade and minimum root length in open field conditions, further support the results of the current study, that shading can positively influence root development. However, our findings do not align with the findings reported by Zhou *et al.* (2018), who found no significant differences in root production and total root length among treatments when using minirhizotron. Such variations highlight the complexity of plant responses to shading and the importance of considering various factors, including plant species, environmental conditions, and experimental methods, when interpreting research findings.

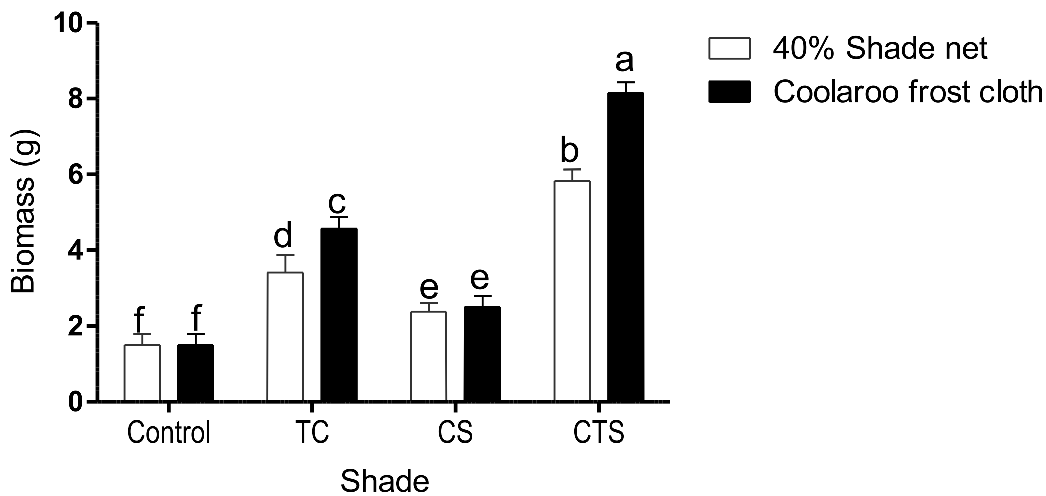

Figure 3.9: The effect of white 40% Shade net and Coolaroo frost cloth on the biomass yield of *Moringa oleifera.* Note: TC (top cover), CS (closed sides) and CTS (closed top and sides).

Higher fresh biomass was significantly observed in plants grown under Coolaroo frost cloth with closed top and sides (CTS), followed by those under 40% shade net with closed top and sides (CTS), while the lowest fresh biomass was observed in control (unshaded plots), recording 8.1 g/plant, 5.8 g/plant, and 1.5 g/plant, respectively. Similarly, Coolaroo frost cloth with top cover (TC) recorded the highest fresh biomass at 4.6 g/plant compared to white 40% shade net with top cover (TC) at 3.4 g/plant. However, there was no significant difference between white 40% shade net with closed sides (CS) and Coolaroo frost cloth with closed sides (CS), with both recording 2.4 g/plant and 2.5 g/plant, respectively. These results suggest that shading, particularly with Coolaroo frost cloth and 40% shade net, positively influences fresh biomass production in *Moringa oleifera* plants. On the contrary, the lower fresh biomass observed in control (unshaded plots) may be attributed to higher temperatures, which could have restricted stomatal conductance and photosynthesis, leading to reduced growth and biomass production.

The alignment of our results with those of Desai *et al.* (2017) underscores the beneficial effect of shading on biomass production, as they found that shaded conditions significantly increased fresh biomass yield per 100m² compared to open-field environments. Their research also supports our findings, indicating that specific colors of shade nets, such as red and green, can enhance fresh biomass yields relative to unshaded conditions. However, our results differ from those of Ramphinwa *et al.* (2022), who reported that unshaded plots yielded the highest fresh biomass, followed by those under white shade nets, with black shade nets producing the least biomass. In summary, while our findings are consistent with some studies that highlight the advantages of shading for fresh biomass yield, the effects can vary based on several factors.

3.4 Conclusion

The findings demonstrate that the use of 40% shade nets and Coolaroo frost cloth significantly enhances the growth and biomass yield of *Moringa oleifera* during the winter season. The shading treatments, particularly the Coolaroo frost cloth with closed top and sides, provided the best protection against cold stress, resulting in greater increases in plant height, stem diameter, and biomass compared the other tested methods. The shading structures created a favorable microclimate that mitigated the effects of low winter temperatures, preventing freezing damage and promoting continuous growth of *Moringa oleifera* in colder climates. These results indicate that shading is a practical and effective strategy for protecting *Moringa oleifera* during the winter season, extending its growing period and improving its overall productivity. While white 40% shade net with closed top and sides (CTS) also led to improved growth and yield compared to unshaded plants, further studies are recommended to investigate the response of *Moringa oleifera* grown under different shade net colors and shade percentages during cold months. Understanding how different shading techniques affect plant physiology and productivity under different environmental conditions can inform more effective strategies for frost protection and overall crop management. The findings suggest that adopting shading structures could be a viable solution for farmers in cold regions who seek to grow Moringa year-round, offering an opportunity to boost agricultural output and improve food security

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Chapter 4: Potential protective effect of the 40% shade net and coolaroo frost cloth shading intensities on Moringa (*Moringa oleifera* lam.) leaf biomass and phytochemicals

Abstract

This chapter explores the effects of 40% shade nets and Coolaroo frost cloth on the phytochemical composition of *Moringa oleifera* during winter. The study aimed to assess how different shading intensities (closed sides, top cover and closed top and sides) affect the production of secondary metabolites, particularly phenolic compounds, flavonoids, and glycosides. Using a split-plot design, seven treatments were applied, and untargeted metabolite profiling was conducted on the leaves, twigs, and roots through UPLC-MS analysis. The Coolaroo frost cloth and 40% shade net significantly ($p \leq 0.05$) affected the phenolic acids, flavonoids, and glycoside compounds in *Moringa oleifera* during winter. Findings indicate that the Coolaroo frost cloth with a closed top and sides was particularly effective, enhancing the concentrations of critical compounds such as flavonoids, glycosyl compounds, phenolic acids, oligosaccharides and lignans across different plant parts (leaves, roots and twigs). The chemometric analysis revealed that shading structures had varying effects on different plant parts. The metabolites in the roots showed no significant variation across shading treatments, indicating minimal impact from shading. However, both the twigs and leaves exhibited distinct metabolome profiles. Twigs under 40% shade net and Coolaroo frost cloth with closed sides retained higher concentrations of flavonoids such as quercetin 3-galactoside, rutin, and astragalin 7-rhamnoside. The leaves showed more significant responses, forming four distinct clusters based on metabolite composition. Shading treatments, particularly the 40% shade net with closed sides and top, resulted in higher concentrations of key flavonoids, including quecetrin and kaempferol derivatives. The results highlight the potential of shading structures to enhance the phytochemical profile of *Moringa oleifera*, particularly in the leaves and twigs, by improving the concentration of bioactive compounds. This suggests the both the 40% shade net and Coolaroo frost cloth are effective in mitigating and boosting the

phytochemical composition of Moringa, especially in cold environments where frost damage can hinder growth and yield.

Key words: *Moringa oleifera*, 40% shade net, Coolaroo frost cloth, Flavonoids, cold stress.

4.1. Introduction

Phytochemicals are non-nutritious secondary metabolites found in plants that accumulate to significant concentrations for improving human immunity through a range of biological activities such as scavenging of free radicals (Singh *et al.,* 2020). In plants, the role of phytochemicals is unlike primary metabolites which are directly involved in essential processes such as growth and development. However, the role of phytochemicals focuses on improving the defense mechanisms of the plant against abiotic and biotic stresses such as drought, salinity, excessive heat, virus infestation, nematodes infestation, pathogenic bacteria and fungi infestation, and insect pests (Piasecka *et al.,* 2019). Furthermore, phytochemicals contributes to colour, aroma, and signaling within the plant (Crozier *et al.,* 2016).

In this study, application of metabolites profiling were used to elucidate the response of Moringa crops grown under an abiotic stress of chilling conditions and protected growth environments (40% shade net and Coolaroo frost cloth). Moringa (*Moringa oleifera* Lam.) is a tropical to subtropical crop which tend to wither its leaves in cold winter due to the development of a dormant condition (Manduwa *et al.,* 2019). Yet, the demand for its fresh leaves which are commonly used as a nutraceutical ingredient for preparation of smoothies or vegetables becomes unsatisfied and ultimately exposes some populations to the consumption of unbalanced diets. According to Amaglo *et al*. (2010), Moringa is rich in a diverse range of phytochemicals, including phenolic acids and flavonoids like glucosides, rutinosides, malonylglucosides, and trace amounts of acetylglucosides derived from kaempferol, quercetin, and isorhamnetin. Moringa also contains a unique type of glucosinolates that are broken down by the enzyme myrosinase, primarily leading to the formation of isothiocyanates and nitriles. These substances can subsequently be metabolized into different derivatives, including nitrile, thiocarbamate, and carbamate compounds (Leuck and Kunz, 1998).

Moringa is recognized for its wide range of phytochemical properties, including analgesic, anti-inflammatory, antipyretic, anticancer, antioxidant, nootropic, hepatoprotective, gastroprotective, anti-ulcer, cardiovascular, anti-obesity, antiepileptic, antiasthmatic, antidiabetic, anti-urolithiatic, diuretic, local anesthetic, anti-allergic, anthelmintic, wound-healing, antimicrobial, immunomodulatory, and antidiarrheal activities (Gupta *et al.,* 2020). Although research on Moringa's potential as a nutraceutical plant to improve human health is available (Leone *et al.,* 2015) and the application of its extract to encourage the development of other crops, such as quinoa (Rady *et al.,* 2015), critical information about improving its productivity has been overlooked. Several studies have explored various phytochemicals present in different parts of Moringa (Anwar *et al*., 2007; Siddhuraju and Becker, 2003), as well as the effects of spacing, harvesting frequency, and melatonin on Moringa's growth and yield (Amaglo *et al.*, 2006). However, this work was undertaken during summer warm season when the weather is optimum for Moringa growth. Application of protection environments such as shade nets and Coolaroo frost net have been used in the commercial sector of agriculture to protect crops against frost and other hazards such as hail occurrence.

Santana *et al.* (2012) demonstrated that shade nets can significantly alter plant physiology and biochemistry by modifying light availability and spectral quality, which in turn affects plant metabolic processes. Likewise, Stamps (2009) noted that the alterations in plant physiology resulting from the application of shade nets can directly influence the production of secondary metabolites. For instance, Ramphinwa *et al.* (2022) found that bush tea plants grown under an 80% white shade net accumulated higher levels of chlorogenic acids (CGAs) compared to those cultivated under 80% black or green shade nets or in direct sunlight. This increase in CGAs is likely due to the lower temperatures and reduced light intensities under the white shade net, which may have allowed for better light penetration and consequently, greater accumulation of chlorogenic acids. However, there is limited data on the effects of Coolaroo frost cloth on plant phytochemical profiles, making this study one of the few to explore its influence in this area. The objective of the study was to investigate the effect of a white 40% shade net and Coolaroo frost cloth with intensities (closed sides, closed top and closed top and sides) on the phytochemical profiles of *Moringa oleifera* trees during winter season.

4.2. Materials and methods

4.2.1 Study site

The study was carried out at the location detailed in section 3.2.1.

4.2.2 Treatments and research design

Seven treatments were arranged in a split-plot design using a two-way factorial, with each treatment replicated three times (7 treatments x 3 replications), the treatments are as follows:

- 40% shade covering netting structure with open sides
- Coolaroo Frost Cloth covering netting structure with open sides
- 40% shade net boxed netting structure with open top
- Coolaroo Frost Cloth boxed netting structure with open top
- 40% shade net boxed netting structure with closed top and sides
- Coolaroo Frost Cloth boxed netting structure with closed top and sides
- Control with no netting structure

Each plot consisted of six plants.

4.2.3 Procedure

Fresh leaves, twigs, and roots were gathered from an experiment carried out at the Green Biotechnology Research Center of Excellence (GBRCE) at the University of Limpopo, South Africa. The shoot samples of *Moringa oleifera*, comprising leaves, roots, and twigs, were collected, dried in an oven at 52 °C, and finely ground into powder for extract preparation.

4.2.4 Extraction of untargeted metabolites profiling

To extract *Moringa oleifera* samples for UHPLC-MS-QTOF untargeted metabolite profiling, 200 mg of dried Moringa was ground with 2 mL of a methanol:HCl:distilled water solution (80:0.5:19.5 v/v/v), maintaining a 1:10 sample-to-solvent ratio. The mixture was then incubated in a thermostatic shaking water bath at 70°C for 15 7uminutes, following the method established by Mpai *et al*. (2018) without alterations, as detailed by Taia *et al.* (2021). A Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system equipped with a PDA detector (Waters, Milford, MA, USA) was used to analyze the metabolites of *Moringa oleifera*. The system included an Acquity UPLC® HSS C18 column (150 mm × 2.1 mm, 1.8 µm particle size, Waters), utilizing water with 0.1% formic acid as mobile phase A and acetonitrile as mobile phase B. The samples were analyzed at the Central Analytical Facility (CAF) at Stellenbosch University. Initial tests were conducted to optimize chromatographic conditions for improved resolution and shorter analysis times. Gradient elution was employed with the following conditions: starting at 95% A and 5% B, transitioning to 85% A and 15% B over one minute, reaching 5% A and 95% B by fifteen minutes, followed by returning to initial conditions. The injection volume was 2.0 µL (full-loop injection), with a flow rate of 0.3 mL min^{-1} at a temperature of 30 °C. The identical column, elution gradient, and flow rate were employed for the UPLC-MS study. In the negative ion electrospray mode, nitrogen was utilized as the desolvation gas. The parameters were as follows: capillary voltage: 2500 V, sampling cone voltage: 45 V, extraction cone voltage: 4 V, desolvation gas flow: 500 L/h, source temperature: 100 °C, and desolvation temperature: 350 °C. Software called Masslynx 4.1 was used to process the chromatographic results. Mass accuracy and repeatability were ensured by centroiding data in the *m/z* 100–1500 range and utilizing independent reference lock-mass ions with the Lock SprayTM interface. Each chromatographic operation was conducted in triplicate at room temperature. The compounds were quantified by comparing them to a calibration curve created using a series of quercetin standards from ranging from 0.5 to 100 mg/L. Tentative compound identifications were performed using an internet database and relevant literature (HurtadoFernandez *et al.,* 2011).

4.2.5 Statistical analysis

Untargeted metabolite data from leaves, roots, and twigs were analyzed using a factorial design and completely randomized setup through MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) 1,2 SWATH-MS/MS, and DIA-MS. MS-DIAL enables data-independent MS/MS deconvolution for a comprehensive metabolome assessment, as described in Nature Methods, 12, 523–526 (2015). The identification of metabolites involves integrating metabolome databases with mass spectrometry cheminformatics, as outlined in Nature Methods, 15, 53-56 (2018). The data were further analyzed using exploratory principal component analysis (PCA).

4.3 Results and discussion

4.3.1 Tentative identification of metabolites identified in *Moringa oleifera* plant tissues (roots, leaves and twigs) cultivated under different shade types and shading intensities.

In this study, metabolites were identified from *Moringa oleifera* tissues (roots, leaves, and twigs). The largest groups of metabolites were flavonoids, followed by phenolic acids, and then other compounds including glycosyl compounds, fatty acids, tannins, lignans, oligosaccharides, and terpenoids were determined (Table 4.1). In the analysis, the retention time, *m/z* ratio of pseudomolecular ion and fragment ions were used to determine for tentative identification Table 4.1. The predominant phenolic acids identified in the *Moringa oleifera* plant tissues (roots, leaves and twigs) were primarily glucoside derivatives, such as salicylic acid beta-D-glucoside, gentesic acid 5-O-glucoside, and O-p-coumaroyl-beta-D-glucose. Salicylic acid beta-D-glucoside was detected at retention time 6.357 min, mass-to-charge ratio (*m/z*) 299.0772, with fragment ion of *m/z*: 299.

Gentesic acid 5-O-glucoside were detected at 6.847 min and *m/z* 315.0721, with fragment ion of *m/z* 315, and O-p-coumaroyl-beta-D-glucose at 10.551 min and *m/z* 325.0929 with fragment ion of 325. Additionally, a peak tentatively identified as paeonovicinoside contributed 48% of the total phenolic acids, detected at retention time 10.762 min and *m/z* 445.1343, with fragment ion at *m/z*: 445. Other phenolic compounds included rhyncoside A, chlorogenic acid, and 3-O-caffeoyl-4-O-methylquinic acid, detected at retention times 7.267 (*m/z* 433.1354), 8.34 (*m/z* 353.0887), and 11.225 (*m/z* 367.1041), with fragment ion at 443, 353 and 367, respectively, contributing to the overall phenolic composition of the samples

The majority of flavonoid compounds identified in the *Moringa oleifera* samples (roots, leaves and twigs) are all part of the flavonol glycoside subgroup, which includes quercetin 3-galactoside, quercetin 3-O-(6''-acetyl-glucoside), quercetin 3-(6''-malonyl-glucoside), quercitrin, quercetin 3-(6''-malonylneohesperidoside) and quercetin 3-glucosyl-(1->2)-galactoside. These compounds are characterized by their structure where a quercetin molecule (a type of flavonol) is bonded to various sugar molecules (such as galactose, glucoside and neohesperidoside) through glycosidic bonds. Flavonol glycosides are commonly found in plants and have been extensively studied for their antioxidant properties and potential therapeutic benefits (Hollman *et al.,* 1992).

For example, quercetin 3-galactoside was detected with a retention time of 16.576 min and a mass-to-charge ratio (*m/z*) of 463.0884, with fragment ion of 463, quercetin 3-O-(6''-acetyl-glucoside) at 17.361 min and *m/z*: 505.0987, with fragment ion of 505, quercetin 3-(6''-malonyl-glucoside) at 17.361 min and *m/z*: 549.0888, with fragment ion at 549, quercetin at 18.223 min and *m/z:* 447.0929, with fragment ion of 447, quercetin 3-(6''-malonylneohesperidoside) at 14.059 min and *m/z* 695.1473, with fragment ion at 695, and quercetin 3-glucosyl-(1->2)-galactoside at rt:14.744 min and *m/z* 625.1403, with fragment ion of 625. Neocarlinoside (rt: 8.82 min and *m/z* 579.1352, with fragment ion of 579), eriodictyol 7-O-sophoroside (rt: 10.963 min and *m/z:* 611.1617 with fragment ion of 611), petiolaroside decaacetate (rt: 11.253 min and *m/z*: 1013.2550, with fragment ion at 1013), pinobanksin 5-[galactosyl-(1->4)-glucoside] (rt: 11.75 min and *m/z:* 595.1657, with fragment ion at 595), astragalin 7-rhamnoside (rt: 13.19 min and *m/z*: 593.1506, with fragment ion at 593), rutin (rt: 16.205 min and *m/z* 609.1468, with fragment ion of 609) are other members of the flavonol glycoside derivative that have been identified. 3-(6-acetylgalactoside) kaempferol 3-(19.292 min and *m/z* 547.1037, with fragment ion of 547, chrysoeriol 7-O-(6''-malonyl-glucoside) identified at rt: 21.642 min and *m/z* 547.1093, with fragment ion of 547, and 6''-O-(3-Hydroxy-3-methylglutaroyl) astragalin detected at rt: 19486 min and *m/z* 591.1344, with fragment ion of 591. Other identified flavonoid compounds include afzelechin-(4alpha->8)-afzelechin and [epiafzelechin-(4beta->8)] 2-epiafzelechin, which are classified under the catechin subclass of flavonoids. Additionally, phlorizin, kaempferol 3-alpha-L-arabinopyranoside,5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl), eujambolin, and alangisesquin A were also identified (Table 4.1).

The identified glycosyl compounds in Moringa tissues during the winter season include zizybeoside I, hydroxytyrosol 1-O-glucoside, benzyl beta-primeveroside, phenylethyl primeveroside, and conferoside. These compounds were detected at retention times (rt), mass-to-charge ratios (*m/z*) and fragment ions as follows: zizybeoside I at rt: 10 min with *m/z* 477.1615 and fragment ion of 477, hydroxytyrosol 1-O-glucoside at rt: 11 min with *m/z* 315.1084 and fragment ion of 315, benzyl beta-primeveroside at rt 11.959 min with *m/z* 447.1509 and fragment ion of 447, phenylethyl primeveroside at rt: 14.1441 min with *m/z* 461.1664 and fragment ion at 461, and conferoside at rt: 21.5 min with *m/z* 551.2383 and fragment ion at 551. Other compounds such as fatty acids, oligosaccharides and lignans were also detected in all the treatments.

4.3.2. Chemometric analysis on the UPLC-MS untargeted metabolites of *Moringa oleifera* plant tissues (roots, leaves and twigs) cultivated under different shade types and shading intensities.

There was no clear clustering pattern on the metabolome profile of the *Moringa oleifera* roots grown under the two shade type and shading intensities (Figure 4.1A). This information was advocating to the no significant difference showed by the biplot on Figure

4.1B. The results therefore suggest that the application of shading type and shading intensity did not affect the metabolites profile in the Moringa roots.

On the other hand, 40% shade net with top cover (TCt), and with closed sides (CSt), and samples grown under Coolaroo frost cloth with closed sides (CSt) and closed sides and top (CSTt) presented similar metabolome profile and were grouped at the same cluster on horizontal PC1 (Figure 4.2A). in contrast, samples grown under the 40% shade net and Coolaroo frost cloth with the closed sides share a similar metabolome profile on the horizontal PC2 (Figure 4.2A). The component of variation within this two clusters were including iridoid, quercetin 3-galactoside, rutin, and astragalin 7-rhamnoside (Figure 4.2B).Therefore, the results showed that both shading types (40% shade net and Coolaroo frost cloth) retained the flavonoids compounds more, when compared to the top cover intensity in the twigs of the Moringa..

Lastly, leaves showed interesting response to the shading types and shading intensities. Four main clusters were observed based on similarities of metabolite. The first cluster grouped samples of the control on the vertical PC 1 (Figure 4.3A) and these sample were predominated by the Iridiod compound as compared to other treatments. The second cluster was observed on the vertical PC 2 and showed that samples grown under the 40% shade net with no cover contained compounds of the rutin, and astragalin 7-rhamnoside as the main makers. Samples grown under the Coolarro frost clost with closed side, top cover, and closed sides and top together with samples grown under the 40% shade net with closed sides were grouped together and showed no significant difference on their metabolites composition. The last cluster contained samples grown under the 40% shade net with closed side and top which exhibited more of the quecetrin and kaempferol 3- (6-acetyl galactoside) (Figure 4.3B).

Table 4.1: Tentative identification of untargeted UHPLC-MS metabolites profiles from *Moringa oleifera's* plant tissues grown under different protected environments.

Retention time (min)	Pseudomolecular ion (*m/z*)	Fragment ions (*m/z*)	Tentative identification	Formula
Phenolic acids				
6.357	299.0772	299.07712:16993 300.08047:2536 301.08383:617	Salicylic acid beta-D-glucoside	$C_{13}H_{16}O_8$
6.847	315.0721	315.07187:16890 316.07522:2727 317.07858:578	Gentesic acid 5-O-glucoside	$C_{13}H_{16}O_9$
7.267	433.1354	433.13538:7123 434.13873:1515 435.14209:346	Rhyncoside A	$C_{18}H_{26}O_{12}$
8.34	353.0887	353.08765:120584 354.091:23014 355.09436:4202	Chlorogenic acid	$C_{16}H_{18}O_9$
9.899	337.0928	337.0928:88366 338.09615:15769 339.09951:2492	3-O-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$
10.762	445.1343	445.13562:12305 446.13897:2570 447.14233:653	Paeonovicinoside	$C_{19}H_{26}O_{12}$
11.225	367.1041	367.1041:69295 368.10745:11537 369.11081:2504	3-O-Caffeoyl-4-O-methylquinic acid	$C_{17}H_{20}O_9$
Flavonoids				
8.82	579.1352	579.13513:13511 580.13848:3994 581.14184:1556	Neocarlinoside	$C_{26}H_{28}O_{15}$
10.859	435.1299	435.12939:36244 436.13274:8668 437.1361:1858	Phlorizin	$C_{21}H_{24}O_{10}$
10.963	611.1617	611.16229:47516 612.16564:14560 613.169:3936	Eriodictyol 7-O-sophoroside	$C_{27}H_{32}O_{16}$
11.253	1013.256	1013.25507:3696 1014.25842:2024 1015.26178:694	petiolaroside decaacetate	$C_{47}H_{50}O_{25}$
11.75	595.1657	595.16669:77007 596.17004:23598 597.1734:5658	Pinobanksin 5-[galactosyl-(1->4)-glucoside]	$C_{27}H_{32}O_{15}$
13.192	593.1506	593.15247:712835 594.15582:228269 595.15918:59318	Astragalin 7-rhamnoside	$C_{27}H_{30}O_{15}$
14.059	695.1473	695.14734:29690 696.15069:10155 697.15405:2606	Quercetin 3-(6''-malonylneohesperidoside)	$C_{30}H_{32}O_{19}$
14.212	545.1451	545.14545:8491 546.1488:3123 547.15216:649	Afzelechin-(4alpha->8)-afzelechin	$C_{30}H_{26}O_{10}$
14.289	507.1874	507.1875:10208 508.19085:3062 509.19421:772	(7'R,8'R)-4,7'-Epoxy-3'-methoxy-4',5,9,9'-lignanetetrol 9'-glucoside	$C_{25}H_{32}O_{11}$
14.643	417.0825	417.0831:4638 418.08645:1038 419.08981:0	Kaempferol 3-alpha-L-arabinopyranoside	$C_{20}H_{18}O_{10}$
14.744	625.1403	625.14111:10528 626.14446:3602 627.14782:938	ercetin 3-glucosyl-(1->2)-galactoside	$C_{27}H_{30}O_{17}$
16.205	609.1468	609.14679:662642 610.15014:212130 611.1535:56081	Rutin	$C_{27}H_{30}O_{16}$
16.456	817.2168	817.21405:3319 818.2174:1881 819.22076:484	[Epiafzelechin-(4beta->8)]2-epiafzelechin	$C_{45}H_{38}O_{15}$

16.576	463.0879	463.08841:778177 464.09176:193834 465.09512:39037	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$
17.361	505.0987	505.09879:884057 506.10214:228984 507.1055:51414	Quercetin 3-O-(6''-acetyl-glucoside)	$C_{23}H_{22}O_{13}$
17.361	549.0888	549.08887:597325 550.09222:166793 551.09558:40694	Quercetin 3-(6''-malonyl-glucoside)	$C_{24}H_{22}O_{15}$
17.628	607.1297	607.13055:151420 608.1339:48426 609.13726:13215	Kaempferol 3-rhamnoside 7-galacturonide	$C_{27}H_{28}O_{16}$
17.829	593.1522	593.15222:683133 594.15557:214828 595.15893:58156	Astragalin 7-rhamnoside	$C_{27}H_{30}O_{15}$
18.233	447.093	447.09296:133042 448.09631:32851 449.09967:6618	Quercitrin	$C_{21}H_{20}O_{11}$
18.908	477.1039	477.10379:65584 478.10714:16224 479.1105:3552	5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)	$C_{22}H_{22}O_{12}$
19.292	489.1038	489.10376:1249326 490.10711:320122 491.11047:72294	Kaempferol 3-(6-acetylgalactoside)	$C_{23}H_{22}O_{12}$
19.486	591.1344	591.13586:222413 592.13921:69841 593.14257:17952	6''-O-(3-Hydroxy-3-methylglutaroyl)astragalin	$C_{27}H_{28}O_{15}$
20.055	519.1144	519.11444:105096 520.11779:28659 521.12115:6712	Eujambolin	$C_{24}H_{24}O_{13}$
21.642	547.109	547.10931:16126 548.11266:4555 549.11602:1033	Chrysoeriol 7-O-(6''-malonyl-glucoside)	$C_{25}H_{24}O_{14}$
22.693	745.2714	745.27216:5802 746.27551:2256 747.27887:832	Alangisesquin A	$C_{37}H_{46}O_{16}$
O-glycosyl compounds				
5.829	415.1462	415.14594:5125 416.14929:1092 417.15265:207	Cellulose, microcrystalline	$C_{14}H_{26}O_{11}$
10	477.1615	477.16092:224384 478.16427:58868 479.16763:12960	Zizybeoside I	$C_{19}H_{28}O_{11}$
11.423	315.1084	315.1088:5485 316.11215:878 317.11551:115	Hydroxytyrosol 1-O-glucoside	$C_{14}H_{20}O_{8}$
11.959	447.1509	447.15054:12096 448.15389:2721 449.15725:572	Benzyl beta-primeveroside	$C_{18}H_{26}O_{10}$
14.441	461.1664	461.16644:22908 462.16979:5170 463.17315:1206	Phenylethyl primeveroside	$C_{19}H_{28}O_{10}$
21.5	557.2383	557.23779:3782 558.24114:1251 559.2445:246	Conferoside	$C_{30}H_{38}O_{10}$
Oligosaccharides				
11.977	449.2027	449.20251:34713 450.20586:8220 451.20922:2314	MINEs-338436	$C_{20}H_{34}O_{11}$
Lignan				
12.55	537.1973	537.19757:22665 538.20092:7259 539.20428:1572	Citrusin A	$C_{26}H_{34}O_{12}$

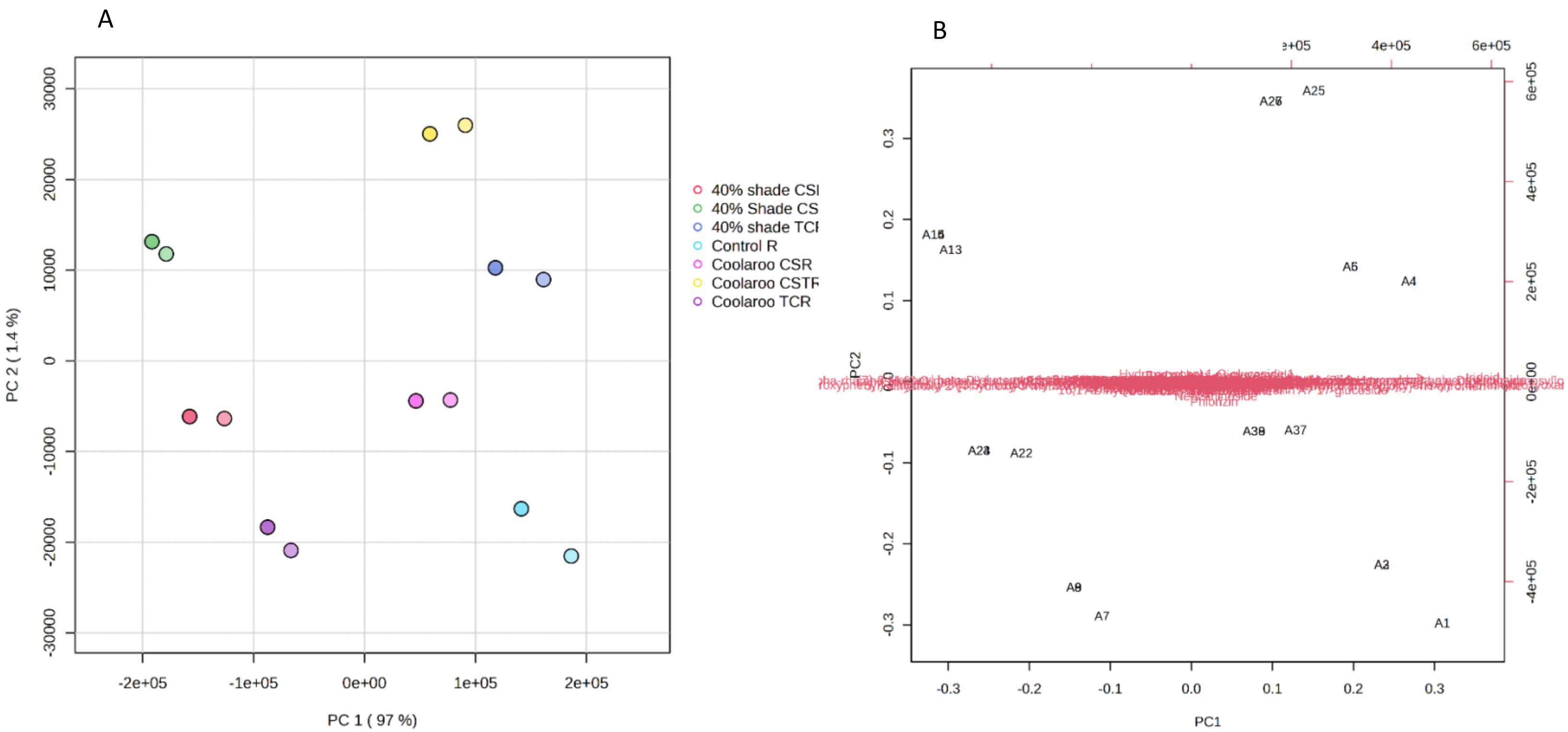

Figure: 4.1: Effect of shading type and shading intensity of the UPLC-MS untargeted metabolites on *Moringa oleifera* roots. The data was mean-cantered and Pareto scaled. The calculated Hoteling's T2 with a 95% confidence interval is represented by the ellipses present in each PCA scores plot. The datasets used to compute these models consisted of 124 features. A= PCA plot, B=biplot

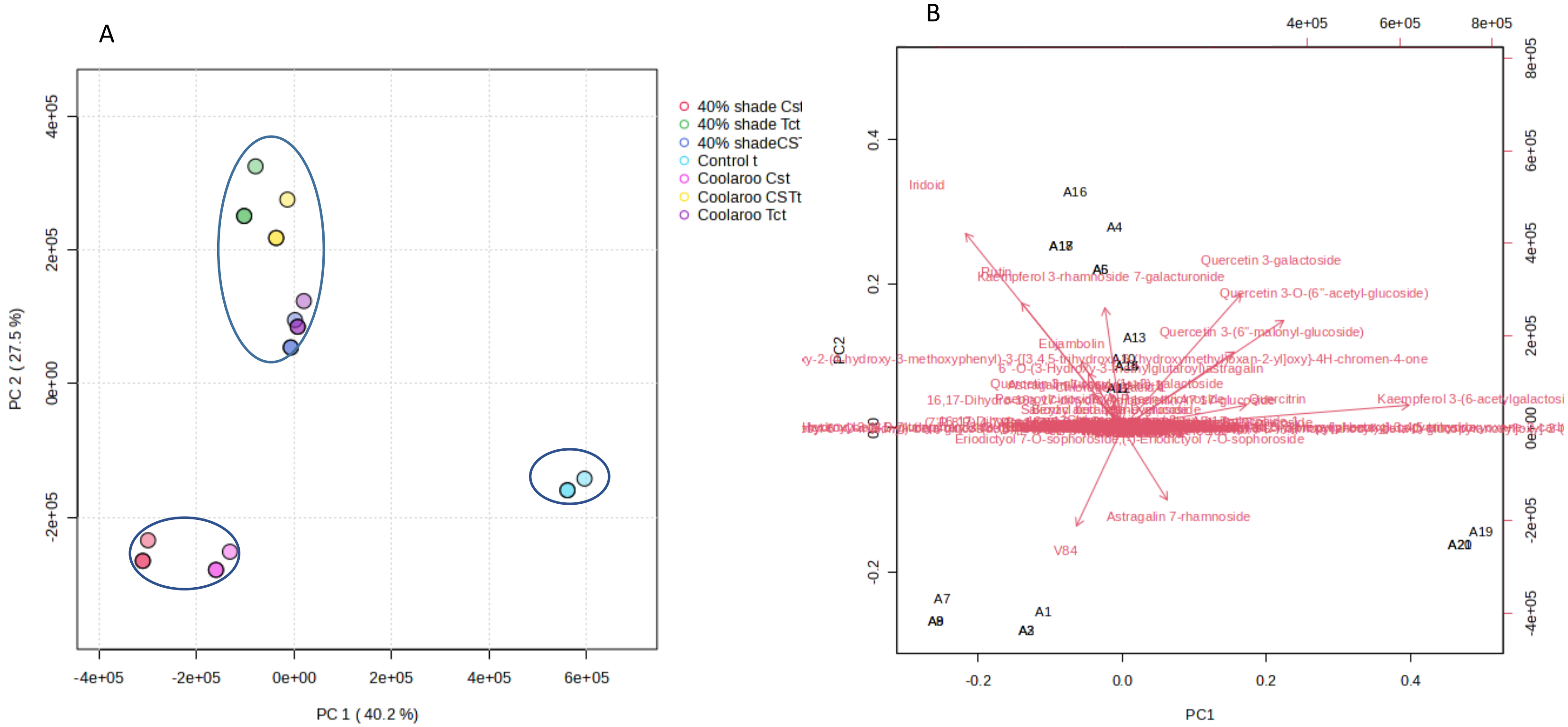

Figure: 4.2: Effect of shading type and shading intensity of the UPLC-MS untargeted metabolites on *Moringa oleifera* twigs. The data was mean-cantered and Pareto scaled. The calculated Hoteling's T2 with a 95% confidence interval is represented by the ellipses present in each PCA scores plot. The datasets used to compute these models consisted of 124 features. A= PCA plot, B=biplot

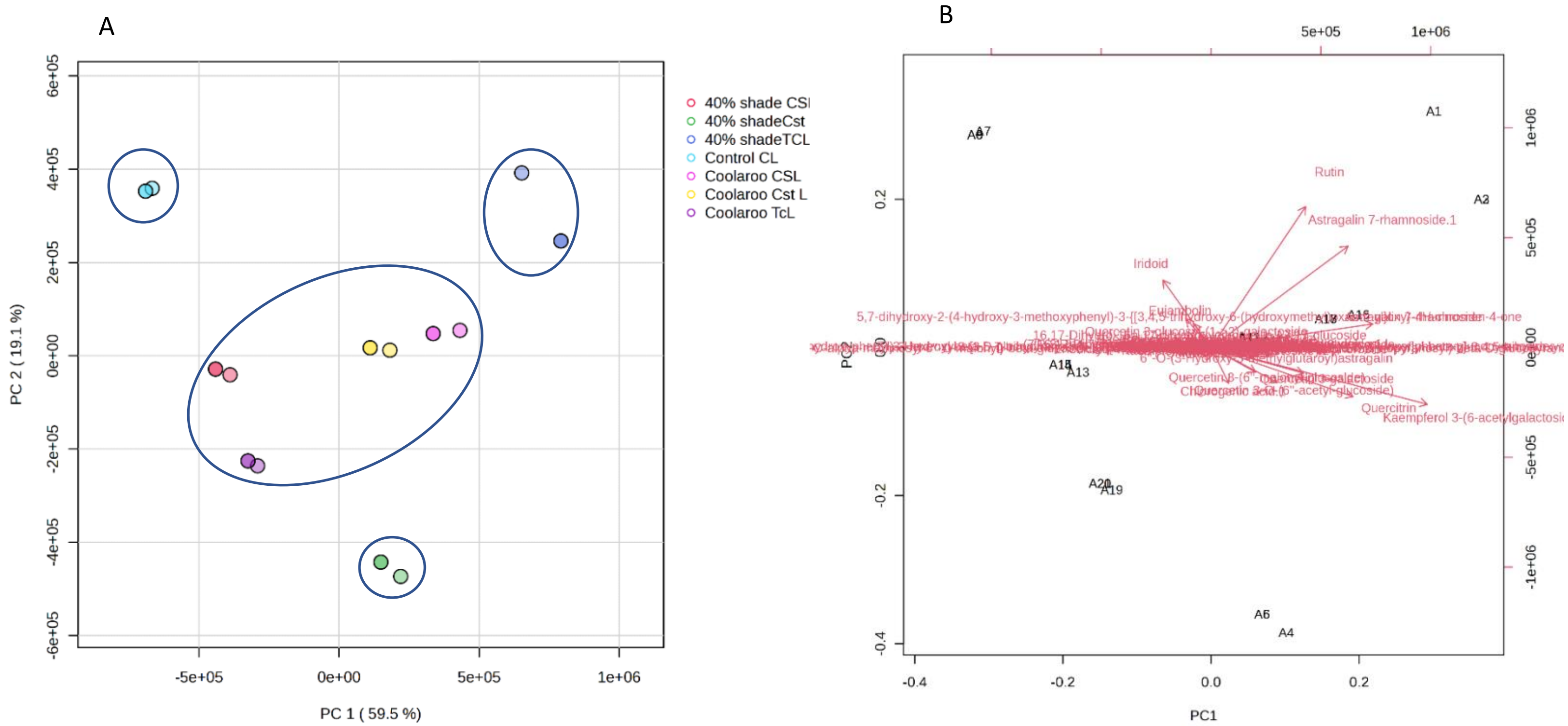

Figure: 4.3: Effect of shading type and shading intensity of the UPLC-MS untargeted metabolites on *Moringa oleifera* leaves. The data was mean-cantered and Pareto scaled. The calculated Hoteling's T2 with a 95% confidence interval is represented by the ellipses present in each PCA scores plot. The datasets used to compute these models consisted of 124 features. A= PCA plot, B=biplot.

4.3.3. Interaction between shading type and shading intensity on the some metabolites in *Moringa oleifera* leaves

There was a significant difference ($p \leq 0.05$) between shading type (40% shade net and Coolaroo frost cloth) and shading intensities (closed sides, closed top and closed top and sides) on the contents of the metabolites found in the roots, twigs, leaves of *Moringa oleifera* as shown in Table 4.2.

Among the phenolic compounds, leaves grown under Coolaroo frost cloth with a top cover exhibited the highest contents of 3-O-p-coumaroylquinic acid (121.32 mg/kg), salicylic acid beta-D-glucoside (80.83 mg/kg), and rhyncoside (14.62 mg/kg). In contrast, leaves grown under control conditions recorded the highest contents of paeonovicinoside (55.39 mg/kg) and gentesic acid 5-O-glucoside (41.85 mg/kg). Notably, chlorogenic acid reached its peak contents (653.90 mg/kg) under a 40% shade net with closed sides. All detected phenolic compounds showed the lowest concentrations under Coolaroo frost cloth with closed sides (Table 4.2). Among the flavonoids, quercetin 3-O-(6''-acetyl-glucoside), quercetin 3-galactoside, and astragalin 7-rhamnoside had the highest contents irrespective of shading type and shading intensity. For example, quercetin 3-O-(6''-acetyl-glucoside) and quercetin 3-galactoside showed the highest contents in leaves grown under a 40% shade net with closed sides, recording 2643.1 mg/kg and 1990.1 mg/kg, respectively (Table 4.2). Conversely, astragalin 7-rhamnoside had the highest contents in leaves grown under Coolaroo frost cloth with closed top and sides (1136.1 mg/kg). On the other hand, neocarlinoside, petiolaroside decaacetate, and kaempferol 3-alpha-L-arabinopyranosid had the lowest overall contents.

Neocarlinoside was not detected in multiple treatments (40% shade net with closed sides, 40% shade net with closed top and sides, Coolaroo frost cloth with closed sides, Coolaroo frost cloth with closed top and sides). Petiolaroside decaacetate was only detected in leaves grown under Coolaroo frost cloth with a top cover (0.26 mg/kg), indicating a very low concentration. Similarly, kaempferol 3-alpha-L-arabinopyranosid was not detected in the majority of treatments (control, 40% shade net with top cover, 40% shade net with closed sides, 40% shade net with closed top and sides, Coolaroo frost cloth with closed sides, Coolaroo frost cloth with closed top and sides).

Among the glycosyl compounds, leaves grown under Coolaroo frost cloth with a top cover showed the highest concentrations of benzyl-beta-primeveroside (62.02 mg/kg), followed by hydroxytyrosol 1-O-glucoside (16.33 mg/kg), cellulose microcrystalline (8.21 mg/kg), and conferoside (6.69 mg/kg) during winter (Table 4.2). Leaves grown under Coolaroo frost cloth with closed top and sides had the highest concentrations of zizybeoside I (415.59 mg/kg) and phenylethyl primeveroside (33.78 mg/kg). In contrast, leaves grown under Coolaroo frost cloth with closed sides recorded the lowest concentrations for all detected glycosyl compounds, with cellulose microcrystalline at 0.35 mg/kg, conferoside at 0.53 mg/kg, hydroxytyrosol 1-O-glucoside at 3.79 mg/kg, benzyl-beta-primeveroside at 5.92 mg/kg, phenylethyl primeveroside at 8.46 mg/kg, and zizybeoside I at 75.34 mg/kg.

Among the phenolic compounds, twigs grown under Coolaroo frost cloth with closed sides had the highest concentrations of chlorogenic acid (218.19 mg/kg), salicylic acid beta-D-glucoside (85.29 mg/kg), and rhyncoside (17.52 mg/kg) (Table 4.2). Conversely, twigs grown under Coolaroo frost cloth with a top cover had the highest concentrations of paeonovicinoside (86.34 mg/kg), 3-O-p-coumaroylquinic acid (84.17 mg/kg), 3-O-caffeoyl-4-O-methylquinic acid (81.03 mg/kg), and gentesic acid 5-O-glucoside (25.17 mg/kg). Notably, twigs grown under Coolaroo frost cloth with closed sides recorded the lowest concentration of gentesic acid 5-O-glucoside (9.17 mg/kg). In contrast, the Coolaroo frost cloth with a top cover was more effective during winter for increasing the concents of paeonovicinoside, 3-O-p-coumaroylquinic acid, 3-O-caffeoyl-4-O-methylquinic acid, and gentesic acid 5-O-glucoside in the twigs.

Among the flavonoids, twigs grown under Coolaroo frost cloth with a top cover exhibited the highest contents of quercetin 3-O-(6''-acetyl-glucoside) (2023.0 mg/kg), followed by quercetin 3-galactoside (1522.7 mg/kg) and astragalin 7-rhamnoside (899.67 mg/kg) (Table 4.2). Similar to the results in leaves, neocarlinoside, petiolaroside decaacetate, and kaempferol 3-alpha-L-arabinopyranosid were found in the lowest concentrations overall.

Among the glycosyl componds, twigs grown under Coolaroo frost cloth with closed sides and top exhibited the highest concentrations of zizybeoside I (471.03 mg/kg), followed by benzyleta-primeveroside (74.69 mg/kg), hydroxytyrosol 1-O-glucoside (16.33 mg/kg), and conferoside (7 mg/kg). Conversely, phenylethyl primeveroside, cellulose microcrystalline, and conferoside achieved their highest concentrations in twigs grown under a 40% shade net with closed sides, recording 40.24 mg/kg, 11.34 mg/kg, and 6.79 mg/kg, respectively (Table 4.3). The lowest concentrations for cellulose microcrystalline and conferoside were observed in other treatments, recording 1.02 mg/kg and 0.65 mg/kg, respectively.

Among the phenolic compounds, roots grown under Coolaroo frost cloth with a top cover exhibited the highest contents of phenolic acids compounds including: paeonovicinoside (62.59 mg/kg), salicylic acid beta-D-glucoside (36.78 mg/kg), 3-O-p-coumaroylquinic acid (6.42 mg/kg), rhyncoside (5.71 mg/kg), and 3-O-caffeoyl-4-O-methylquinic acid (2.96 mg/kg). Conversely, roots grown under the Coolaroo closed sides, showed lower concentrations of these compounds: paeonovicinoside (21.97 mg/kg), rhyncoside (1.47 mg/kg), 3-O-p-coumaroylquinic acid (1.20 mg/kg), 3-O-caffeoyl-4-O-methylquinic acid (0.52 mg/kg), and chlorogenic acid (0.43 mg/kg) (table 4.1). Futhermore, gentesic acid 5-O-glucoside reached its highest contents under control conditions (10.57 mg/kg), while the lowest contents was observed under a 40% shade net with closed top and sides (1.74 mg/kg).

Among the flavonoids, roots grown under a 40% shade net with closed sides exhibited the highest contents of quercetin 3-O-(6''-acetyl-glucoside) and quercetin 3-(6''-malonyl-glucoside), recording 65.54 mg/kg and 45.19 mg/kg, respectively. In contrast, the lowest contents were observed for eriodictyol 7-O-sophoroside and alangisesquin A, both recording 0.01 mg/kg (Table 4.1). Eriodictyol 7-O-sophoroside was found at this low level under Coolaroo top cover, Coolaroo with closed top and sides, and control conditions, while alangisesquin A was recorded at this low level under control and Coolaroo with closed top and sides conditions. The significantly higher contents of quercetin 3-O-(6''-acetyl-glucoside) and quercetin 3-(6''-malonyl-glucoside) in roots grown under 40% shade net with closed sides suggest that the shade net environment creates optimal conditions for the synthesis or retention of these compounds (Table 4.1). The regulated light exposure and possibly moderated temperature provided by the closed sides could be contributing factors to these elevated levels. On the other hand, the consistently low concentrations of eriodictyol 7-O-sophoroside and alangisesquin A across various treatments, including Coolaroo top cover, Coolaroo with closed top and sides and control, indicate that these compounds are either not prominently synthesized or are quickly degraded in these environments.

Among the glycosyl compounds, roots grown under Coolaroo with a top cover showed the highest contents of zizybeoside I (95.28 mg/kg), followed by benzyl-beta-primeveroside (54.24 mg/kg), cellulose microcrystalline (35.45 mg/kg), phenylethyl primeveroside (14.44 mg/kg), and conferoside (3.47 mg/kg) (Table 4.1). In contrast, the lowest contents were found in Coolaroo with closed sides (conferoside at 1.08 mg/kg) and in the 40% shade net with closed top and sides (cellulose microcrystalline at 0.64 mg/kg). Additionally, roots grown under 40% shade net with a top cover had the highest contents of hydroxytyrosol 1-O-glucoside (30.26 mg/kg), while the lowest contents was observed in Coolaroo with closed sides (15.67 mg/kg). The higher contents of glycosyl compounds in roots grown under Coolaroo with a top cover could be due to environmental factors such as light intensity, temperature regulation, and humidity levels provided by the Coolaroo frost cloth with a top cover. On the other hand, the lowest concentrations of these compounds were found in conditions with more restricted airflow and light, such as Coolaroo with closed sides and 40% shade net with closed top and sides.

Discussion

The results showed that *Moringa oleifera* (leaves, roots, and twigs) is a rich source of flavonoid metabolites and flavonol glycosides, which were the most predominant metabolites compared to other compounds (Tables 4.1–4.3). These findings align with the study by Makita *et al.* (2016), which reported that *Moringa oleifera* contained higher levels of flavonoids than *Moringa ovalifolia*.

Several studies have suggested that *Moringa oleifera* does not produce rutin, a highly bioavailable flavonoid. However, in this study, rutin was detected in the roots, twigs, and leaves of *Moringa oleifera* across all treatments, as shown in Tables 4.2, 4.3 and 4.4. The highest concentration of rutin was found in leaves grown under a 40% shade net with a top cover, measuring 799.67 mg/kg (Table 4.2). Rutin is well known as one of the most powerful natural antioxidants, offering a broad range of pharmacological activities, including antibacterial, anti-inflammatory, antiallergic, antiviral, antiprotozoal, and antitumor properties. It also has cytoprotective, vasoactive, antiplatelet, hypolipidemic, and antihypertensive effects (Patel and Patel, 2019). Our findings contradict earlier studies, such as those by Habtemariam *et al.* (2016), which reported that *Moringa oleifera* does not produce the bioavailable flavonoid rutin. However, they are consistent with a follow up study by Makita *et al.* (2017), which compared 12 cultivars of *Moringa oleifera* and found that some cultivars contained rutin.

The results demonstrated that leaves, roots, and twigs grown under 40% shade with closed sides had higher flavonoid content (Table 4.2-4.4). This could be attributed to the fact that the closed sides allow the plants to still receive some solar radiation. Although shaded, the partial exposure to sunlight may be sufficient to stimulate flavonoid production. According to reviewed literature (Silva-Beltrán *et al.,* 2015; Toor *et al.,* 2005), an increase in flavonoids, particularly rutin, is largely driven by exposure to intense solar radiation, which is in line with the results obtained in this experiment.

The Coolaroo frost cloth with a top cover significantly increased the content of glycosyl compounds, oligosaccharides, and lignans across various plant parts, including leaves, twigs, and roots. During winter, the Coolaroo frost cloth with top cover was particularly effective in boosting concentrations of compounds like paeonovicinoside, 3-O-p-coumaroylquinic acid, 3-O-caffeoyl-4-O-methylquinic acid, and gentesic acid 5-O-glucoside in twigs. The benefits likely stem from improved light penetration and favorable temperature control under the top cover, which creates an optimal environment for these compounds in colder months.

This finding differs from the observations made by Karimi *et al.* (2013), who reported that exposing three varieties of *Labisia pumila* Benth to high light intensities increased the levels of phenolic compounds, including gallic acid, caffeic acid, and flavonoids such as quercetin, rutin, myricetin, kaempferol, and naringin. In contrast, Abreu *et al.* (2019) found, consistent with the current study, that shading led to increased phenolic content in tomato fruits. In that study, control conditions with high direct solar radiation caused greater phenolic accumulation in the leaves, likely because the leaves were directly exposed to the light stressor. Additionally, the observed increase in flavonoids may not only result from reduced light exposure but potentially other regulatory factors as well. The comparison of results from *Moringa oleifera* with those from other plant species under shade net conditions is necessitated by the limited availability of specific research on Moringa's response to such environments.

Table 4.2 Identification and quantification of metabolites identified in *Moringa oleifera* roots grown 40% shade net and Coolaroo frost cloth.

	Control	40% top cover	40% closed sides	40% closed top and sides	Coolaroo top cover	Coolaroo closed sides	Coolaroo closed top and sides
Salicylic acid beta-D-glucoside	27.88±0.92b	20.61±0.39d	26.31±1.56c	8.90±0.39g	36.78±0.83a	10.81±0.49f	15.83±0.69e
Gentesic acid 5-O-glucoside	10.57±0.62a	5.16±0.17d	9.53±0.57b	1.74±0.17g	7.69±0.47c	3.06±0.16f	4.36±0.25c
Rhyncoside A	2.40±0.16c	3.24±0.56b	5.50±0.46a	1.60±0.06d	5.71±0.23a	1.47±0.05d	2.22±0.11c
Chlorogenic acid	0.59±0.02d	0.89±0.05d	7.19±0.37a	0.59±0.03d	3.91±0.04b	1.00±0.07c	0.43±0.02e
3-O-p-Coumaroylquinic acid	47.27±2.13c	48.82±1.21b	38.99±2.24d	18.83±0.35g	62.59±1.70a	21.97±0.49f	35.77±1.26e
Paeonovicinoside	1.57±0.01b	1.51±0.03b	2.90±0.01a	0.76±0.03c	2.96±0.14a	0.52±0.02c	0.81±0.02c
3-O-Caffeoyl-4-O-methylquinic acid	3.06±0.11e	3.14±0.09c	6.14±0.37b	2.02±0.02f	6.42±0.26a	1.20±0.06g	1.91±0.03f
Flavonoids	Flavonoids						
Neocarlinoside	46.27±2.38b	40.79±1.30c	39.66±2.40c	12.43±0.10f	64.11±1.31a	26.87±0.74d	16.28±0.50e
Phlorizin	75.87±4.48b	52.82±1.80c	108.70±4.15a	42.57±1.98d	109.50±5.49a	37.42±1.04e	33.09±1.02f
Eriodictyol 7-O-sophoroside	0.04±0.01c	0.09±0.01c	0.71±0.06b	0.49±0.02c	0.01±0.01c	0.91±0.01a	0.05±0.02c
petiolaroside decaacetate	10.50±0.49b	9.94±0.19c	10.94±0.55b	3.59±0.09e	13.97±0.52a	7.19±0.19d	3.57±0.11e
Pinobanksin 5-[galactosyl-(1->4)-glucoside]	1.45±0.09b	1.42±0.02b	2.15±0.05a	0.64±0.04d	2.49±0.05a	0.78±0.01d	1.02±0.01c
Astragalin 7-rhamnoside	7.43±0.19d	15.9±0.16b	18.44±0.88a	9.23±0.10c	18.97±0.43a	6.45±0.14d	6.69±0.22d
Quercetin 3-(6''-malonylneohesperidoside)	ND	ND	0.19±0.02a	ND	0.06±0.04a	ND	ND
Afzelechin-(4alpha->8)-afzelechin	14.50±0.09c	16.82±0.10b	26.17±0.26a	6.87±0.11d	25.88±0.27a	7.19±0.03d	5.99±0.05e
(7'R,8'R)-4,7'-Epoxy-3'-methoxy-4',5,9,9'-lignanetetrol 9'-glucoside	9.84±0.49b	7.33±0.29c	9.99±0.47b	3.65±0.09e	10.35±0.29a	3.29±0.09e	5.31±0.07d
Kaempferol 3-alpha-L-arabinopyranoside	5.05±0.14b	4.78±0.07c	3.86±0.03d	1.05±0.09g	6.04±0.15a	3.31±0.07e	1.86±0.06f
ercetin 3-glucosyl-(1->2)-galactoside	2.04±0.05b	0.31±0.01c	3.16±0.22b	0.39±0.01c	11.21±0.18a	0.23±0.01c	0.49±0.01c
Rutin	3.68±0.18b	4.83±0.55b	16.81±0.95a	2.39±0.09c	1.34±0.02d	1.13±0.03d	0.65±0.03e

[Epiafzelechin-(4beta->8)]2-epiafzelechin	3.87±0.19b	0.42±0.13e	5.91±0.02a	1.41±0.07d	5.94±0.14a	1.88±0.06c	1.45±0.01d
Quercetin 3-galactoside	8.39±0.09c	5.69±0.90d	55.02±1.27a	6.42±0.13d	52.41±1.34b	6.52±0.17d	5.85±0.03d
Quercetin 3-O-(6''-acetyl-glucoside)	4.53±0.01d	3.92±0.05d	65.54±2.51a	6.05±6.30c	34.12±0.57b	7.53±0.23c	5.19±0.26c
Quercetin 3-(6''-malonyl-glucoside)	3.06±0.09c	2.69±0.01c	45.19±1.83a	4.03±4.11c	23.11±0.05b	5.20±0.08c	3.51±0.09c
Kaempferol 3-rhamnoside 7-galacturonide	4.19±0.07c	1.34±0.01d	16.24±0.81a	2.06±0.36d	12.55±0.36b	1.71±0.10d	1.79±0.03d
Astragalin 7-rhamnoside	2.61±0.12c	5.15±0.07b	8.18±0.42a	0.65±0.02d	0.18±0.06d	0.05±0.01d	0.73±0.05d
Quercitrin	6.58±0.09b	7.03±0.18b	17.79±0.72a	4.39±0.11c	16.08±0.30a	3.66±0.23c	5.71±0.14b
5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)	11.64±0.69c	8.24±0.24e	18.81±1.09b	5.20±0.14f	42.73±1.28a	5.06±0.12f	9.97±0.37d
Kaempferol 3-(6-acetylgalactoside)	2.47±0.01c	12.1±0.05b	28.69±1.54a	4.75±0.23c	11.36±0.26b	2.87±0.15c	4.44±0.21c
6''-O-(3-Hydroxy-3-methylglutaroyl)astragalin	5.55±0.25b	2.47±0.02c	6.53±0.23b	8.04±0.02a	10.16±0.46a	1.42±0.05c	3.33±0.18c
Eujambolin	11.34±0.69c	8.54±0.33c	22.45±1.26b	4.89±0.08m	35.15±1.63a	4.18±0.13d	9.39±0.19c
Chrysoeriol 7-O-(6''-malonyl-glucoside)	ND	ND	0.14±0.14a	ND	ND	ND	ND
Alangisesquin A	0.01±0.01a	ND	ND	ND	ND	ND	0.01±0.01a
O-glycosyl compounds	O-glycosyl compounds						
Cellulose, microcrystalline	26.04±1.09b	4.59±0.11e	9.12±0.55c	0.64±0.02g	35.45±1.07a	2.45±0.06f	8.35±0.42d
Zizybeoside I	90.29±4.81b	69.18±2.53d	59.59±3.99e	41.51±0.69f	95.28±3.80a	42.03±0.73f	81.99±1.42c
Hydroxytyrosol 1-O-glucoside	24.80±0.01c	30.26±0.04a	26.33±1.07b	18.89±0.21e	23.21±0.13d	15.67±0.43f	25.10±0.59c
Benzyl beta-primeveroside	50.59±1.97b	51.61±1.09b	51.69±3.49b	30.08±0.46d	54.24±0.13a	29.20±1.01e	43.43±1.09c
Phenylethyl primeveroside	12.48±0.54b	10.82±0.28c	12.24±0.70b	4.70±0.23e	14.44±0.28a	6.51±0.22d	6.63±0.30d
Conferoside	2.59±0.03c	2.26±0.06cd	2.78±0.18b	1.19±0.05d	3.47±0.28a	1.08±0.08f	1.59±0.08e
Oligosaccharides	Oligosaccharides						
MINEs-338436	0.07±0.07a	0.07±0.03a	0.17±0.11a	0.04±0.06a	0.06±0.06a	0.04±0.02a	0.06±0.02a
Lignan	Lignan						
Citrusin A	0.17±0.06a	0.15±0.04a	0.21±0.04a	ND	0.04±0.04a	0.03±0.01a	0.16±0.02a

Values are presented as means ± standard error. Columns with different letters (a-g) indicate significant differences ($p \leq 0.05$). ND stands for Not Detected.

Table 4.3: Identification and quantification of metabolites identified in *Moringa oleifera* leaves grown 40% shade net and Coolaroo frost cloth.

	Control	40% top cover	40% closed sides	40% closed top and sides	Coolaroo top cover	Coolaroo closed sides	Coolaroo closed top and sides
Salicylic acid beta-D-glucoside	59.67±2.02b	22.14±0.63c	17.23±0.79e	19.66±0.63d	80.83±2.71a	8.29±0.48f	17.74±0.65e
Gentesic acid 5-O-glucoside	41.85±1.06a	20.31±0.56e	25.26±1.51c	36.01±0.62b	19.37±0.86f	9.76±0.48g	23.57±0.42d
Rhyncoside A	6.92±0.36b	0.56±0.01c	ND	0.11±0.02d	14.62±0.91a	0.05±0.01e	0.07±0.01e
Chlorogenic acid	79.92±1.35e	145.61±3.43d	653.90±21.85a	449.02±9.41b	244.78±6.37c	59.34±2.22f	238.30±7.75c
3-O-p-Coumaroylquinic acid	55.39±1.59a	15.12±0.09c	11.09±0.74de	12.35±0.57d	51.27±1.65b	2.62±0.15f	16.16±0.36c
Paeonovicinoside	26.18±0.43e	82.35±3.29b	89.58±4.04a	81.09±2.31b	37.94±1.17c	23.07±1.12e	82.45±2.41b
3-O-Caffeoyl-4-O-methylquinic acid	43.68±1.25f	105.91±3.30b	98.65±4.25c	78.97±2.19d	75.03±2.70fe	19.97±1.10g	121.32±3.13a
Flavonoids	Flavonoids						
Neocarlinoside	0.43±0.01b	0.11±0.02b	ND	ND	1.50±0.14a	ND	ND
Phlorizin	0.38±0.01b	0.37±0.02b	ND	0.10±0.10b	3.32±0.06a	ND	ND
Eriodictyol 7-O-sophoroside	19.72±0.78e	56.63±2.09c	94.86±25.81a	62.47±3.71b	45.01±2.35d	15.85±0.92f	99.15±3.72a
petiolaroside decaacetate	ND	ND	ND	ND	0.26±0.06a	ND	ND
Pinobanksin 5-[galactosyl-(1->4)-glucoside]	31.56±0.47g	93.63±1.55a	70.18±2.05d	81.22±2.49c	36.88±1.24f	55.88±2.02e	90.64±3.16b
Astragalin 7-rhamnoside	421.52±9.69f	858.17±22.31c	910.33±23.48b	798.21±15.64d	505.63±12.55e	491.35±13.55e	1136.1±26.06a
Quercetin 3-(6''-malonylneohesperidoside)	3.77±0.23f	35.96±0.74a	26.46±0.99c	31.50±0.76b	7.19±0.10e	3.10±0.25g	24.64±1.09d

Afzelechin-(4alpha->8)-afzelechin	0.06±0.01a	0.01±0.01a	ND	0.03±0.03a	0.58±0.01a	ND	ND
(7'R,8'R)-4,7'-Epoxy-3'-methoxy-4',5,9,9'-lignanetetrol 9'-glucoside	16.02±0.48a	0.80±0.01d	1.08±0.08b	1.33±0.07c	16.78±0.39a	0.37±0.04d	1.03±0.12b
Kaempferol 3-alpha-L-arabinopyranoside	ND	ND	ND	ND	0.04±0.04a	ND	ND
ercetin 3-glucosyl-(1->2)-galactoside	119.61±1.67b	12.86±0.15e	17.05±1.1d	11.72±0.33e	163.18±3.07a	22.30±1.03c	2.08±0.13f
Rutin	465.27±6.14d	799.67±19.32a	535.11±16.28c	5.30±0.02f	232.06±3.90d	166.31±10.01e	713.71±20.78b
[Epiafzelechin-(4beta->8)]2-epiafzelechin	ND	0.05±0.01a	ND	ND	0.07±0.01a	ND	ND
Quercetin 3-galactoside	752.30±13.10f	944.41±17.37e	1990.1±46.76a	1294.±11.91c	1232.2±25.37d	414.37±10.12g	1595.0±44.11b
Quercetin 3-O-(6''-acetyl-glucoside)	890.93±19.80f	1057.0±35.65e	2643.1±96.26a	1419.4±35.77d	1499.9±19.22c	419.97±8.71g	1769.7±41.88b
Quercetin 3-(6''-malonyl-glucoside)	620.25±9.74f	725.45±12.81e	1852.5±67.76a	984.24±22.35d	1035.4±18.32c	293.36±9.92g	1223.9±33.92b
Kaempferol 3-rhamnoside 7-galacturonide	242.11±2.68g	182.87±4.28d	141.81±5.04e	329.28±6.04b	437.87±10.95a	56.64±2.28f	315.37±7.51c
Astragalin 7-rhamnoside	67.92±0.73e	831.62±12.69a	462.20±18.39b	0.24±0.02g	52.11±0.89f	134.08±2.88d	400.86±9.80c
Quercitrin	217.13±0.18e	612.49±401.45e	625.83±10.74c	978.70±12.67a	262.14±2.76d	266.75±9.41d	772.56±12.77b
5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)	279.66±1.67b	80.50±0.56f	103.36±3.32c	89.86±1.72e	334.30±6.51a	26.18±0.64g	96.38±2.13d
Kaempferol 3-(6-acetylgalactoside)	382.87±10.99f	1490.4±53.71d	1669.3±50.29a	1602.7±56.75b	535.16±10.77e	317.76±9.16f	1485.9±37.54d
6''-O-(3-Hydroxy-3-methylglutaroyl)astragalin	99.05±1.94e	265.39±9.49b	95.69±3.84e	360.15±7.78a	127.39±3.07d	48.41±1.80f	195.53±6.14c
Eujambolin	373.08±6.51b	124.89±5f	206.31±8.48c	136.48±4.28e	460.45±10.21a	26.84±1.09g	161.84±4.29d
Chrysoeriol 7-O-(6''-malonyl-glucoside)	2.81±0.05e	20.32±0.39a	17.93±0.05c	18.46±0.20b	4.95±0.08d	1.89±0.07e	16.35±0.34c
Alangisesquin A	0.83±0.02f	7.09±0.08a	7.15±0.45a	6.53±0.03c	1.07±0.02e	3.05±0.19d	6.87±0.28b
O-glycosyl compounds	O-glycosyl compounds						
Cellulose, microcrystalline	3.50±0.19b	0.99±0.03d	1.29±0.07c	0.98±0.08d	8.21±0.36a	0.35±0.02e	1.22±0.12c
Zizybeoside I	292.13±5.06b	268.61±8.72c	213.70±6.41e	295.25±11.55b	243.71±3.39d	75.34±2.35e	415.59±10.76a

Hydroxytyrosol 1-O-glucoside	14.68±0.36b	6.64±0.14c	5.38±0.16e	6.87±0.30c	16.33±0.11fa	3.79±0.20f	6.20±0.31d
Benzyl beta-primeveroside	55.65±0.86b	14.63±0.31e	18.94±0.76c	17.37±0.75d	62.02±0.11a	5.92±0.28f	17.97±0.53c
Phenylethyl primeveroside	31.26±0.69c	27.91±0.40e	23.72±0.88f	29.31±1.11d	31.83±1.01b	8.46±0.59g	33.78±1.46a
Conferoside	6.02±0.26b	0.69±0.03d	0.67±0.13d	0.73±0.04d	6.89±0.20a	0.53±0.04e	0.86±0.02c
Oligosaccharides	Oligosaccharides						
MINEs-338436	3.72±0.13f	41.78±1.13c	41.42±2.49c	56.84±1.78a	5.58±0.25e	28.37±0.99d	52.10±2.08b
Lignan	Lignan						
Citrusin A	1.79±0.11f	27.25±0.77b	27.39±1.50b	26.22±1.33c	2.38±0.23e	13.46±0.87d	35.44±1.23a

Values are presented as means ± standard error. Columns with different letters (a-g) indicate significant differences ($p \leq 0.05$). ND stands for Not Detected.

Table 4.4: Identification and quantification of metabolites identified in *Moringa oleifera* twigs grown 40% shade net and Coolaroo frost cloth.

	Treatments detection as per treatment (mg/kg)						
	Control	40% top cover (twigs)	40% closed sides (twigs)	40% closed top and sides (twigs)	Coolaroo top cover (roots)	Coolaroo closed sides (twigs)	Coolaroo closed top and sides (twigs)
Salicylic acid beta-D-glucoside	4.73±0.96f	55.24±2.93d	85.29±4.11a	65.39±1.92c	22.62±0.94e	21.86±0.92ef	71.56±2.80b
Gentesic acid 5-O-glucoside	6.63±0.95g	15.57±0.94e	20.77±1.33d	21.93±0.69c	25.17±0.62a	9.17±0.25f	24.42±0.39b
Rhyncoside A	0.80±0.06e	8.83±0.44c	17.52±0.74a	13.22±0.57b	ND	4.57±0.14d	13.33±0.47b
Chlorogenic acid	71.19±3.23f	87.75±3.72e	218.91±4.92a	207.70±6.54b	99.89±0.92d	36.77±1.17g	198.41±7.45c

3-O-p-Coumaroylquinic acid	20.93±2.75e	66.51±1.77b	55.55±2.29d	64.59±2.51c	9.99±0.09g	15.22±0.65f	86.34±1.64a
Paeonovicinoside	1.37±0.71f	30.04±1.46c	30.73±1dc	35.38±0.70b	81.03±1.73a	16.40±0.83e	34.22±1.57bc
3-O-Caffeoyl-4-O-methylquinic acid	9.69±1.31g	74.29±2.96c	48.66±1.72f	76.70±2.00b	84.17±1.33a	63.90±2.64e	69.13±1.15d
Neocarlinoside	2.69±1.31a	0.41±0.03d	0.91±0.01b	0.66±0.01c	0.03±0.03d	0.68±0.02c	1.07±0.07b
Phlorizin	1.63±0.32b	0.12±0.06c	1.56±0.08b	0.72±0.01b	0.19±0.04c	0.90±0.04c	2.03±0.06a
Eriodictyol 7-O-sophoroside	18.63±1.60g	27.17±1.74e	22.40±0.68f	32.12±1.64c	80.71±2.05a	50.25±3.37b	28.55±0.96d
petiolaroside decaacetate	ND	ND	0.02±0.02a	ND	ND	0.12±0.02a	0.08±0.02a
Pinobanksin 5-[galactosyl-(1->4)-glucoside]	41.33±3.76b	34.93±1.59d	27.78±1.39e	31.43±0.77d	96.13±1.04a	20.17±1.02f	39.62±1.39c
Astragalin 7-rhamnoside	318.38±29.15f	414.25±14.29d	350.23±9.70e	493.94±8.49c	899.67±23.46a	557.45±28.73b	408.54±8.32d
Quercetin 3-(6''-malonylneohesperidoside)	18.96±1.67b	4.19±0.21d	3.75±0.02e	4.45±0.09c	41.32±1.21a	0.80±0.02f	4.44±0.09c
Afzelechin-(4alpha->8)-afzelechin	1.56±0.38a	ND	0.06±0.06c	0.09±0.03c	0.05±0.05c	0.04±0.04c	0.45±0.06b
(7'R,8'R)-4,7'-Epoxy-3'-methoxy-4',5,9,9'-lignanetetrol 9'-glucoside	6.89±0.91e	12.77±0.86d	21.28±0.95a	17.29±0.70c	0.56±0.01g	5.53±0.07f	19.19±0.58b
Kaempferol 3-alpha-L-arabinopyranoside	0.71±0.09a	0.08±0.02b	ND	ND	ND	0.01±0.01b	0.04±0.04b
ercetin 3-glucosyl-(1->2)-galactoside	82.92±6.19d	88.48±3.89c	90.93±2.75c	146.43±1.74a	18.61±0.74f	109.04±3.56b	18.06±0.27e
Rutin	117.07±4.90d	707.81±15.28a	354.95±11.44b	25.86±0.55e	26.76±1.08e	201.90±7.89c	349.56±8.55b

[Epiafzelechin-(4beta->8)]2-epiafzelechin	1.15±0.79a	ND	ND	ND	0.04±0.03b	ND	0.03±0.03b
Quercetin 3-galactoside	108.33±7.23f	747.04±21.94e	1095.4±23.08c	968.25±20.63d	1522.7±10.57a	332.57±9.85f	1143.4±29.04b
Quercetin 3-O-(6''-acetyl-glucoside)	500.15±100.01f	918.98.04±21.94d	1341.5±47.17b	1144.0±17.00c	2023.0±33.41a	523.33±23.36e	1334.3±40.73b
Quercetin 3-(6''-malonyl-glucoside)	738.31±43.02e	647.94±20.61f	972.07±22.60b	785.74±13.17d	1402.4±18.23a	358.04±7.71g	919.03±16.46b
Kaempferol 3-rhamnoside 7-galacturonide	40.11±10.01g	301.51±1.66c	276.95±8.35d	641.67±6.58a	200.46±3.19e	82.48±3.38f	474.36±13.22b
Quercitrin	345.67±48.00b	235.21±5.98e	173.45±4.42f	317.49±0.04c	807.22±4.26a	73.95±1.93f	254.38±3.32d
5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)	215.01±17.35c	214.57±6.96c	336.62±4.89a	334.73±3.74a	112.33±1.34d	90.05±1.74e	287.68±6.14b
Kaempferol 3-(6-acetylgalactoside)	414.67±67.03e	484.75±21.39d	330.99±9.56f	672.19±11.95b	1840.4±42.05a	194.07±8.76g	509.73±14.62c
6''-O-(3-Hydroxy-3-methylglutaroyl)astragalin	48.96±1.28e	131.38±4.59c	71.50±1.11d	363.13±11.87a	133.90±2.27c	30.75±1.13f	149.80±5.17b
Eujambolin	114.40±1.88g	303.36±12.92d	472.47±15.56a	444.63±13.44b	207.23±6.69e	153.51±7.89f	413.09±14.97c
Chrysoeriol 7-O-(6''-malonyl-glucoside)	1.11±0.84e	3.61±0.18c	3.49±0.10d	5.77±0.09b	18.64±0.57a	1.09±0.01e	3.86±0.01c
Alangisesquin A	3.11±1.01b	0.65±0.07d	0.79±0.03d	1.14±0.02c	6.79±0.19a	0.42±0.08d	0.87±0.22d
Cellulose, microcrystalline	0.24±0.16g	3.34±0.30d	11.34±0.51a	4.59±0.06c	1.20±0.02f	2.89±0.03e	9.66±0.27b
Zizybeoside I	72.05±7.06f	340.40±9.77b	272.33±10.72d	320.89±10.44c	319.95±13.57c	90.85±3.99e	471.03±14.03a
Hydroxytyrosol 1-O-glucoside	7.96±1.51e	15.03±0.66d	16.59±0.50b	15.90±0.37c	5.48±0.17f	5.55±0.09f	19.35±0.38a

Benzyl beta-primeveroside	8.07±1.68g	55.56±2.19d	71.66±2.92b	59.00±1.83c	16.02±0.24f	22.58±0.51e	74.69±2.28a
Phenylethyl primeveroside	42.68±2.57a	31.04±2.13e	40.24±1.16a	35.32±0.72b	30.48±1.17e	16.41±0.45f	31.86±0.99c
Conferoside	0.45±0.01f	5.71±0.41b	6.79±0.44a	5.36±0.05c	0.65±0.06e	2.46±0.04d	7.0±0.32a
MINEs-338436	1.87±0.04e	3.14±0.23d	4.83±0.28c	4.83±0.28c	49.21±2.58a	2.86±0.01d	5.74±0.22b
Citrusin A	0.01±0.01f	1.37±0.14d	1.77±0.06c	1.77±0.06c	28.02±1.25a	0.87±0.01e	2.39±0.13b

Values are presented as means ± standard error. Columns with different letters (a-g) indicate significant differences ($p \leq 0.05$). ND stands for Not Detected

4.4 Conclusion

Ths study indicates that the use of Coolaroo frost cloth and 40% shade netting significantly enhances the accumulation of valuable phytochemicals, such as phenolic compounds, flavonoids, and glycosyl compounds, in *Moringa oleifera* leaves, twigs, and roots during winter. Among the treatments, the Coolaroo frost cloth with a closed top and sides had the most pronounced impact, increasing concentrations of essential compounds like 3-O-p-coumaroylquinic acid, salicylic acid beta-D-glucoside, and various quercetin derivatives. Chemometric analysis further revealed that while root metabolites were minimally affected, twigs and leaves showed significant variation, with shading structures enhancing key metabolites in twigs and increasing important compounds such as rutin and kaempferol derivatives in leaves. These findings underscore that shading structures, particularly those with closed configurations, can provide a favorable environment to enhance the nutritional and medicinal value of *Moringa oleifera,* making them a viable solution for year-round cultivation and offering sustainable agricultural benefits in colder regions.

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Chapter 5: Summary of findings, significance, recommendations and conclusions

5.1 Summary of findings

The study evaluated the effectiveness of Coolaroo frost cloth and 40% shade net in protecting *Moringa oleifera* from cold stress during winter, using seven treatments: Control, 40% shade net with top cover, 40% shade net with closed sides, 40% shade net with closed top and sides, Coolaroo frost cloth with top cover, Coolaroo frost cloth with closed sides, and Coolaroo frost cloth with closed top and sides. These techniques resulted in increased plant height (cm), stem diameter (mm), chlorophyll content, root length (cm), number of leaves, and biomass (g) production regardless of the environmental conditions. However, the study highlighted that using Coolaroo frost cloth with closed top and sides (CTS) during winter led to a two-fold increase in biomass (g), stem diameter (mm), plant height (cm), leaf count, root length (cm), and chlorophyll content. Furthermore, the Coolaroo frost cloth with top cover improved the concentration of key phytochemicals such as flavonoids, phenolic and glycosyl compounds in the leaves, roots, and twigs. Chemometric analysis of the UHPLC-MS data revealed distinct effects of shading on different plant tissues. While the root metabolites showed no significant variation across shading treatments, the twigs and leaves exhibited pronounced differences. Twigs grown under 40% shade net and Coolaroo frost cloth with closed sides retained higher concentrations of key flavonoids such as quercetin 3-galactoside, rutin, and astragalin 7-rhamnoside. The leaves formed distinct clusters based on metabolite composition, with shading structures, especially the 40% shade net with closed top aand sides, enhancing the accumulation of important phytochemicals like quecetrin and kaempferol derivatives.

5.2 Significance

The findings in the current study demonstrated that the Coolaroo frost cloth could be used to protect *Moringa oleifera* against cold temperatures. By creating a more favorable microclimate, shade nets effectively mitigate cold stress, allowing *Moringa* to thrive in regions with colder climates, which would otherwise be unsuitable for its cultivation. The findings documented in this study have the potential to offer a low-cost, easy to implement solution through the use of shade nets and frost cloths for small-scale farmers. This makes it feasible for small-scale farmers to protect their crops without a large upfront investment, improving their livelihoods. The ability to grow *Moringa* during winter using these protective techniques extends the farming season, allowing farmers to produce crops year-round. This not only increases their income potential but also ensures a steady supply of *Moringa*, which is highly nutritious and has a growing demand in both local and international markets.

5.3 Recommendations

The findings of this study suggest that Coolaroo frost cloth has the potential to improve the growth, yield, and phytochemical composition of *Moringa oleifera* during winter. However, a thorough cost-benefit analysis is essential before any firm recommendations can be made. This method has demonstrated effectiveness in enhancing these key factors. Furthermore, it is a cost-effective, easy-to-implement, and environmentally sustainable cold-protection strategy, making it well-suited for smallholder farmers with limited resources. Given the positive results with Moringa, additional research should explore the application of shade nets and frost cloths for protecting other cold-sensitive crops during winter, expanding the benefits to a wider range of crops and regions. It is also recommended that agricultural policies promote the use of shading structures by small-scale farmers, offering subsidies or training programs to support the adoption of these techniques for improved off-season production.

5.4 Conclusions

The study concluded that shading, particularly with Coolaroo frost cloth, is a viable method for protecting *Moringa oleifera* from cold temperatures, improving both its growth and phytochemical properties during the winter season. The findings confirm that shading structures create a favorable microclimate for the plant, leading to enhanced biomass yield and phytochemical accumulation. This research provides a practical and accessible solution for year-round *Moringa* cultivation, contributing to food security, improved livelihoods, and increased agricultural productivity in cold-prone regions. The phytochemical compounds identified in this study supports its historical use in various herbal remedies for treating numerous ailments, including malnutrition, diabetes, high cholesterol, inflammatory conditions, digestive disorders, respiratory issues, skin conditions, anemia, cancer, cardiovascular health, liver health, and infections owing to its antimicrobial proper.