INFLUENCE OF SUBSTRATE AND LED LIGHT SPECTRUM ON GROWTH, YIELD, AND PHYTOCHEMICAL CONTENT OF ETHIOPIAN KALE (*BRASSICA CARINATA* A. BRAUN*)* MICROGREENS

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2024

Influence of Substrate and LED Light Spectrum on Growth, Yield, and Phytochemical Content of Ethiopian Kale (*Brassica carinata* A. braun*)* Microgreens

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Horticulture of the Jomo Kenyatta University of Agriculture and Technology

2024

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

To my beloved father, mother, siblings and friends for their unending love and encouragement during the entire study period.

ACKNOWLEDGEMENT

Firstly, I would like to thank God for His unconditional love and grace that enabled completion of my MSc studies. Secondly, I would like to thank my supervisors Dr. Agnes Kavoo, Dr. Johnstone Neondo, Prof. John Wesonga and Prof. Dr. Hiromu Okazawa, for their guidance, encouragement, support and availability throughout the study period. A special mention goes to Prof. Dr. Hiromu Okazawa for his support, guidance and hosting me in his laboratory. Many thanks go to all the postgraduate students of Hydro Structure Engineering Laboratory at Tokyo University of Agriculture (Tokyo Nodai) for their support and encouragement. I am so grateful to Prof. Satoko Akiyama, Dr. Ayako Sekiyama and Prof. Shotaro Kawakami of Tokyo University of Agriculture. I also recognize and thank technicians in the Food Science Department at JKUAT for their support in phytochemical analysis. I thank Prof. Francesco Orsini of the University of Bologna, Italy for support with publication of part of the work. I am grateful to Mr. Samuel Mburu Kamau of Little Farm for sharing knowledge and technical assistance in the establishment of microgreen production system. I would like to express my sincere gratitude to the Inter-University Exchange Programme of Tokyo University of Agriculture and Jomo Kenyatta University of Agriculture and Technology. I also thank Japan Student Services Organization (JASSO) for the scholarship and Tokyo University of Agriculture for hosting me during my research in Japan. Part of the work was supported by the Africa ai Japan project under JICA capacity building programme at JKUAT to which I am grateful.

TABLE OF CONTENTS

DECLARATION ..... **ii**

DEDICATION ..... **iii**

ACKNOWLEDGEMENT ..... **iv**

TABLE OF CONTENTS ..... **v**

LIST OF TABLES ..... **ix**

LIST OF FIGURES ..... **x**

ABBREVIATIONS ..... **xi**

ABSTRACT ..... **xii**

CHAPTER ONE **1**

INTRODUCTION **1**

1.1 Background Information 1

1.2 Statement of the Problem 5

1.3 Justification 6

1.4 Objectives 8

1.4.1 General Objective 8

1.4.2 Specific Objectives 8

1.5 Research Hypotheses 8

CHAPTER TWO **9**

LITERATURE REVIEW **9**

2.1 Vegetable and Vegetable Production Systems 9

2.2 Background Information on Microgreens 10

2.3 Production of Microgreens 11

2.4 Mineral Composition and Health Benefits of Microgreens 13

2.5 Phytochemical Composition of Microgreens 13

2.6 Description of Ethiopian kale, Brassica carinata 14

2.7 Substrates Used in Production of Microgreens 15

2.8 Light Quality on Growth and Yield on Microgreens 16

2.9 Light Quality Effect on Phytochemical Content 18

CHAPTER THREE 23

MATERIALS AND METHODS 23

3.1 Experimental Set Up, Site and Source of Materials 23

3.2 Growth Environment and Experimental Set Up 24

3.3 Determination of the Effect of Substrate and LED Light Spectra on Growth and Yield of Ethiopian Kale Microgreens 27

3.3.1Growth Measurements 27

3.3.2 Yield and Biomass Analysis of Brassica carinata Microgreens 27

3.4 Determination of the Effect of Substrate and LED Light Spectra on Phytochemical Content of Ethiopian Kale Microgreens 28

3.4.1 Determination of Flavonoids of Brassica carinata Microgreens 28

3.4.2 Determination of Carotenoids of Brassica carinata Microgreens 29

3.4.3 Determination of Nitrates of Brassica carinata Microgreens 29

3.4.4 Determination of Chlorophyll Content of Brassica carinata Microgreens 30

3.4.5 Determination of Total Phenols of Brassica carinata Microgreens 31

3.4.6 Determination of Vitamin C content of Brassica carinata Microgreens ... 31

3.5 Data Analysis 32

CHAPTER FOUR 33

RESULTS 33

4.1 Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover of *Brassica carinata* Microgreens 33

4.2 Effect of LED Light and Substrate on Yield and Biomass of *Brassica carinata* Microgreens 34

4.3 Effect of LED Light and Substrate on Phytochemical Content of *Brassica carinata* Microgreens 35

4.3.1 Carotenoids 35

4.3.2 Flavonoids 35

4.3.3 Total Chlorophyll 37

4.3.4 Nitrates 37

4.3.5Total Phenols 37

4.3.6 Vitamin C 39

CHAPTER FIVE 40

DISCUSSION 40

5.1 Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover of *Brassica carinata* Microgreens 40

5.2 Effect of LED Light and Substrate on Yield and Biomass of *Brassica carinata* Microgreens 41

5.3 Effect of LED Light and Substrate on Phytochemical Content of *Brassica carinata* Microgreens 42

5.3.1 Carotenoids 42

5.3.2 Flavonoids 43

5.3.3 Chlorophyll 44

5.3.4 Total Phenols 45

5.3.5 Vitamin C 46

5.3.6 Nitrates 47

5.4 Interactive Effects of Light and Substrate on Phytochemicals of *Brassica carinata* Microgreens 49

CHAPTER SIX 50

CONCLUSIONS AND RECOMMENDATIONS 50

6.1 Conclusions 50

6.2 Recommendations 51

REFERENCES 52

LIST OF TABLES

Table 4.1: Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover of *Brassica carinata* Microgreens..... 34

Table 4.2: Effect of LED Light and Substrate on Yield and Dry Weight of *Brassica carinata* Microgreens..... 36

LIST OF FIGURES

Figure 1.1: Various Stages of Plants Utilized as Food by Human Beings..... 3

Figure 2.1: Examples of Common Microgreens..... 11

Figure 3.1: A Section of the Chamber and Different Compartments Separated by a Black Opaque Material. 23

Figure 3.2: Spectral Distribution of LEDs Recorded from a Portable Spectroradiometer..... 24

Figure 3.3: Experimental Layout 25

Figure 3.4: Flow Chart Showing Key Stages/Steps during Experimentation. 26

Figure 3.5: Irrigation by Capillary Wick Technology 26

Figure 4.1: Effect of LED Light on Phytochemicals (A: Carotenoid, B: Flavonoid, C: Chlorophyll and D: Nitrates) Under Different Substrates (Coco peat+ Sand, Sand and Coco peat). Bars Represent Standard Errors of Means38

Figure 4.2: Effect of LED Light on Phytochemicals (Phenols and Vitamin C) under Different Substrates (Coco peat+ Sand, Sand and Coco peat). Bars Represent Standard Errors of Means. 39

ACRONYMES AND ABBREVIATIONS

ANOVA	Analysis of Variance
CO_2	Carbon Dioxide
DPPH	2, 2-Diphenyl-1-picryl hydrazyl
DW	Dry Weight
GAE	Gallic Acid Equivalents
HPLC	High Performance Liquid Chromatography
HSD	Tukey's Honestly Significant Difference
HY5	Hypocotyl 5
LED	Light Emitting Diodes
NCDs	Non communicable diseases
RE	Rutin Equivalent
UV	Ultra Violet
UV-VIS	Ultra Violet Visible Spectroscopy
WHO	World Health Organization
TUA	Tokyo University of Agriculture

ABSTRACT

Microgreens are plant products harvested shortly after the first true leaves emerge, usually between 7 and 21 days. Microgreens are rich in nutrients and other beneficial phytochemicals that play a major role in alleviating diet related illnesses. Therefore, they can play a role in addressing malnutrition and lifestyle diseases in Kenya. Microgreens are gaining popularity in human diets as functional foods that deliver superior nutritional value and health benefits to consumers compared to their mature counterparts. In Kenya awareness of microgreens, their production and utilization is extremely low due to limited information on microgreens. As a result, their benefits have not been fully harnessed. Since substrates and light conditions influence the quality of microgreens in terms of nutrients and phytochemicals content, it is necessary to determine appropriate substrates and optimum lighting for microgreen production. Therefore, the present study aimed at providing insights on the influence of different lighting treatments provided by LEDs, including Blue (B, 450nm), Red (R, 650nm), a cool White (W) and a combination of three color diodes (B+R+W) and substrates Cocopeat, Sand and Cocopeat-Sand mix (v:v) (1:1) on growth, yield and phytochemical content of *Brassica carinata* microgreens. The research was carried out at Tokyo University of Agriculture, Japan. *Brassica carinata* seeds were germinated in dark chambers and cultivated in growth chambers equipped with LED lighting systems in a factorial experimental setup in a split-plot design for 14 days. The plants were exposed to a fixed light intensity of 160 ± 2.5 µmol m^{-2} s^{-1} with a photoperiod of 12 h d^{-1}. Light was considered as the main plot while substrate as the subplot. There were three replications for light spectra and twelve for substrate treatments. Growth parameters assessed included plant height, leaf area, canopy cover, fresh weight and dry weight of *B. carinata* microgreens. Selected phytochemicals including ascorbic acid (Vitamin C), chlorophyll and total flavonoids, carotenoids and total polyphenols all of which are associated with antioxidant activities were assessed. Anti-nutrients (nitrates) were also assessed. All data collected were subjected to ANOVA in R software at $P \leq 0.05$. Significant means were separated by Tukey's HSD (Honestly Significant Difference). Best performance including statistically higher average yield (19.19 g $plant^{-1}$), higher plant height (9.94 cm), leaf area (68.11 mm^2) and canopy cover (55.9%) were found under combined Blue + Red + White (B+R+W) LEDs and Cocopeat + Sand mix. B+R+W LEDs enhanced carotenoids and flavonoid content, while Blue LED alone (B) increased total amount of chlorophyll (11880 mg kg^{-1}). *Brassica carinata* microgreens grown using Red LED alone (R) and in cocopeat +Sand mix recorded the highest total phenols (8.1 mg kg^{-1}). In addition, B+R+W LED and in cocopeat enhanced accumulation of Vitamin C content of *B. carinata* microgreens (1155.1 mg kg^{-1}). For plants grown under B+R+W LED in cocopeat, high nitrate levels were observed. The results therefore suggest that substrate and LEDs are important factors for the growth, development and accumulation of secondary metabolites of *B. carinata* microgreens specifically supplemental irradiation with combined (B+R+W) LED or Blue LED alone and using combined sand and cocopeat substrates can improve growth and nutritional quality of *B. carinata* microgreens.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Food and nutrition insecurity is among the major challenges prioritized by Government of Kenya and the United Nations under SDG 2 (Macharia, 2019). The urban population is particularly vulnerable with reports showing 80% of residents being food insecure and 50% malnutrition rates among children (Mutoro, 2017). In 2021, Kenya had a Global Hunger Index of 23 which is classified as serious (Global Hunger Index, 2021). Of particular concern is the high level of diet related illnesses such as obesity and overweight (Ayeni et al., 2021; Bryant et al., 2019) caused by poor diets. Notably, there is low consumption of vegetables and fruits among Kenyans (Keding, 2016), which are major sources of vitamins and minerals. Furthermore, cooking, which is the most common method of preparing vegetables has been shown to reduce phytochemicals including flavonoids, glycosides, hydroxycinnamic acid derivatives and carotenoids (Odongo et al., 2017). Microgreens which are consumed raw with their richness in nutrients and other beneficial phytochemicals can play a major role in alleviating diet related illnesses. They can therefore contribute to reduction of hidden hunger which is prevalent in Kenya.

Microgreens are plant products from normal plants sown at a medium to high density and harvested shortly after the first true leaves emerge, usually between 7 and 21 days by cutting the stem just above the substrate or above the roots for soilless cultivation (Verlinden, 2020). They are highly nutritious with high amounts of antioxidants and are considered feasible options for addressing high levels of malnutrition and dietary illnesses (Rouphael et al., 2021). They are considered to have more nutrients compared to seeds or mature plants that are commonly consumed (Choe et al., 2018; Johnson et al., 2021). The superiority of microgreens over other plant stages of the same species is attributed to the germination process from dry seeds to growing plants which involves many metabolic activities and *de novo* synthesis of nutrients (Loedolff et al., 2017). Consequently, microgreens are

reported to have higher concentrations of functional components such as vitamins, minerals and antioxidants (Zhang et al., 2021). As a result of their nutrient dense properties and hence the capacity to supply nutrients at relatively small consumption quantities compared to their mature counterparts, microgreens are gaining wide recognition globally. In the United States of America, microgreens interest has increased by 96% since 2004 (Bunning, 2019; Ebert, 2022). Furthermore, growers both greenhouse and indoor have recently become interested in microgreen production due to their short production cycles, low cost of production, nutrient density leading to high market value (Treadwell et al., 2020). In Kenya awareness, production and utilization of microgreens is extremely limited. Consequently, the benefits of microgreens have not been harnessed.

Morphologically, microgreens are plant seedlings that fall between cotyledonary and the first fully formed primary (true) leaf stages of growth or between sprout and baby leaf vegetable stages (Figure 1.1) (Treadwell et al., 2020). The young, tender greens are used to enhance the color, texture, or flavor of salads, or to garnish a wide variety of main dishes. They can also be used to fortify other products such as smoothies, yoghurts and ice cream among others. Microgreens are gaining attention and recognition as a new class of food due to their unique characteristics such as flavour, tenderness, colour among others (Bulgari et al., 2021; Appolloni et al., 2022) and nutrient density (Zhang et al., 2021).

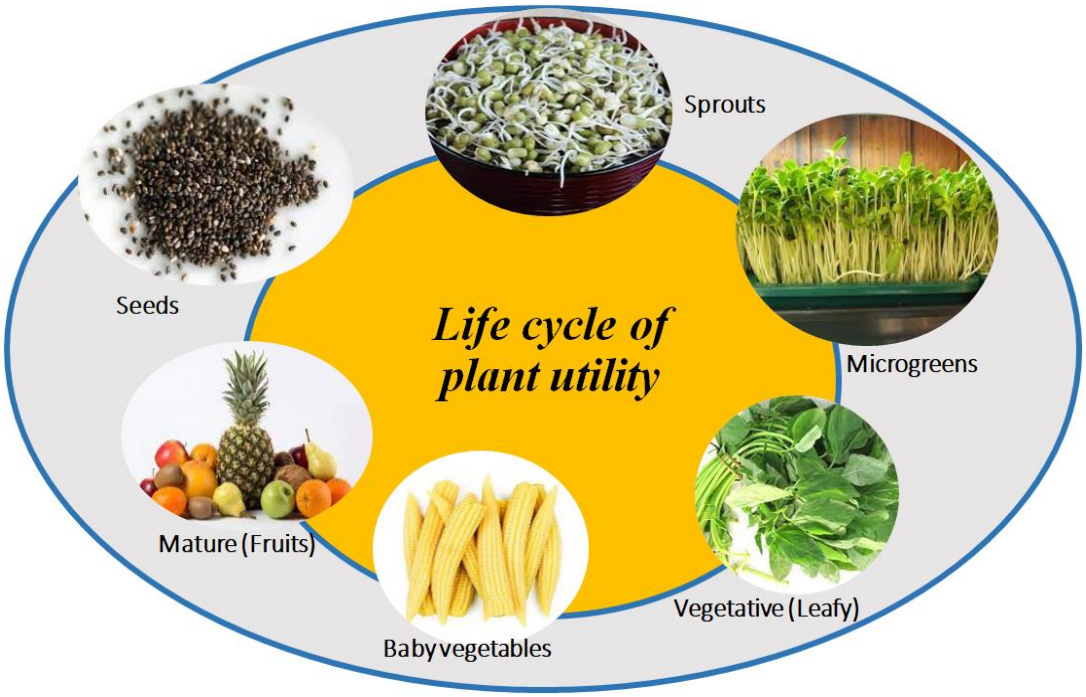

Figure 1.1: Various Stages of Plants Utilized as Food by Human Beings; Source **(**Maru, 2024**)**

Common plant species used as microgreens are from the families of Asteraceae, Apiaceae, Amaryllidaceae, Amaranthaceae, Lamiaceae and Brassicaceae (Rouphael et al., 2021). Brassicaceae family are the most popularly grown microgreens due to their distinct colors, ease of germination, short growing cycles, unique flavors and their high concentration of phytochemicals (Xiao et al., 2012).

Apart from amaranth, other African indigenous vegetables have not been utilized as microgreens. It is therefore necessary to evaluate the possibility of utilizing African indigenous vegetables as microgreens. Ethiopian kale, *Brassica carinata*, belongs to the *Brassicaceae* family whose members are known to be rich in bioactive metabolites (Peña et al., 2022; Zhang et al., 2022; Zhu et al., 2022). It is an indigenous African leafy vegetable (ALVs) that is rich in nutrients and health-promoting secondary plant metabolites (Neugart et al., 2017) with potential for use against non-communicable diseases such as cancer. The leaves and seeds of *B. carinata* are rich in nutrients with high concentrations of glucosinolates, especially 2-propenyl glucosinolate (sinigrin), as well as phenolic compounds. *Brassica. carinata*

has been reported to reduce aflatoxin B1-induced DNA damage (Odongo et al., 2017). *Brassica. carinata* microgreens have been shown to contain flavonoids, phenols, tannins, saponins, alkaloids, and terpenoids but not glycosides (Nakakaawa et al., 2023).

Substrates are among the important factors to be considered in the production of microgreens. Some of the substrates used for microgreen production include soil, vermiculite, perlite, peat moss and cocopeat. Substrates vary in conditions such as pH and salinity levels which in turn influence seed germination and growth of microgreens (Thuong et al., 2020; Wieth et al., 2019). For radish microgreens, growing substrates varied in salinity and pH which significantly affected its fresh weight (Thuong et al., 2020). In addition to their effects on plant performance, some substrates such coco peat are expensive, not readily available and contain high amounts of salt. They therefore present challenges in their utilization making it necessary to explore alternative substrates for production of microgreens. For *B. carinata* microgreens, growing substrate for enhanced growth and phytochemical content is yet to be determined hence the need to investigate it to determine the best growing substrate.

Light plays a major role in plants influencing production of phytochemical and bio-active compounds (Ying, 2020; Ying et al., 2020,). Light quality (Wavelength), light quantity (intensity), direction, and photoperiod (duration) are vital components of light conditions. In plants such as lettuce, high light intensity resulted in production of high amounts of phenolic, anthocyanins, carotenoids among other phytochemicals which could be beneficial to human health (Craver et al., 2017). The use of artificial light sources such as light emitting diodes (LED) grow lights as a source of supplemental lighting in controlled environments such as indoor spaces and greenhouses has been used in production of microgreens (Brazaityte et al., 2015).

Although LEDs and substrate have been proven to influence the growth and synthesis of bioactive compounds of microgreens, their influence on *B. carinata* microgreens is still unknown. This study was therefore aimed at providing insights on the influence of LEDs: Blue (B), Red (R), White (W) and a combination of

B+R+W on yield, growth and phytochemical content of Ethiopian kale microgreens grown in Cocopeat, Sand and in combination of Cocopeat and Sand (v:v).

1.2 Statement of the Problem

Brassica carinata is mainly utilized as a mature leafy vegetable. It faces various challenges such as early maturity which limits its full utilization. There is limited information on its growth and utilization as a microgreen, particularly on production conditions in Kenya. The knowledge on optimum conditions such as substrate and light conditions for growth, yield, nutritional and phytochemical content of *B. carinata* microgreens is not documented anywhere. The substrate that is currently used in production of microgreens is coco peat due to its good physicochemical properties. However, it is expensive, not easily available and requires treatments for its concentrated salts before use, which increases costs. (Di Gioia et al., 2017; Kyriacou et al., 2020; Poudel et al., 2023; Thepsilvisut et al., 2023). This leads to increased cost of production of microgreens. Accordingly, the exploration of alternative substrates or additives enabling to reduce the amount of coco peat needed may lead to the identification of sustainable, cheaper and renewable growing substrates for microgreens. Light quality is a major factor in plant growth and development. It has an influence on the phytochemical biosynthesis in plants and this affects the phytochemical content (Ying et al., 2020). Regarding the effects of light on microgreen growth, research results vary across studies and for different vegetable species. For example, it has been found that growth and phytochemical accumulation in *Brassica juncea* and *Brassica napus* using different R and B ratios, differed depending on species (Brazaityte et al., 2015). Notably, there is no information on how LED light spectrum influences the growth, yield and phytochemical profile of *B. carinata*. In addition, it is also unclear how plants respond to LEDs in combination with substrates since most of the previous studies assessed either LEDs or substrates alone. This information is critical in developing a system for microgreen production and to contribute to increased production and consumption of microgreens in Kenya.

1.3 Justification

Microgreens are nutrient dense (Deepa & Malladadavar, 2020) and are consumed without cooking hence can contribute to reduction of malnutrition (Ilakiya et al., 2020) including "hidden hunger" that is increasing globally (Hoffman et al., 2018). *Brassica* vegetables such as kale, broccoli and radish are good sources of health-promoting phytochemicals with high antioxidant capacities. According to various studies the nutritional value and phytochemical content may vary with plant growth stage and development and are found in higher concentrations at the microgreen stage (Bulgari et al., 2021; Appolloni et al., 2022).

Microgreens are easy to grow indoors or in small spaces making it accessible for urban farmers with limited gardening spaces (Bulgari et al., 2021). Compared to traditional vegetables they are considered sustainable as they require less water, space, growing cycle (between 7 and 21 days) and can be a feasible option for growing nutrient dense vegetables with limited resources (Bulgari et al., 2021). Their high market value makes them economically viable option to their producers making them a profitable crop. Microgreens are beneficial to both the producers and the consumers due to their high nutrient dense characteristics (Deepa & Malladadavar, 2020).

Growth substrate is critical in the production of microgreens as it is a major contributor to the production costs (Chen et al., 2020). Substrates affect growth, yield and the environmental sustainability of microgreens production (Craver et al., 2017; Wieth et al., 2019; Thuong et al., 2020; Poudel et al., 2023). Locally available and inexpensive substrates with good water holding capacity and providing aeration are ideal for microgreen production. Those derived from renewable resources and/or that can be recycled are to be preferred (Di Gioia et al., 2017). According to previous reports, cocopeat is one of the most used substrates for microgreen production due to its favorable physicochemical properties. However, it is expensive, not easily available and requires treatments for its concentrated salts before use, which increases costs (Di Gioia et al., 2017; Kyriacou et al., 2020; Gbollie et al., 2022; Poudel et al., 2023; Thepsilvisut et al., 2023). Accordingly, the

exploration of alternative substrates or additives that enable reduction of the amount of cocopeat needed may lead to the identification of sustainable, cheaper and renewable growing substrates for microgreens.

Light is another major factor in plants growth and influences development and production of phytochemical and bio- active compounds (Ying et al., 2020). Light quality (its composition in the spectral regions), light quantity (intensity), direction, and duration (photoperiod) are vital components in microgreen production. In plants such as lettuce, high light intensity results in production of high amounts of phenolic, anthocyanins, carotenoids among others which could be beneficial to human health (Craver et al., 2017). Regarding the effects of light on microgreen growth, research results vary across studies and for different vegetable species. For example, Brazaityte et al., (2015) found that growth and phytochemical accumulation in *Brassica juncea* and *Brassica napus* using different R and B ratios, differed depending on species. The chlorophyll, carotenoid and soluble protein contents depended on photoperiod in other *Brassica* species (Liu et al., 2022).

In addition, inconsistencies in results on the effect of different spectral regions across plant species and phenological stages have been acknowledged as gray areas requiring further research (Naznin et al., 2019). Similarly, there are no studies on the effects of quality of light either alone or in combination with substrate on the growth and yield of *B. carinata* microgreens hence the need to investigate it. This study seeks to contribute to information for *B. carinata* microgreens production optimization by investigating suitable substrates and LEDs alone or in combination for high quality traits and enhanced phytochemical accumulation in *B. carinata* microgreens.

1.4 Objectives

1.4.1 General Objective

To evaluate the effect of substrate and LED light spectrum on growth, yield, and phytochemical content of Ethiopian kale microgreens.

1.4.2 Specific Objectives

1. To determine the effects of substrate and light spectrum on the growth and yield of Ethiopian kale microgreens.
2. To determine the effects of substrate and LED light spectrum on the phytochemical content of Ethiopian kale microgreens.

1.5 Research Hypotheses

1. Substrate and LED light spectrum do not have significant influence on the growth and yield of Ethiopian kale microgreens.
2. Substrate and LED light spectrum do not have significance influence on the phytochemical content of Ethiopian kale microgreens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Vegetable and Vegetable Production Systems

Vegetables in general are considered essential for well-balanced diets since they supply essential micronutrients and health promoting phytochemical needed by human beings. Vegetables may be grown outdoors or indoors, and commercial production is mainly done in greenhouses. Each vegetable group contains a unique combination of phytonutrients that distinguishes them from other groups. For example, *Brassicaceae* vegetables are known to provide ascorbate, chlorophyll, carotenoids, total phenolic (for flavonoids and anthocyanin) (Zhang & Jing, 2022). These are, in general, found in higher concentrations at the sprout and microgreen stage than in the respective adult (vegetable) edible plant organs (Ebert, 2022).

In addition, consumption of vegetables which are nutritious, and health promoting is 268g per person per day is low and remains well below the WHO recommendation of 400 g per day per person (Ebert, 2022). The low consumption has been associated with high rate of malnutrition which has increased non-communicable diseases (NCDs) such as diabetes, cardiovascular disease; hypertension, stroke, cancer and obesity (Ebert, 2022), which are all diet related. Vegetables are mainly prepared by cooking, a process that destroys some active metabolites (Francisco et al. 2010; Odongo et al., 2017). Utilization of vegetables without destructive processing methods such as cooking would therefore enable exploitation of the potential of such vegetables.

Vegetables are produced in various ways such as open field production where vegetables are grown directly in the soil without any protective structures. Vegetables can also be produced hydroponically where they are grown in nutrient rich water rather than soil in a protected environment. They can also be grown in vertical gardens, and this is where vegetables are grown in stacked layers or in vertically inclined areas and this is mainly done indoors. Vegetables can also be

produced aquaponically where plants use wastewater from the fish as the plants help with filtering the water used by the fish.

2.2 Background Information on Microgreens

Microgreens are small salad greens of vegetables and herbs that are harvested with two fully developed cotyledon leaves with or without the emergence of a rudimentary first pair of true leaves. Microgreens developmentally occur between "sprouts" and "baby leaf" stages (Murphy et al., 2010; Verlinden, 2020). Development of cotyledon leaves occurs between 10 to 14 days from seedlings emergence. Compared to mature greens, the length of the growing period to harvesting of microgreens is relatively short (between 7-21 days) and varies depending on the species and growing conditions. The height of microgreens ranges between 5 and 10 cm and are sold with the stem and attached cotyledons (seed leaves). They are grown in substrates and light (sunlight or artificial) and are harvested by cutting the stem at the base above the growing substrate.

Microgreens can provide a large array of intense flavors, vivid colors and tender textures. Therefore, they can be served as ingredient in salad, soups and sandwiches enhancing their color, texture, and/or flavor, and can be used as edible garnish to brighten up a wide variety of main dishes (Jungsoo et al., 2009; Murphy et al., 2010; Treadwell et al., 2020). Microgreens have been proven to have a higher amount of health promoting nutrients such as phytochemical content and are thus referred to as functional foods (food enriched with health promoting additives) (Liu et al., 2022). According to research they are proposed as an alternative possible solution to malnutrition that is facing many people globally (Ilakiya et al., 2020). Although microgreens have been claimed as nutritionally beneficial, no data is available on Ethiopian kale microgreens production including substrate and effects of LED light spectrum on growth, quality and phytochemical content.

There are many species that can be cultivated as microgreens including vegetable species, herbaceous plants, aromatic herbs (Figure 2.1). Other species belong to the *Amaranthaceae, Asteraceae, Apiaceae, Chenopodiacea and Lamiaceae* family (Rouphael et al., 2021). Many vegetable species that have been studied and

cultivated as microgreens belong to the *Brassicaceae* family because they contain high phytochemical content (Björkman et al., 2011) some of which have medical benefits such cancer prevention (Peña et al., 2022). The most found phytochemical in *Brassicacea*e crops include ascorbate, chlorophyll, carotenoids, total phenolic (e.g., flavonoids and anthocyanin) (Zhang & Jing, 2022).

Red amaranth microgreen

Sunflower microgreens

Radish microgreens

Figure 2.1: Examples of Common Microgreens

Microgreens are often confused with sprouts. Sprouts differ from microgreens in their development stage, growing and harvesting methods. Sprouts refer to the initial stage of development that occurs prior to the complete development of cotyledons. Unlike microgreens, they are grown directly in water without any growing substrates and light and are consumed whole with the rootlets. Both sprouts and microgreens are considered more nutrient-dense than ungerminated seeds or mature vegetables (Ebert, 2022). In addition, studies comparing microgreens and sprouted seeds found that there was no pathogenic microbial contamination in both spouts and microgreens (Bergšpica et al., 2020).

2.3 Production of Microgreens

Microgreens may be grown in greenhouses, or indoors, with artificial light sources, in the soil or, most commonly, in soil-less systems, using organic or inorganic solid growing media or hydroponics. Despite the short growing cycle, the commercial production of microgreens requires particular attention, and the choice of the growing medium represents one of the most critical aspects of the production process (Di Gioia et al., 2017). They can be grown at home by individuals because they require relatively small spaces for growth. According to research, commercial production of high value microgreens is difficult (Di Gioia et al., 2017). Commercial

production of microgreens is usually done in controlled environments such as greenhouses, indoor with vertical spaces depending on the quantity of microgreens required to meet the demand and climatic conditions (Di Gioia et al., 2017).

Microgreens are less affected by pest and diseases, however due to high seed density, relatively high amount of water required during germination, fungal diseases likely occurred. Like any other crops, microgreens require adequate light for their growth and development. Light is important for photosynthesis and thus contributes to the growth, yield and nutritional content of microgreens. It has been shown that varieties grown under sufficient light have relatively higher nutrients than varieties grown under darkness (Xiao et al., 2012). Water is important throughout all the growth stages of microgreens. During the seeding and germination stage misting using nozzles is normally used to avoid displacing of seeds. Bottom watering is used during other stages of growth (Thuong et al., 2020).

Microgreens are normally harvested before or after development of the first two true leaves and are at a height of 5- 10 cm. Harvesting is done by hand for small producers which are a difficult task and commercially it is done by machine to save labor and time costs (Riggio et al., 2019). The main aim of microgreens production is to achieve higher amount of fresh weight accumulation as they are normally sold on fresh weight basis, therefore production factors and conditions that lead to increased fresh weight are normally enhanced. The color, smell and flavor of the microgreens stalk and leaves are a great factor that is considered by consumers (Riggio et al., 2019). Red and darker colored leafy microgreens are normally more appealing to the consumers as they are known to have more nutrient content as compared to the light-colored microgreens (Ying et al., 2020). Microgreens have a relatively short shelf life; therefore, they are packed in modified atmosphere package that maintain freshness and prolong shelf life of microgreens. Some research show that this method of packaging has been successful in fresh produce such as Lettuce, Broccoli, Spinach and Mushrooms (Kalal et al., 2021).

2.4 Mineral Composition and Health Benefits of Microgreens

Microgreens contain various phyto-nutrients and minerals required for normal growth and development in the human body. Some of the phyto-nutrients include ascorbic acid, β-carotene, α-tocopherols, and phylloquinone (Kalal et al., 2021). Minerals contained in microgreens include Ca, Mg, Fe, Mn, Zn, Se, and Mo. These mineral and phyto-nutrients are relatively higher than their mature counterparts (Ebert, 2022). Due to their dense nutrients and distinctive characteristics, they can therefore be used by consumers such as vegetarians to enrich their diets with the available microgreens.

Essential elements are nutrients that the human body requires for normal growth and development. There are two classes of essential elements; macro elements which are required in relatively higher amount (Ca, Mg, P, K, and Na) and micro elements which are required in small amount (Fe, Zn, Cu, and Mn) (Ebert, 2022). Both macro and micronutrients are important in helping the body in various biological processes. Deficiency of these elements lead to metabolic disorders leading to organ damage and some may play a role in development of acute and chronic diseases and may even lead to death (Kalal et al., 2021). Sufficient dietary intake of mineral nutrients is therefore important for human health and wellness. Malnutrition is still a major problem in Kenya and worldwide and is considered one of the global challenges.

2.5 Phytochemical Composition of Microgreens

Phytochemicals are plant based bioactive compounds produced by plants mainly for their protection. They include flavonoids, carotenoids, polyphenols, tannins, saponins, anthocyanins, ascorbate among others (Björkman et al., 2011). Vegetable crops are a major source of phytochemicals that promote good human health. These phytochemicals are good sources of antioxidants that promote good human health by inhibiting or delaying oxidative damages and preventing some chronic diseases (Alrifai et al., 2019). Recent research shows the importance of antioxidants in human diet in controlling inflammation and immune system responses at the cellular level in animal models and human trials (Reuter et al., 2010). Due to the increasing cases of

health issues, microgreens are gaining attention because they have higher content of phytochemicals compared to their mature counterparts (Xiao et al., 2012).

Over the past decade, crops in the Brassicaceae family have been extensively investigated and cultivated because of their phytochemical profiles (Björkman et al., 2011). Most common phytochemicals found in the Brassicaceae family include chlorophyll, carotenoids, ascorbate, phenolic such as flavonoids and anthocyanins as well as glucosinolates. Environmental factors such as light (intensity, quality and duration), CO_2 concentration, temperature, water availability among others greatly affect the biosynthesis of the phytochemical therefore affecting the content of phytochemical. Responses vary with the agronomic factors such as the species, developmental stage, plant density, and fertilization (Björkman et al., 2011).

2.6 Description of Ethiopian kale, *Brassica carinata*

Ethiopian kale, *Brassica carinata* A. Braun is one of the indigenous African leafy vegetables (ALVs) widely grown and consumed in East and Southern Africa. They are rich in nutrients and health-promoting secondary plant metabolites (Neugart et al., 2017) with potential for use against non-communicable diseases such as cancer that are prevalent in many parts of the world. The leaves and seeds of *B. carinata* are rich in nutrients with high concentrations of glucosinolates, especially 2-propenyl glucosinolate (sinigrin), as well as phenolic compounds. *Brassica carinata* has been reported to reduce afb1-induced DNA damage (Odongo et al., 2017). The research recommends consumption of Ethiopian kale, *B. carinata* as part of chemo-preventive measures to combat prevalence of aflatoxin-induced diseases. According to world vegetable center, Ethiopian kale, *B. carinata* contain high amount of beta-carotene, Vitamin E, ascorbic acid, folic acid, calcium, iron and leaves contain high levels of glucosinolates (The World Vegetable Center, 2009). Consequently, its consumption was recommended as a means of curbing prevalence of aflatoxin induced diseases. *Brassica carinata* microgreens have been shown to contain flavonoids (124.20 ± 0.78mg [Rutin equivalent]/g), phenols (98.13 ± 1.91mg [Gallic Acid Equivalent]/g mg (TAE)/g), tannins (50.63 ± 0.25 mg [Tannic Acid equivalent]/g, saponins, alkaloids, terpenoids but not glycosides (Nakakaawa et al., 2023).

Concentration of secondary metabolites such as 2-Propenyl and 3-Indolylmethyl glucosinolates in *B. carinata* is affected by moisture stress (Schreiner et al., 2009). It is therefore possible that substrate used in growing *B. carinata* can influence production of secondary metabolites through the effects on availability of moisture.

Brassica carinata, like other leafy vegetables, is prepared by cooking, a process that destroys some active metabolites (Odongo et al., 2017). For example, conventional boiling and high-pressure cooking were shown to result in 70% and 64% loss of phenolic compound and glucosinolates respectively as well 64% loss in some Brassicaceae. In the same study high nutrient retention was observed where steaming was used in vegetable preparation and low nutrient retention was observed where high pressure-cooking and conventional boiling method was used. Utilization of *B. carinata* and other African vegetables without destructive processing methods such as cooking would therefore enable exploitation of the potential of such vegetables. In addition, utilization of *B. carinata* as a microgreen could result in higher nutrient content and novel phytochemicals as reported in other plant species (Deepa & Malladadavar, 2020).

2.7 Substrates Used in Production of Microgreens

Microgreens can be grown in several substrates. In the past soil was the main medium used for microgreens production but currently soilless- systems, using organic and inorganic media or hydroponics has been adopted (Thuong et al., 2020). Growth substrate is critical in the production of microgreens as it is a major contributor to the production costs (Chen et al., 2020). Substrates will affect growth, yield and environmental sustainability of microgreens production (Poudel et al., 2023). Locally available and inexpensive substrates with good water holding capacity and providing aeration are ideal for microgreen production. Those derived from renewable resources and/or that can be recycled are to be preferred (Di Gioia et al., 2017). According to several authors, peat and peat-based mixes represent the most used growing substrates for production of microgreens because of their good physicochemical properties, but coconut coir (also referred to as cocopeat) is common as well (Di Gioia et al., 2017; Kyriacou et al., 2020; Thepsilvisut et al.,

2023). However, these substrates are quite expensive, and when they are not locally available, they require importation. The use of peat poses environmental concern due to its continuous extraction which contributes to emission of carbon dioxide. On the other hand, cocopeat (derived from coconut processing industry and its discarded fibers) is a renewable resource and could be used as an alternative to peat (Di Gioia et al., 2017). However, it can also be an expensive material and requires treatment for removal of its concentrated salts before use which increases costs. Accordingly, the exploration of alternative substrates or additives enabling to reduce the amount of coco peat needed may lead to the identification of sustainable, cheaper and renewable growing substrates for microgreens.

2.8 Light Quality on Growth and Yield on Microgreens

Light is one of the most important environmental factors in plants production including microgreens. It plays a major role in providing a source of energy for photosynthesis, also a signal for most physiological responses and influence the production of secondary metabolites such as phytochemical in plants (Brazaityte et al., 2015). LED lighting provides a good opportunity to exploit its potential in producing crops with horticultural benefits. The narrow emission spectra of LEDs allow lighting systems to be designed to stimulate specific plant photoreceptors, allowing plants to be manipulated to produce desirable characteristics (Samuolienė et al., 2017). Lighting systems can therefore be designed to maximize growth, control morphology, and optimize yield (Davis & Burns, 2016).

Yield is an important parameter in plant production especially in vegetable and fruit because most of these are sold on fresh weight basis (Ying et al., 2020). Use of artificial lights such as Red LED grow lights was done for the first time in 1962 for plant production after development of super bright LED grow lights in 1980s (Ying et al., 2020). Previous studies show that use of 100% Red LED light resulted to “R light syndrome” for example dysfunctional photosynthetic operations, undesirable growth characteristics, and the translocation of photosynthates out of the leaves might also be inhibited (Hogewoning et al., 2010).

Blue LED light that was developed later was found to prevent the "R light syndrome" (decrease in photosynthetic capacity, reduced stomata opening and decrease in leaf thickness in plants), and it was therefore used to prevent certain plant growth characteristics such as down-rolled leaf margins, low photosynthetic rate and low biomass (Yeh et al., 2009; Hogewoning et al., 2010; Ying et al., 2020). Previous study indicated that use of a combination of red and blue LED light increased dry matter accumulation of spinach, lettuce and radish as compared to plants cultivated under red LED light alone (Meas et al., 2020). Red-blue LED combination was also shown to have increased fresh weight and dry weight of chili pepper (*C. annum*), lettuce and *Phaelenopsis* × *Doritis*, and fresh weight of sprouting broccoli (*Brassica oleacea* L.) (Yeh et al., 2009). An early study done showed that blue LED alone and in combination with red enhanced the yield of broccoli microgreens more than Red LED alone (Madar et al., 2022). Furthermore, a study done by Di Gioia et al. (2023) indicated that low yield was recorded for broccoli microgreens grown in hydroponics in a tunnel covered with polythene film. In addition, basil microgreens grown using UV-A supplemental light had higher fresh weight while the same was shown to have decreased fresh weight in beet microgreens (Brazaityte et al., 2015). Blue light is required for phototropism, photo morphogenesis, stomatal opening, and increasing photosynthetic capacity of the leaf and this result in increased fresh weight and biomass accumulation in plants (Davis & Burns, 2016).

In the case of growth, one study indicated that lettuce grown using increased ratio of red radiation had increased shoot height and shoot: root ratio compared to those grown using a blue light source (Son et al., 2015). In addition, monochromatic B and in combination with far-red were found to increase mustard (*Brassica juncea*) and arugula (*Eruca sativa*) microgreens elongation (as defined as plant height) (Ying et al., 2020). In addition, Inconsistencies in results on the effect of different spectral regions across plant species and phenological stages have been acknowledged as gray area requiring further research (Naznin et al., 2019). Similarly, there are no studies on the quality of light on the growth and yield of *B. carinata* microgreens hence the need to investigate it.

2.9 Light Quality Effect on Phytochemical Content

Phytochemicals are secondary metabolites compounds that occur naturally in plants. They are not involved in growth and development of plants but protect the plant against biotic and abiotic stress by delaying or inhibiting oxidative damage (Agati et al., 2012). In human body they are important for modulating inflammation in the immune system when there is oxidative stress therefore protecting the body from various chronic diseases (Alrifai et al., 2019). The biosynthesis of secondary metabolites is controlled internally by plant hormones and by external factors such as light (Craver et al., 2017). However, there are no studies on the effect of light quality on the phytochemical content of *B. carinata* microgreens hence the need to investigate it.

Chlorophyll and carotenoids are vital components in plant photosynthesis. The biosynthesis of chlorophyll occurs in the chloroplast and is initiated from glutamate. Other chlorophyll precursors like 5-aminolevulinic acid (ALA) and protochlorophyllide are involved during synthesis of Chlorophyll (Hogewoning et al., 2010). Chlorophyll pigment absorbs light cross the photo synthetically active radiation spectrum at wavelength between 400-700nm. Chlorophyll a and b play a predominant role in photosynthetic light absorption with absorption peak occurring at 453 and 642 respectively. In addition, blue and red light are absorbed more as compared to others (Ying et al., 2020).

The biosynthesis of chlorophyll requires transcriptional factors that mediate light-induced responses therefore, light is an important component involved in chlorophyll biosynthesis (Meas et al., 2020). Previous study indicated that blue LED and combined blue and red LED increased chlorophyl content of red cabbage and broccoli compared to red LED (Madar et al., 2022). Furthermore, B LED light used alone or in combination with Red LED was found to enhance Chl and total Chl of amaranth microgreens but did not affect Chl accumulation in turnip greens (Toscana et al., 2021). Another study found that photoperiod and light intensity affected yield and phytochemical content of Beet Microgreens (Hernández-Adasme et al., 2023). Longer (16 hr Light) photoperiod raised phenolic compounds, total betalains, and

antioxidant capacity but reduced microgreens yield compared shorter 12 hr Light photoperiod. Additionally, low (120 ± 2 µmol m^{-2} s^{-1}) and medium (160 ± 2 µmol m^{-2} s^{-1}) light intensities promoted yield relative to high (220 ± 2 µmol m^{-2} s^{-1}) light intensity.

Furthermore, mustard and Kohlrabi microgreens grown using increased ratio of R to blue combination had increased chlorophyll accumulation compared to those grown using lower rations of combined Red and Blue LED (Craver et al., 2017). However, Ying et al. (2020) found that changing the percentage of blue LED concentration did not affect the concentration of chlorophyl and carotenoid pigments in *Brassicaceae* family investigated.

Carotenoids which are important components of all photosynthetic organisms belong to a group of tetraterpenes and are derived from mevalonate pathway (Alcaíno et al., 2016). Various physiological properties such as antidiabetic, antioxidant, anti-inflammation, and anti-obesity activities have been ascribed to carotenoids thus being presented among important nutrients in the human diet (Saleh, 2023). They are also light harvesting pigments that absorb light photons ranging from approximately 350–500 nm. They also play an important role in protecting the plant from oxidative damages through the xanthophylls cycle when plants are exposed excessive light (Meas et al., 2020).

Previous study by (Meas et al., 2020) indicate that phytochrome- mediated transcription factors PIF and Long Hypocotyl 5 (HY5) are involved in biosynthesis of carotenoids. Previously, blue LED light has been shown to enhance carotenoid accumulation in red cabbage and combined blue and red LED enhanced accumulation in broccoli (Madar et al., 2022). Additionally, Brassicaceae family microgreens cultivated in soilless media under white LED showed higher carotenoid accumulation. Specifically, higher accumulation was recorded for broccoli microgreens compared to red cabbage microgreens (Kowitcharoen et al., 2021). This study also indicated that carotenoid accumulation did not only depend on the type of LED used but also highly depended on the species studied and therefore it is

important to study how light quality influences carotenoids accumulation in *B. carinata* microgreens.

Vitamin C, also known as ascorbate, is an essential nutrient that is found in citrus fruits and in vegetables. It is a good source of antioxidants required by the human body. Plants produce ascorbate to prevent themselves against biotic and abiotic stress by delaying or inhibiting oxidative damage and limit oxidative stresses (Agati et al., 2012). Previous study indicate that the biosynthesis of ascorbate is modulated by light at the transcriptional level (Metallo, 2017). In microgreen production, light, particularly light-emitting diodes (LEDs) treatments, act as elicitors that trigger various biosynthetic pathways associated with different phytochemicals such as Vitamin C.

Previous study indicates that lentil microgreen cultivated under white LED presented high accumulation of vitamin C content (Kowitcharoen et al., 2021). Additionally, LED illumination has been shown to increase ascorbic acid accumulation. Specifically, research by (Brazaityte et al., 2015) indicated that using red LED alone or in combination with other LEDs enhanced ascorbic acid accumulation in Brassicaceae family microgreens studied. Similarly, a combination of red and blue LED was found to enhance accumulation of Vitamin C content of two amaranth microgreens (Meas et al., 2020). From these studies, it is demonstrated that light optimization is crucial for enhanced Vitamin C content therefore, it is important to investigate how LED would influence Vitamin C content of *B. carinata* microgreens.

Phenolic compounds are a group of secondary metabolites that are derived from secondary pathways of plants. They include water soluble compounds such as flavonoids and water insoluble compounds such as lignins (Zhang & Jing, 2022). These compounds are a good source of antioxidants and protect plants from oxidative stress. They play a vital role in plants' taste, smell, and color. In addition, they are involved in growth, development and defense mechanisms (Saleh, 2023). In the human body they are a source of vital nutrients that contribute to human health. Phenolic compounds possess various anti-aging, anti-inflammatory, and antioxidant

functions, which can decrease the risk of acute diseases like diabetes, various types of cancer, and cardiovascular diseases (Lin et al., 2016).

A study shows that flavonoids absorb shorter wavelength of light including UV light, although the energy cannot be used for photosynthesis (Agati et al., 2012). In plants such as lettuce high light intensity resulted in production of high amounts of phenolic, anthocyanins, carotenoids among others which could be beneficial to human health (Craver et al., 2017). The accumulation of phenolic compounds, like other secondary metabolites, are greatly affected by environmental factors, including light intensity and spectra (Zhang & Jing, 2022), therefore the need to investigate it. An early study indicated that Blue LED enhanced accumulation of phenolic compounds in both turnip and amaranth microgreens while Red LED depressed phenolic content in the same species (Toscana et al., 2021).

Similarly, broccoli microgreens grown using Red LED was shown to have lower accumulation of phenolic compound as compared to those grown using White, Blue or a combination of Red and Blue LEDs (Liang et al., 2022). Additionally, (Kowitcharoen et al., 2021) study indicated that the amount of phenolic compound accumulation also depends not only on the LED used for production but also on the species studied. The study found that buckwheat microgreens had higher amount of phenolic compound accumulation compared to morning glory microgreens both produced under white LED.

Furthermore, mustard and Kohlrabi microgreens grown using increased ratio of R to blue combination had increased phenolic compound accumulation compared to those grown using lower rations of combined Red and Blue LED (Craver et al., 2017). From the studies, it is evident that light quality affects the overall accumulation of phenolic compounds and may depend on the species, however studies on how LED affect accumulation of phenolic compounds in *B. carinata* microgreens remains a gray area and therefore the need to investigate it.

Nitrates are among the main compounds that may negatively affect food safety. Vegetables can accumulate nitrates which are associated with harmful effects on human health, with toxic effects of methemoglobinemia and the possibility of

causing an endogenous formation of carcinogenic N-nitroso compounds. Accumulation of nitrates in vegetables may vary depending on the species, the substrate used for production or the stage of plant growth at harvest.

Several studies reported that microgreens recorded lower levels of nitrates compared to their mature counterparts (Pinto et al., 2015; Ferron-Carrillo et al., 2021) therefore microgreens are commonly considered safe to consume within a healthy diet. As reported by (Ferron-Carrillo et al., 2021) lighting conditions can influence accumulation of nitrates in vegetables thus affecting their quality. The study indicated that white LED induced higher concentration of nitrates more than Blue and Red LED when they were used in production of lettuce microgreens.

Furthermore, in all their experiments Red LED was shown to reduce the concentration of nitrate content in lettuce microgreens. In contrast, a study conducted indicate that amaranth microgreens cultivated using R LED had higher amount of nitrate content accumulation while no difference in nitrates accumulation was noted for turnip microgreens grown using Red and Blue LED (Toscana et al., 2021). In addition, Brazaitytė et al., (2021) indicated that there was lower nitrate accumulation in mustard microgreens grown using B50R50 ratio and B100 R0 ratio combined LED. Similarly, Red LED was found to enhance nitrate content accumulation of beet, mustard, basil and parsley microgreen (Brazaityte et al., 2016). These research shows inconsistencies of the influence of LED on microgreen species indicating that nitrate accumulation is also species dependent hence the need to investigate the influence of LED on *B. carinata* microgreens.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Set Up, Site and Source of Materials

Experiments were conducted in a controlled environment in a locally fabricated walk-in growth chamber at Tokyo University of Agriculture in Japan (35.6411° N, 139.6321° E) between April and December 2023. The chamber was divided into four compartments using black opaque fabric as shown in figure 3.1 to prevent light interferences across the compartments. Each compartment measured 100 cm by 100 cm. In each compartment, a LED fixture was placed such that it was 50 cm above the surface of the substrate. Ethiopian kale (*Brassica carinata*) seeds used in the study were sourced from a commercial vendor in Kenya. Phytosanitary certificate to allow entry of seeds to Japan was obtained from the Kenya Plant Health Inspectorate Service (KEPHIS). *Brassica carinata* was identified by a taxonomist at JKUAT GoK laboratories and a voucher specimen (JMW/JKUAT/BOT/H001) is maintained at the JKUAT herbarium.

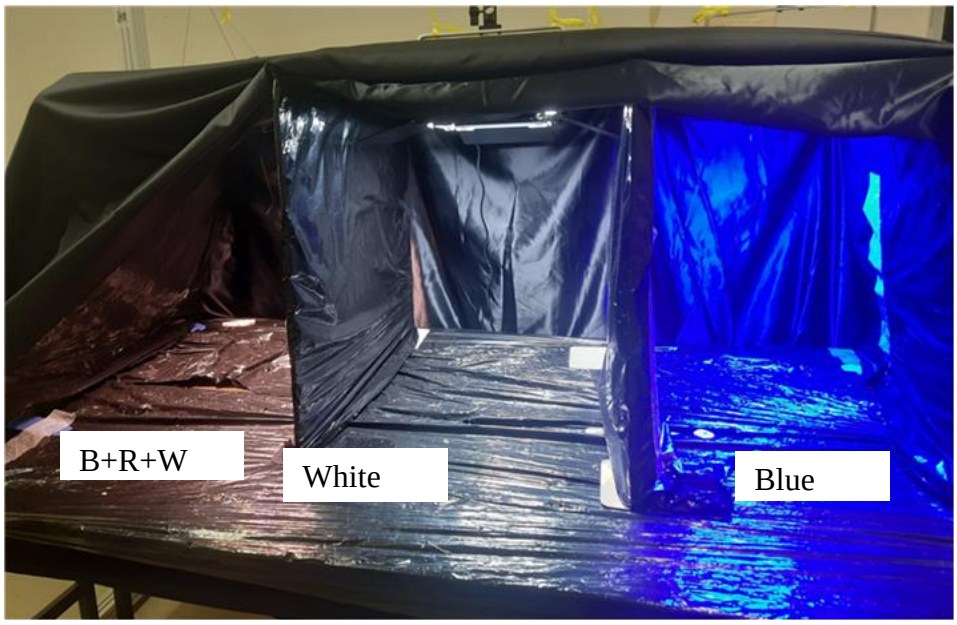

Figure 3.1: A Section of the Chamber and Different Compartments Separated by a Black Opaque Material

3.2 Growth Environment and Experimental Set Up

Seeds of *B. carinata* were sown and grown using three substrates (coco peat, sand and a mix of sand and coco peat in the ratio of 1:1 (v:v) under four LED light spectra in a factorial experiment. The light spectra used were blue (with the peak at 450 nm), red (with the peak at 650 nm), cool white light, and a B+R+W LED (Figure 3.2) in each compartment. The three substrate types (cocopeat, sand and a mix of cocopeat and sand) and one LED light were randomly placed in each compartment to give a split plot design with light being the main plot factor and substrate the subplot factor. There were three replicates for light spectra and twelve for the substrate as shown in the experimental layout (figure 3.3). The plants were allowed to grow until the development of the first set of two true leaves (14 days) and key stages are shown in figure 3.4. The lights had a fixed light intensity of 160 ± 2.5 µmol $m^{-2}s^{-1}$) and a 12h photoperiod was applied. The intensity was chosen based on the recommendation from a previous study on the best intensity for microgreen production (Adasme et al., 2023). The air temperature in the walk-in growth chamber was set and maintained at 26 °C± 2 while relative humidity was maintained at approximately 60% during the experimental period. Temperature and relative humidity were monitored using a data logger (HOBO, Onset Data Logging Solutions, Bourne, MA, USA). Irrigation was done using capillary wick technology (Semananda et al., 2018) as shown in figure 3.5.

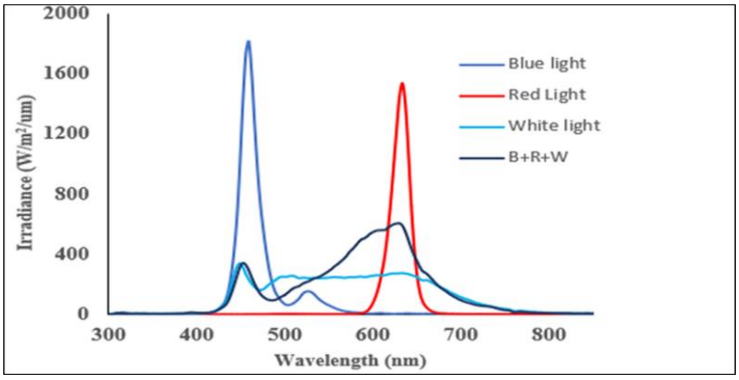

Figure 3.2: Spectral Distribution of LEDs Recorded from a Portable Spectroradiometer

.Red R1	CO. R1	White. R2	SA. R5	Blue.R3	CO. R9
	CS. R1		CO. R5		CS. R9
	SA. R1		CS. R5		SA. R9
Blue.R1	SA. R2	Red.R2	CO. R6	B+R+W.R3	CS. R10
	CS. R2		SA. R6		CO. R10
	CO. R2		CS. R6		SA. R10
White. R1	CS. R3	B+R+W.R2	CO. R7	Red.R3	SA. R11
	SA. R3		CS. R7		CO. R11
	CO. R3		SA. R7		CS. R11
B+R+W.R1	CS. R4	Blue. R2	CO. R8	White. R3	CS. R12
	CO. R4		CS. R8		CO. R12
	SA. R4		SA. R8		SA. R12

Key

Light Spectra

1. Red LED light (650 nm)
2. Blue LED light (450 nm)
3. White LED light
4. B+R+W LED light

Substrate

1. CO: Cocopeat
2. SA: Sand
3. CS: Cocopeat +Sand (1:1)

R: Replication

Figure 3.3: Experimental Layout

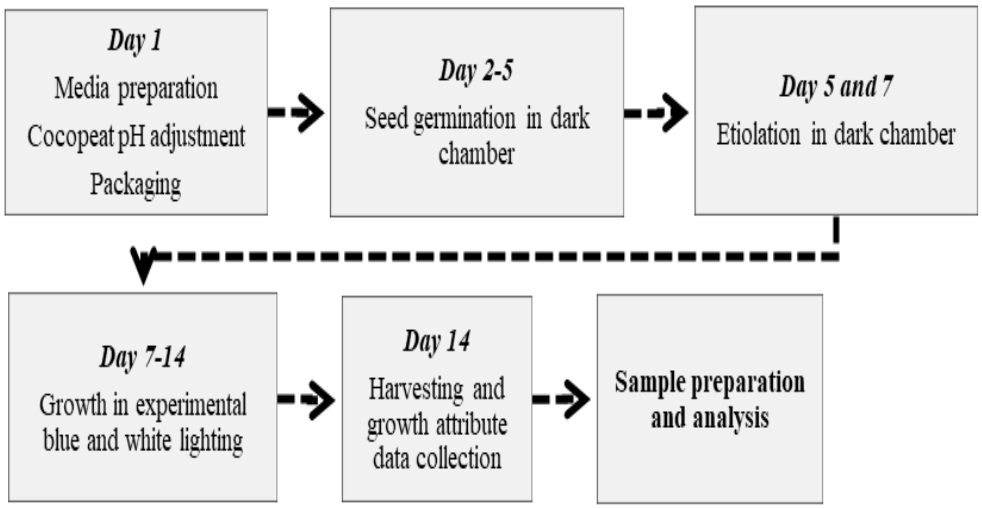

Figure 3.4: Flow Chart Showing Key Stages/Steps during Experimentation

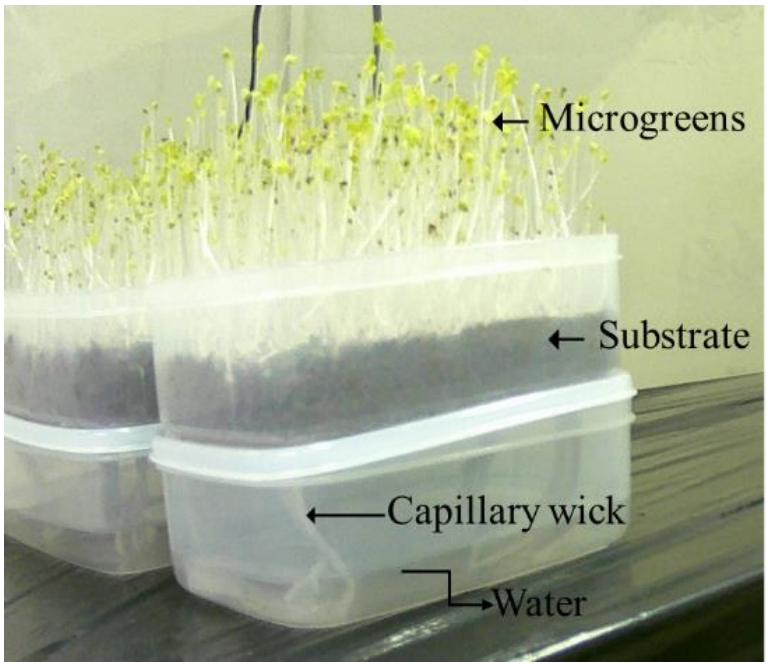

Figure 3.5: Irrigation by Capillary Wick Technology

3.3 Determination of the Effect of Substrate and LED Light Spectra on Growth and Yield of Ethiopian Kale Microgreens

3.3.1Growth Measurements

Growth was assessed at the end of the experiment (14 days after sowing following the appearance of the first two true leaves) (Nakakaawa et al., 2023) in terms of height, leaf area and canopy cover. Ten plants were randomly selected from each treatment and harvested for height and leaf area measurements. The plants were harvested by cutting at the base and above the substrate. The individual height of each plant was measured using a ruler.

Leaf area values were estimated using ImageJ v.1.5 software (Schneider, 2012). Leaves from the ten randomly selected plants per treatment were spread on a clean white sheet of paper and photographs were taken against a ruler as reference. Additionally, a square paper of known area (2×2 mm) was included for verification of the measurements obtained.

Canopy cover was estimated using Canopeo software (Patrignani & Ochsner, 2015). This was done by taking aerial photographs of all the above-ground plant materials in each treatment. To achieve uniformity in all the photographs, a 30 cm distance from the camera to the treatment was maintained. The photographs were processed with Canopeo software version (1.1.7), and canopy cover was calculated as a percentage of the total surface area. Image J and Canopeo software are non-destructive methods for assessing growth and therefore were used for this study.

3.3.2 Yield and Biomass Analysis of *Brassica carinata* Microgreens

Yield and dry biomass were obtained by weighing the whole 10 plants per treatment harvested microgreen shoots 14 days after sowing (DAS) which was their horticultural maturity stage. All above-ground parts including the leaves, stems and the cotyledons were harvested by cutting them at the base and fresh weight (yield) and dry biomass weighed (after freeze drying at -41^{o}C for 24 hours) using a weighing balance. The samples were further ground to powder and used for phytochemical analysis.

3.4 Determination of the Effect of Substrate and LED Light Spectra on Phytochemical Content of Ethiopian Kale Microgreens

Samples from the experiment in objective 1 were used to determine phytochemical content of the microgreens following the procedure outlined by Nyonje et al. (2014). The quantified phytochemicals included ascorbic acid, total chlorophyll, total flavonoids, carotenoids and total polyphenols all of which are associated with non-enzymatic antioxidant activities (Björkman et al., 2011). The total nitrate, an anti-nutrient was also determined.

3.4.1 Determination of Flavonoids of *Brassica carinata* Microgreens

The estimation of total flavonoids in the sample was done using the Aluminum chloride method. Rutin was used as the standard according to Baba and Malik (2014). Sample (0.1ml) and standards were prepared in triplicates vortexed and incubated for 5mins at room temperature. Aluminum chloride (10%) was then added after sample incubation. Afterwards, the sample was vortexed and incubated for 6 minutes at room temperature. The absorbance was measured against the blank at 510 nm using a spectrophotometer (Shimadzu model UV-Vis 1601 PC, Kyoto, Japan). The standard curve was plotted, and the regression equation used to determine the total amount of flavonoids in the sample. The total amount of flavonoids in the sample were expressed as milligram of Rutin equivalent (RE)/g of dry weight of sample. Flavonoids content was determined using equation 3.1 below.

$$Flavonoids\ (mg/100g) = 0.0001 * \frac{(A_s - A_b)}{0.0018 * W} * D \qquad \text{Equation 3.1}$$

Where: A_b = absorbance of blank, A_s = absorbance of sample, D = dilution factor (30), W = weight of sample (g). 0.0018 is the slope of the standard curve while 0.0001 is the factor for conversion to mg/100g.

3.4.2 Determination of Carotenoids of *Brassica carinata* Microgreens

Total carotenoids were extracted using acetone and analyzed using column chromatography and UV Spectrophotometer, according to Nyonje et al., (2014). Approximately 0.08g of dried sample was weighed in triplicates and ground in a mortar containing 10mL acetone and extraction repeated until the residue turned colorless. 25 mL of the extract was evaporated to dryness using rotary evaporator and the residue dissolved in 10 mL petroleum ether and the solution introduced into a chromatographic column (Rodriguez-Amaya and Kimura, 2004; AOAC, 1996). Absorbance was read at 450 nm in a UV-Vis spectrophotometer (Shimadzu model UV-Vis 1601 PC, Kyoto, Japan). Equation 3.2 shown below was used to calculate carotenoids from absorbance.

$$Carotenoids\ (mg/100g) = 0.001 * \frac{A}{2592 * W} \qquad \text{Equation 3.2}$$

Where: A = absorbance and W = weight of sample (g), 2592 is the absorption coefficient of β-carotene in petroleum Ether.

3.4.3 Determination of Nitrates of *Brassica carinata* Microgreens

The nitrate content in the test samples was determined by the calorimetric method using salicylic acid according to Nyonje et al. (2014). Dried samples of *B. carinata* (0.3g) were weighed in triplicates and put in a test tube. Hot (90-95°C) distilled water measuring 10 ml was added. The closed tubes were placed in a water bath at 80 °C and shaken for 30 minutes. The samples were then cooled and centrifuged at 4500 rpm. Chlorophyll in the sample was be removed by adding 0.5 g $MgCO_3$ to the supernatant and centrifuged again. The supernatant containing the nitrate extract was then treated with NaOH and a combination of salicylic acid and H_2SO_4. Nitrate standards were prepared using sodium nitrate calibration curve. Absorbance was read at 410 nm in UV-V is spectrophotometer (Shimadzu model UV-Vis 1601 PC, Kyoto,

Japan). Nitrate concentration was expressed on dry weight basis (mg/100g DW). Equation 3.3 shown below was used to calculate nitrates from absorbance.

$$Nitrates\ (mg/100g) = 0.1 * \frac{A_s - A_b}{0.0078 * W} * D \qquad \text{Equation 3.3}$$

Where: A_b = absorbance of blank, A_s = absorbance of sample, D = dilution factor (30), W = weight of sample (g). 0.0078 is the slope of the standard curve while 0.1 is the factor for conversion to mg/100g.

3.4.4 Determination of Chlorophyll Content of *Brassica carinata* Microgreens

Chlorophyll was extracted using acetone and analyzed using column chromatography (AOAC, 1990; Rodriguez-Amaya & Kimura, 2004) and UV Spectrophotometer (Shimadzu model UV-Vis 1601 PC, Kyoto, Japan), according to Wellburn et al., (1994). Approximately 0.08g dry sample was weighed and ground in a mortar containing 10mL acetone. The extraction was repeated until the residue turned colorless. An aliquot of 25 mL of the extract was evaporated to dryness using a rotary evaporator and the residue dissolved in10mL petroleum ether. The solution was introduced into a chromatographic column and absorbance read at 645 nm and 663 nm in a UV-Vis spectrophotometer (Shimadzu model UV-Vis 1601 PC, Kyoto, Japan). Chlorophyll A and B were determined by computation from the absorbance using equations 3.4a, 3.4b and 3.4c as shown below.

For Chlorophyll A (mg/100g)

$$ChlA = 0.1 * (9.93 * A_{663} - 0.78 * A_{645}) * \frac{D}{W} \qquad \text{Equation 3.4a}$$

For Chlorophyll B (mg/100g)

$$ChlA = 0.1 * (17.60 * A_{645} - 2.81 * A_{663}) * \frac{D}{W} \qquad \text{Equation 3.4b}$$

For Total Chlorophyll (mg/100g)

$$ChlA = 0.1 * (7.12 * A_{663} + 16.8 * A_{645}) * \frac{D}{W}$$ Equation 3.4c

Where: A = absorbance at indicated wavelength (645 or 663), D = dilution factor (25), W = weight of sample (g)

3.4.5 Determination of Total Phenols of *Brassica carinata* Microgreens

Total phenols were determined using the Folin-Ciocalteau method using Gallic acid as standard according to Meas et al., (2020). Approximately 0.08g dry sample of *B. carinata* was prepared and weighed. Standards and blank solutions were also prepared. In addition, Folin-Ciocalteau reagent was then added to all the samples including the blank and vortexed. Further, 5% sodium carbonate was added and left to sit for 40mins in the dark at room temperature. The absorbance was observed and measured at 725 nm using a UV-VIS spectrophotometer (Shimadzu model UV-Vis 1601 PC, Kyoto, Japan). The standard curve was then plotted and the total phenolic content in the sample expressed as mg of gallic acid equivalents (GAE) /g of dry weight extract (DW). Equation 3.5 shown below was used to calculate total phenols from the absorbance.

$$Total\ Phenols\ (mg/100g) = 0.1 * \frac{A_s - A_b}{0.0177 * W} * D$$ Equation 3.5

Where: A_b = absorbance of blank, A_s = absorbance of sample, D = dilution factor (40), W = weight of sample (g). 0.0177 is the slope of the standard curve while 0.1 is the factor for conversion to mg/100g.

3.4.6 Determination of Vitamin C content of *Brassica carinata* Microgreens

Vitamin C content was analyzed using rapid reflectometric test (Reflect quant ascorbic acid test) using a RQflex hand-held reflectometer (Merk, Darmstadt, Germany). Approximately 0.2g of dried *B. carinata* sample extracts were prepared.

Metaphosphoric acid (15%) was then added to the sample and a homogenizer (polyvinylpolypyrrolidone) was added as well. The sample was mixed and centrifuged at 6000rpm for 5 minutes to allow separation of the supernatant that was used to measure Vitamin C content using Reflect quant ascorbic acid test strips. Equation 3.6 shown below was used to compute Vitamin C content.

$$Vitamin\ C\ (mg/100g) = \frac{RQ*W*M.A}{W} * 0.1 \qquad \text{Equation 3.6}$$

Where RQ is the reading from Reflect quant ascorbic acid test strips, W= weight of the sample (g), M.A is the metaphosphoric acid weight (g) and 0.1 is the factor for conversion to mg/100g

3.5 Data Analysis

Statistical analysis was performed using R software, version (4.3.2). Growth measurements (leaf area and plant height) were analyzed based on the individual values of the 10 sampled plants from each subplot while (canopy cover, yield and dry weight) were analyzed at the sub- plot level. All data were subjected to Analysis of Variance (ANOVA) and significant differences among means were determined by Tukey's multiple comparison test at $P \leq 0.05$. Tukey's test is a post hoc test recommended for pairwise comparison that controls for overall treatment error rate hence prevents type I error.

CHAPTER FOUR

RESULTS

4.1 Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover of *Brassica carinata* Microgreens

There were no significant ($P > 0.05$) interactions between substrates and LED light treatments on height, leaf area and canopy cover while each factor affected the parameters significantly. Height differed significantly in response to both different substrates and LED light treatments (Table 4.1). The microgreens grown using monochromatic R were significantly shorter compared to those grown using other LEDs. More specifically, microgreens grown under monochromatic R were 7.6% shorter compared to those under monochromatic B. Microgreens grown under Blue, White and in combination of Blue Red and White did not differ significantly in height.

Microgreens grown in either sand alone or cocopeat-sand mix were significantly taller than those grown in cocopeat alone ($F(3,108) = 11.86$, ($P \leq 0.001$). Microgreens grown in cocopeat were shorter than those grown in sand and cocopeat-sand mix by 8%. Both substrate and LED treatment had a significant effect on leaf area (Table 4.1). Microgreens grown under B+R+W had significantly higher leaf area (68.11 mm^2) compared to microgreens grown under W (63.43mm^2) and both under monochromatic B and R (57.62 mm^2 and 57.36mm^2) respectively. Leaf area of microgreens grown in cocopeat-sand mix was significantly higher by 22 % (65.6 mm^2) compared to microgreens produced using cocopeat alone (59.1 mm^2).

Both the growing substrate and LED treatments had significant effect on canopy cover (Table 4.1). Canopy cover values under B+R+W treatment was significantly higher (55.15%) than those produced in monochromatic R (44.45%). On the other hand, microgreens grown in sand had a significantly higher canopy cover (56.0%) compared to those in cocopeat (47.1%).

Table 4.1: Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover of *Brassica carinata* Microgreens

Treatment	Height (cm)	Leaf Area (cm^2)	Canopy Cover (%)
LED Lights			
B	9.9 (0.16) [a]	57.62 (1.40) [c]	50.68 (4.51) [a]
R	9.2 (0.16) [b]	57.36 (1.46) [c]	44.45 (2.66) [b]
W	9.7 (0.18) [a]	63.43 (1.56) [b]	56.39 (2.85) [a]
B+R+W	9.8 (0.11) [a]	68.11 (1.96) [a]	55.15 (2.76) [a]
P	0.011	< 0.001	<0.001
$LSD_{0.05}$	0.39	4.32	5.87
F Value	F (3,108) =3.92	F (3,108) =11.18	F (3,33) =13.12
Substrates			
Sand	9.8 (0.13) [a]	60.0 (1.36) [b]	56.0 (3.26) [a]
Cocopeat	9.2 (0.12) [b]	59.1 (1.44) [c]	47.1 (2.07) [b]
Sand + Cocopeat	9.9 (0.14) [a]	65.6 (1.66) [a]	51.9 (3.394) [ab]
P	<0.001	0.001	0.005
$LSD_{0.05}$	0.34	3.74	5.08
F Value	F (3,108) =11.86	F (3,108) =7.28	F (3,33) =12.02

Mean separation by the Tukey test at the 5% significant level. Values in brackets are standard errors of means. Values labeled with different letters in a column within a factor are significantly different ($P \leq 0.05$).

4.2 Effect of LED Light and Substrate on Yield and Biomass of *Brassica carinata* Microgreens

There was no significant interaction between substrates and LED light on yield and biomass. Similarly, no significant differences in yield were noted among LEDs. Regarding the effects of LEDs on dry weight, significant differences were noted between monochromatic R and all other LEDs (Table 4.2). No significant ($P>0.05$) differences were noted between B+R+W and monochromatic B while R differed significantly ($P \leq 0.05$) from B, W and B+R+W (Table 4.2). Dry matter among the

substrates ranged from about 1.0g (cocopeat) to 1.3g (Sand). The microgreen yield in sand and cocopeat-sand mix differed significantly from cocopeat alone (Table 4.2). Sand alone had dry weight that was not significantly different from cocopeat-sand mix but from cocopeat alone (Table 4.2).

4.3 Effect of LED Light and Substrate on Phytochemical Content of *Brassica carinata* Microgreens

4.3.1 Carotenoids

There were significant differences for carotenoids among LED lights ($F_{(3,24)} = 1270.56$, $P \leq 0.001$), substrates ($F_{(2,24)} = 50.24$, $P \leq 0.001$) and their interactions ($F_{(6,24)} = 1814.12$, $P \leq 0.001$). Microgreens under B+R+W light in cocopeat had the highest carotenoids (644.4 mg kg^{-1} DW). Under monochromatic B and R, more carotenoids were found in sand compared to cocopeat and in cocopeat-sand mix. Under W and B+R+W in cocopeat had higher carotenoids relative to those in sand alone and cocopeat-sand mix (Fig.4.1A).

4.3.2 Flavonoids

Flavonoids similarly showed significant differences among LED lights ($F_{(3,24)} = 100.77$, $P \leq 0.001$), substrates ($F_{(2,24)} = 98.23$, $P \leq 0.001$) and interactions ($F_{(6,24)} = 105.09$, $P \leq 0.001$). Monochromatic B and B+R+W had higher flavonoids in sand than in cocopeat alone as well as in cocopeat-sand mix. Under monochromatic B in sand, flavonoids were 16.8% higher than in cocopeat and 32.4 % higher than in cocopeat-sand mix. For B+R+W in sand, flavonoids were 11.5% higher than in cocopeat and 12.0 % higher than in cocopeat-sand mix. Monochromatic R had higher flavonoid in sand alone than in cocopeat alone by 4.6% but less than in cocopeat-sand mix by 15.7%. Similarly, under W in sand had 9.8 % more flavonoids than in cocopeat alone but less by 6.3% in sand alone than in cocopeat-sand mix (Fig.4.1B).

Table 4.2: Effect of LED Light and Substrate on Yield and Dry Weight of *Brassica carinata* Microgreens

Treatment	Yield (g)	Dry weight (g)
LED Lights		
B	17.9 (1.94) [a]	1.2 (0.11) [b]
R	16.0 (0.86) [a]	1.0 (0.06) [c]
W	18.8 (2.36) [a]	1.3 (0.17) [a]
B+R+W	19.5 (2.22) [a]	1.2 (0.10) [b]
P	0.339	0.053
$LSD_{0.05}$	3.73	0.23
F (3, 33)	1.28	5.38
Substrates		
Cocopeat	15.2(1.75) [b]	1.0 (0.08) [a]
Sand	19.2(1.54) [a]	1.3 (0.10) [b]
Cocopeat + Sand	19.8 (1.76) [a]	1.2 (0.13) [ab]
P	0.013	0.016
$LSD_{0.05}$	3.23	0.20
F (2, 33)	11.29	13.14

Mean separation by the Tukey test at the 5% significant level. Values in brackets are standard errors of means. Values labelled with different letters in a column within a factor are significantly different ($P \leq 0.05$).

4.3.3 Total Chlorophyll

Total chlorophyll content differed significantly among LED light ($F_{(3,24)} = 2690.47$, $P \leq 0.001$) and substrates ($F_{(2,24)} = 6647.47$, $P \leq 0.001$). In addition, the interaction between substrate and lights was significant ($F_{(6,24)} = 2957.42$, $P \leq 0.001$). Except for W, total chlorophyll content under monochromatic B, R and B+R+W was higher in sand compared to cocopeat. The highest total chlorophyll content (11880 mg kg^{-1}) was observed under monochromatic B in sand while the lowest (3100 mg kg^{-1}) was under monochromatic B in cocopeat, a reduction by 73.9%. The chlorophyll content under B+R+W was higher in sand by 26.1% compared to B+R+W in cocopeat substrate while for monochromatic R it was 34.5% higher in Sand than in cocopeat (Fig. 4.1C).

4.3.4 Nitrates

There were significant differences for nitrates among LED lights ($F_{(3,24)} = 1696.07$, $p < 0.001$), substrates ($F_{(2,24)} = 110.47$, $P \leq 0.001$) and interactions (($F_{(6,24)} = 983.54$, $P \leq 0.001$). Microgreens under B+R+W in cocopeat had extremely higher nitrates (966.2 mg kg-1 DW) compared to other treatments. Except under W and B+R+W, nitrates were higher in sand than in cocopeat. Under monochromatic B, nitrates in sand were higher by 53.4% compared to cocopeat while for monochromatic R it was 30.3% higher in sand compared to cocopeat (Fig.4.1D).

4.3.5Total Phenols

There were significant differences for phenols among the substrates used for growing *B carinata* microgreens ($P \leq 0.001$). Specifically, microgreens grown using Cocopeat-sand mix a showed significantly higher amount of phenol content compared to those grown using cocopeat alone. There were significant differences for phenols or the interactions among LED lights ($P \leq 0.001$), substrates ($P \leq 0.001$) and their interactions ($P \leq 0.001$). Microgreens grown under monochromatic R LED and in cocopeat-sand mix had the highest phenols (8.1 mg kg-1 DW). Under monochromatic B, W and B+R+W LED more phenols were found in *B carinata* grown sand compared to those grown using cocopeat (Fig. 4.2A).

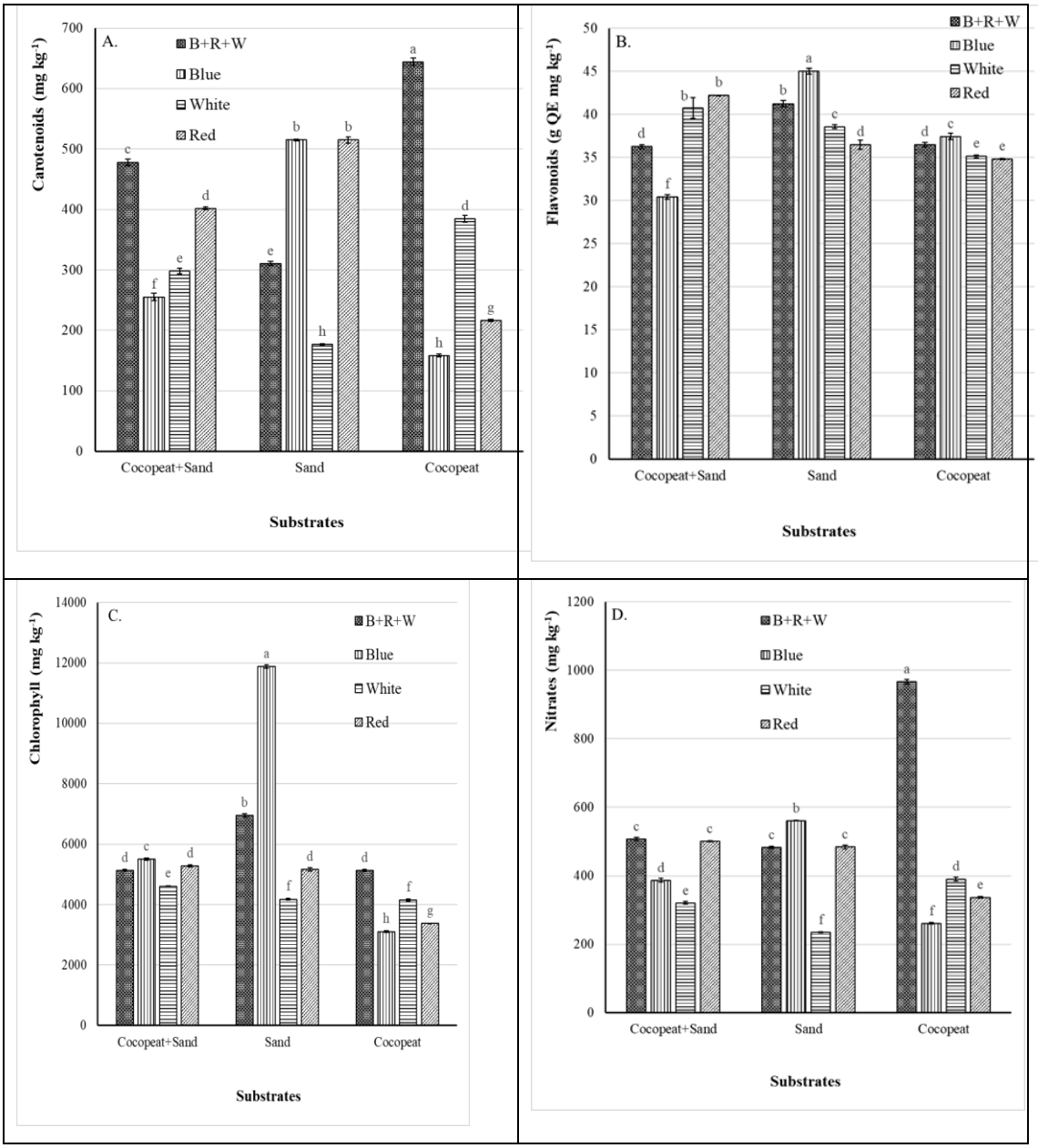

Figure 4.1: Effect of LED Light on Phytochemicals (A: Carotenoid, B: Flavonoid, C: Chlorophyll and D: Nitrates) Under Different Substrates (Coco peat+ Sand, Sand and Coco peat). Bars Represent Standard Errors of Means

4.3.6 Vitamin C

Vitamin C content differed significantly among LED light ($P \leq 0.001$) and substrates ($P \leq 0.001$). In addition, the interaction between substrate and lights was significant ($P \leq 0.001$). The highest Vitamin C content (1155.1 mg kg^{-1}) was observed under B+R+W in cocopeat while the lowest content (472.8 mg kg^{-1}) was under monochromatic B in cocopeat substrate. Except for *B. carinata* microgreens grown using monochromatic B and in sand, B+R+W showed higher Vitamin C content in cocopeat-sand mix and in cocopeat. For *B. carinata* microgreens grown using W LED low Vitamin C content was observed in those produced using sand substrate (Fig. 4.2B).

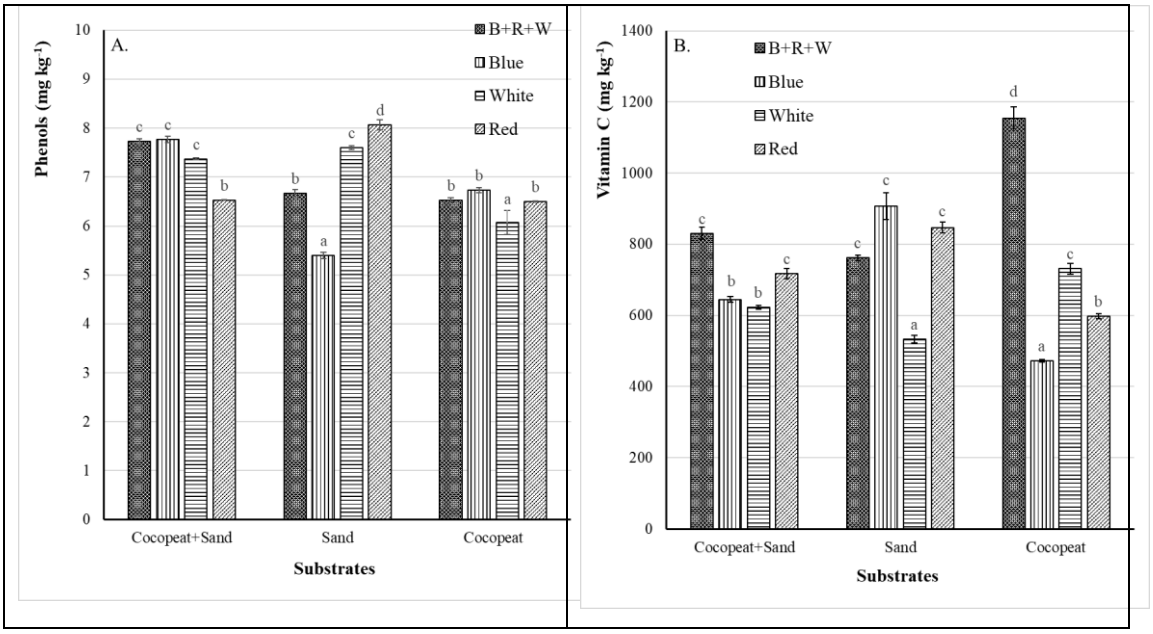

Figure 4.2: Effect of LED Light on Phytochemicals (Phenols and Vitamin C) under Different Substrates (Coco peat+ Sand, Sand and Coco peat). Bars Represent Standard Errors of Means

CHAPTER FIVE

DISCUSSION

5.1 Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover of *Brassica carinata* Microgreens

In recent years, several scientific reports addressed the role of light in stimulating specific plant photoreceptors, allowing plants to be manipulated to produce desirable phytochemicals and nutrients. Lighting systems for indoor farming can therefore be designed to maximize growth, control morphology, and optimize yield (Davis & Burns, 2016). This study established that *B. carinata* grown under monochromatic B were significantly taller compared to those grown using a monochromatic R source contrary to earlier scientific reports where, monochromatic B has been documented to decrease hypocotyl elongation. For example, stem length of baby lettuce decreased by 33% when a supplemental B treatment was provided (Qian et al., 2016). Furthermore, lettuce grown using an increased ratio of red radiation had increased shoot height and shoot: root ratio compared to those grown using a blue light source (Son et al., 2015). Inconsistencies in results on the effect of different spectral regions across plant species and phenological stages have been acknowledged as gray area requiring further research (Naznin et al., 2019). Monochromatic B and in combination with far-red were found to increase mustard (*Brassica juncea*) and arugula (*Eruca sativa*) microgreens elongation (defined as plant height) (Ying et al., 2020). Therefore, the effect of LED lights depends on the species and the light combinations.

The results of this study show that sand alone or in cocopeat-sand mix had better growth than cocopeat indicating that these substrates provided a better growing environment. This could be due to the physiochemical properties such as low water retention capacity hence allowing good aeration as compared to cocopeat which could have retained excessive moisture potentially leading to anoxia conditions. Similarly, Awang et al. (2009) reported that using cocopeat based mixes with other coarser materials such as burnt rice hull improved growth of *Celosia cristata.*

The present research also found that B+R+W and white light resulted in better yield performances than monochromatic red or blue. This was previously associated with synergistic effects of the different spectral regions (Naznin et al., 2019). Red light combined with varying ratios of blue has been reported to enhance growth characteristics of lettuce, spinach, kale, basil, and sweet pepper compared to red light alone (Naznin et al., 2019). Similarly, leaf area among other growth parameters of lettuce increased with increasing the proportion of red light in combination with blue (Son et al., 2015). For leaf area and canopy cover, B+R+W LED in the ratio of 1:1:1 and cocopeat-sand mix enhanced the leaf growth of *B. carinata* microgreens. In this study, cocopeat based substrate (cocopeat-sand mix), showed increased leaf area of *B carinata* microgreens. Similar results were reported by (Gunjal et al., 2024) who found that cocopeat based substrate increased plant growth, yield, nutritional, biochemical composition, and antioxidant activity of various microgreens species. These positive effects were attributed to enhanced nutrient acquisition, water retention and root development.

5.2 Effect of LED Light and Substrate on Yield and Biomass of *Brassica carinata* Microgreens

Yield is an important parameter in microgreen production because they are sold on fresh weight basis (Ying, 2020). One of the limiting factors in microgreen production remains to be low yield due to various elements (Bulgari et al., 2017). Microgreen yield can be affected by seed quality (Nolan, 2018), growing media (Thuong and Minh, 2020), and light quality and intensity (Jones-Baumgardt et al., 2019) among others. In this study, both substrate and light spectrum significantly affected the yield and dry matter accumulation for *B. carinata.* Notably, the yield of microgreens varied across the different light spectra used, being highest under W. Results obtained are similar to those reported in literature where fresh weight which was used as a measure of yield responded differently in plants grown using different light spectra. On the other hand, in the hereby presented experiments, increase in yield also depended on the substrate used.

For *Brassica carinata* microgreens, higher yield was recorded in sand alone or in cocopeat-sand mix. In previous research comparing different substrates, yield of sunflower microgreens was significantly affected by the type of substrate used (Thepsilvisut et al., 2023). Dry mass yield is a good indicator of crop productivity and photosynthetic efficiency in microgreens (Liu et al., 2010). In this study highest dry matter accumulation was in microgreens grown using W. Conversely, microgreens grown in cocopeat using R had the lowest dry matter accumulation. Therefore, a significant effect resulting from substrate was observed in the trial indicating the importance of substrate and lighting on the yield of *B. carinata* microgreens. Other studies on dry matter assessment of microgreens seem to indicate interspecies variability. For example, Demir et al., (2023) found differences in dry mass accumulation within W and R for broccoli, cabbage, and radish microgreens.

5.3 Effect of LED Light and Substrate on Phytochemical Content of *Brassica carinata* Microgreens

5.3.1 Carotenoids

Carotenoid compounds namely lutein and β-carotene are vital components of microgreens. Lutein has been associated with macular protection against oxidative damage and degeneration while β-carotene is a precursor of vitamin A, essential for growth, visual and immune functions (Kyriacou et al., 2020). Therefore, higher carotenoid concentration in microgreens is mainly appreciated by consumers due to their nutritional value.

Microgreens grown using B+R+W and in cocopeat had higher amounts of carotenoids. This is consistent with previous observations on the effect of light treatments on carotenoid accumulation in plants, where R + B combination increased carotenoid accumulation in lettuce, spinach and pepper (Naznin et al., 2019) while in kale and basil carotenoids accumulation was increased in monochromatic B. Earlier studies also demonstrated that R:B combinations positively influenced carotenoid accumulation in lettuce (Son et al., 2015). Conversely, however, enzymatic activities involved in the metabolic pathways of carotenoid pigments were largely increased

under monochromatic B, resulting in higher carotenoid accumulation in Chinese cabbage (Qian et al., 2016).

For *Brassica* sprouts, carotenoid transcription genes, namely, PSY, βLCY and βOHASE1 was enhanced when higher B percentage compared to R LED (Frede et al., 2023) therefore increasing the carotenoid accumulation in the sprouts. Similar results associated with combined spectrum (resulting from integration of blue, red and amber diodes), that enhanced transcription of a gene involved in carotenoid biosynthesis (PSY), leading to higher carotenoid accumulation in various *Brassica* plants (Alrifai et al., 2021).

In the present study, the results are consistent where the treatment B+R+W often presented higher amounts of carotenoids. Such findings corroborate the concept that combined light spectra are superior to monochromatic B or R light supply. On sand substrate carotenoids were higher under monochromatic R and monochromatic B. These two spectra may have boosted photosynthesis, and therefore leaf transpiration, a scenario that could have led to drought stress ultimately inducing carotenoids biosynthesis and accumulation. Further studies on water retention in sand (compared to other substrates) and how it influences carotenoid accumulation are needed to provide conclusive explanation.

5.3.2 Flavonoids

Flavonoids are important plant compounds that are produced because of stress to prevent DNA damage (Samuoliene et al., 2012). Light quality trigger different transcriptional genes that are used for biosynthesis of flavonoids and could cause differences in the levels of flavonoid accumulation in plants (Harbart et al., 2023).

In the current study, both monochromatic B and B+R+W enhanced the accumulation of flavonoid content in *B. carinata* microgreens grown on sand and cocopeat substrates, just as did monochromatic R and W in cocopeat-sand mix. An earlier study indicates that monochromatic B highly influenced accumulation of flavonoid by modulating phenylpropanoid pathway, a pathway in which most plant secondary metabolites are synthesized (Landi et al., 2020).

The adoption of R: B combinations at low intensities was formerly found to increase accumulation of flavonoids in lettuce (Jiang et al., 2022). This could have resulted from the influence of different R: B ratios on the phenylalanine ammonia lyase (PAL), chalcone synthase (CHS) and other enzymes involved in the flavonoid biosynthesis, ultimately leading to accumulation of flavonoids (Wu et al., 2020). Similarly, a study conducted on the effect of combined ratios of B and R LEDs on bioactive compounds of two lettuce cultivars found that increasing ratios of B LED increased accumulation of flavonoid content as compared to R (Son et al., 2013).

In addition, for *Scrophularia kakudensis*, flavonoid accumulation was higher in monochromatic B and R than in W (Manivannan et al., 2021). Furthermore, these effects of light were also influenced by the substrate used (although different from those adopted in this study). While monochromatic B enhanced flavonoids accumulation in cocopeat and sand, R and W enhanced the same phytochemical in cocopeat-sand mix. These subtle differences point toward a substrate-light interaction, as also previously hypothesized by Saleh (2023).

5.3.3 Chlorophyll

Besides its role as photosynthetic pigment, total chlorophyll content is also one of the key indicators of quality in vegetables, as the green color indicates freshness which leads to product acceptability or rejection by consumers. In microgreens, vivid and intense colors are particularly appreciated, and tend to influence consumer preference (Barrett et al., 2010). Chlorophylls represent part of light-harvesting complex and therefore play a significant role in photosynthesis. As reported in the literature, significant genotypic variations were observed for chlorophyll content in microgreens, with their level also being highly dependent on the lighting conditions (Bulgari et al., 2021; Lobiuc et al., 2017).

In the present study, monochromatic B increased chlorophyll biosynthesis and accumulation in plant tissues. The role of B in boosting chlorophyll accumulation was evidenced in previous studies, both due to increased photosynthetic efficiency as well as a concentration factor (e.g., as a consequence of lower leaf extension as compared with spectra with a higher R fraction (Lobiuc et al., 2017; Pennisi et al.,

2019). Blue light improves expression of genes such as MgCH, GluTR and FeCH, involved in chlorophyll biosynthesis, while red light may lead to a reduction in 5-aminolevulinic acid, a tetrapyrrole precursor required for chlorophyll synthesis (Fan et al., 2013). Similarly, a study conducted on the effect of combined ratios of B and R LEDs on bioactive compounds of two lettuce cultivars found that increasing ratios of B LED increased accumulation of flavonoid content as compared to R (Son et al., 2013).

Furthermore, when a monochromatic R, a monochromatic B and a combination of R and B ratio (with R:B=6), were alternatively applied to Chinese cabbage, a lower chlorophyll content was associated with monochromatic R, as a result of reductions in the synthesis of chlorophyll precursors including ALA, Proto IX, Mg-Proto IX and protochlorophyllide (Fan et al., 2013). Analyzing the effect of the tested substrates, higher chlorophyll content was observed in *B. carinata* grown using sand compared to those grown using cocopeat which could have contributed to the higher yield observed for the same treatments. The use of sand for microgreen production is not common. Elsewhere the use of sand as substrate is reported as an additive to another substrate (Thuong and Minh, 2020). The effects of sand as a microgreen substrate may thus require some further investigation, e.g., by using different mixture combinations.

5.3.4 Total Phenols

The content of phenolic compounds is an important quality index of microgreens, and the accumulation of phenolic phytochemicals can be stimulated by cultivation under different LEDs (Fig 2). The effect of light quality in the synthesis of phenolic compounds in our present study was different depending on the substrate and LED used for growing *B. carinata* microgreens. Light quality has been shown to induce phenolic compound accumulation especially in the blue region as a key enzyme phenylalanine ammonia lyase (PAL) in the synthesis of phenolics is highly influenced. Specifically, phenolic synthesis control is done by the transformation of hydroxycinnamic acids, from the *trans* form, strong inhibitors of PAL, to the *cis* form, less inhibitory by blue light (Lobiuc et al., 2017).

In this study, monochromatic R enhanced phenol content accumulation of *B. carinata* microgreens grown in cocopeat-sand mix, just as monochromatic B and B+R+W LEDs in sand substrate. Similarly, in a pervious study that evaluated effect of light treatment in two different cultivars found that higher ratios of R to B LEDs increased phenolic compound accumulation in the green onion cultivar while decreased ratios of R to B recorded higher accumulation in the red cultivar (Lobiuc et al., 2017). In addition, a previous study found that higher phenolic compound accumulation was associated with higher ratio of R:B LEDs in broccoli microgreens and lower phenolic compounds were found in those grown using monochromatic R only (Liang et al., 2022).

In contrast, monochromatic B was found to enhance phenolic compound accumulation in both turnip and amaranth microgreens while monochromatic R and W LEDs were shown to decrease phenolic compounds synthesis in both species (Toscano et al., 2021). Furthermore, increased B ratios was also found to increase phenolic compounds of lettuce as compared to R LED (Son et al., 2015). Moving to the effects of substrates on phenolic compound accumulation, higher accumulation was found in substrate that mixed cocopeat and sand. Such results have not been found before and it can only be hypothesized that it was due to lack of physiological stress afforded by optimal root development in Cocopeat-sand mix. Elsewhere, when cocopeat was used as a substrate it resulted in higher accumulation of phenolic compounds in coriander, pakchoi and Kohlrabi microgreens compared to synthetic substrates (Kyriacou et al., 2020). However, the influence substrate and light interactions on phenolic compounds accumulations remains a gray area and therefore the need to be investigated further.

5.3.5 Vitamin C

Vitamin C, also known as ascorbic acid, is a vital health promoting compound which is necessary for growth, development and repair all body tissues beside being a co factor for many enzymes. Beside other factors, artificial light has been shown to influence Vitamin C accumulation in microgreens and sprouts. Specifically, Chinese kale microgreens grown using a combination of R and B LEDs of different ratios

under lower intensities were found to accumulate higher Vitamin C content than those grown in the dark (Liu et al., 2022). Similarly, our current study found that B+R+W LED in cocopeat enhanced Vitamin C content accumulation in *B. carinata* microgreens while those grown using monochromatic B and in cocopeat accumulated lower Vitamin C content. In contrast, a previous study found that turnip microgreens grown using B LED accumulated more Vitamin C content compared to those grown using R and W LEDs.

In addition, turnip microgreens accumulated more Vitamin C than amaranth microgreens grown using the same LEDs (Toscano et al., 2021). This study also indicated that Vitamin C concentration is also highly dependent on the species studied. A record of inconsistences has been shown by a previous study that evaluated different broccoli microgreens under monochromatic B, R and W LED and found no differences in Vitamin C accumulation in broccoli microgreens grown in all the three different LEDs (Liang et al., 2022). Analyzing the effect of the substrates tested, Vitamin C accumulation was enhanced when *B. carinata* microgreens was produced using cocopeat substrates. Production of microgreens using cocopeat is common as it is associated with good physiochemical characteristics such as good water holding capacity and aeration which contribute to optimal root development (Awang et al., 2009).

In contrast, a previous study found that Kohlrabi microgreens grown using cocopeat and peat moss substrates recorded the lowest Vitamin C accumulation compared to synthetic substrates such as cellulose sponge and agave fiber (Kyriacou et al., 2020). The study indicates that the concentration of Vitamin C among other phytochemicals was primarily influenced by the species. The inconsistencies in the reports on effect of light quality on microgreens alone and the inadequate information on the interactions between substrates and LEDs effects on Vitamin C accumulation require further investigation.

5.3.6 Nitrates

Nitrates are among the main compounds that may negatively affect food safety. Vegetables can accumulate nitrates which are associated with harmful effects on

human health, with toxic effects of methemoglobinemia and the possibility of causing an endogenous formation of carcinogenic N-nitroso compounds. Accumulation of nitrates in vegetables may vary depending on the species, the substrate used for production or the stage of plant growth at harvest. In the case of species, *Brassicaceae* have been found to be hyper-accumulators of nitrates (Kyriacou et al., 2020). Several studies reported that microgreens recorded lower levels of nitrates compared to their mature counterparts (Ferrón-Carrillo et al., 2021; Pinto et al., 2015). Therefore, microgreens are commonly considered safe to consume within a healthy diet.

Lighting conditions can influence accumulation of nitrates in vegetables thus affecting their quality (Ferrón-Carrillo et al., 2021). The current study found that B+R+W enhanced the accumulation of nitrates content in *B. carinata* microgreens grown using cocopeat while W LED depressed the accumulation of the same. Our results contrast with a previous study that examined lettuce microgreens grown using different LEDs and found that lettuce microgreens grown using W LED accumulated higher levels of nitrates compared to those grown using B and R LEDs. Furthermore, lettuce produced using R LED recorded the lowest amount of nitrate levels (Ferrón-Carrillo et al., 2021). Regarding the substrates, the result contrasts with what was reported before, that evaluated microgreens grown on different substrates and found significantly lower concentration of nitrate in microgreens grown using cocopeat substrate (Bulgari et al., 2021; Poudel et al., 2023).

In this study, cocopeat showed a higher amount of nitrate content compared to the other substrates. This could possibly be because of the differences in the lighting sources during cultivation. Notably, no such results have been reported on microgreens and possibly this assumption could be further investigated. However, a study that evaluated microgreens grown using synthetic and natural fiber substrates found relatively higher nitrates accumulation in *Brassicaceae* and coriander microgreens grown using natural fiber substrates such as cocopeat compared to synthetic substrates (Kyriacou et al., 2020). This could have been due to the presence of abundance macropores in the natural fibers that exert lower water suction

facilitating transpiration and therefore cause increased nitrate uptake (Colla et al., 2018).

5.4 Interactive Effects of Light and Substrate on Phytochemicals of *Brassica carinata* Microgreens

This study reports significant interaction between lighting treatments and substrate composition on phytochemical content of *Brassica carinata* microgreens. For example, interaction between cocopeat and B+R+W and the interaction between sand and B enhanced production of all phytochemicals investigated here. Further, cocopeat-sand mix and R exhibit strong interaction except in the accumulation of carotenoids. This suggests that the effect of light was dependent on the substrate. No such results have been previously reported on microgreens. Possibly, the cause of these interactive effects may be associated with either reflective or absorptive attributes of the substrates (Hanrahan & Krueger, 2023). This could be better studied, for example by measuring the light intensity in a sealed box with light turned on and only one substrate at a time.

The incident radiation could be absorbed or reflected depending on the substrate leading to differences in lighting conditions experienced by the microgreens. Sand for instance is known to have the capacity to cause light scattering (Hanrahan & Krueger, 2002) while cocopeat due to its color and texture would be expected to absorb light. The light absorption and reflection are further affected by moisture content which varies across different substrates. It will be good to test this assumption to understand the mechanisms involved in noted interactive effects.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study aimed to investigate the influence of LED light spectrum and different substrates on growth, yield and accumulation of selected phytochemical and bioactive compounds of *Brassica carinata* microgreens. From the results, it can be concluded that suitable substrate and light environment could influence the growth, yield and concentration of bioactive compounds of *B. carinata* microgreens. Specifically, combining various light spectra (B+R+W) and using substrates resulting from cocopeat-Sand mix were beneficial to improve yield, biomass and morphological qualities such height, leaf area and canopy cover. In addition, phytochemical content such as carotenoids, Vitamin C and flavonoid accumulation were also promoted under the same conditions. Monochromatic B LED could promote the accumulation of chlorophyl content of *B. carinata* microgreens while monochromatic R LED could offer a better chance of obtaining higher accumulation of total phenols in *B. carinata* microgreens. Moving to anti-nutrient content of *B. carinata* microgreens, a combination of various light spectra (B+R+W) and in cocopeat tend to promote nitrate accumulation and monochromatic LEDs were more effective in producing microgreens with lower levels of nitrate content. Thus, results of this study suggest that substrate and LEDs are important factors for the growth, development and accumulation of secondary metabolites of *B. carinata* microgreens specifically supplemental irradiation with combined (B+R+W) LED or Blue LED alone and using combined sand and cocopeat substrates can improve growth and nutritional quality of *B carinata* microgreens. In addition, a combination of cocopeat with sand is a viable alternative to cocopeat considering the addition benefits of lower costs and ubiquitous availability.

6.2 Recommendations

Based on the findings it is recommended that:

1. Combining various light spectra (B+R+W) together with a combination of sand and cocopeat could be used in production of *B. carinata* microgreens for better growth and nutrient quality.
2. Substrates and LEDs promoting growth and accumulation of phytochemical (B+R+W and cocopeat) also tend to promote nitrate accumulation in *B. carinata* microgreens, especially in brassicaceous ones that are known to be nitrate hyper-accumulators. Therefore, nitrate deprivation practices should be considered for microgreens grown using such substrates and LEDs to minimize consumer exposure to nitrates.
3. To elucidate media-related physical and bio-chemical dynamics that could potentially influence how different lighting systems lead to the varied accumulation of phytochemicals further studies are recommended. Since *B. carinata* microgreens have not been extensively studied (compared to other species), the studies should first focus on the most studied microgreen taxa first. Such an understanding would help to explain the specific influence of the interactions between substrate and light spectrum on quality traits (nutritional value, color, texture, taste, etc.) of microgreens.
4. Some genes could be switched on and off during microgreens production enhancing the growth and accumulation of phytochemicals and therefore further studies on how genes are expressed in microgreens should be done

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