Growing Media Amendments and LED Light Interaction effect on Microgreens Plant Growth and Biochemical Composition

by

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Abstract

The key preharvest factors that can affect the edible quality of microgreens are genotypic characteristics, growing media, climate, and management practices. Microgreens are immature plants of vegetables, herbs, or even wild species grown for their high amount of biochemical compounds, mineral nutrients, and high potential biological functions. Microgreens are delicate and responsive to varying cultivation conditions. The presented study focuses on the formulation and assessment of the impact of different proportions of mixed growing media amendments and the interactive effects of different blue: red ratio of LED light on plant growth indices and bio functional properties of different microgreen species. In the first three experiments, the optimum mixed growing media for microgreens production was developed and the efficacy of two different sources of mushroom compost including White oyster mushroom compost (MC1) and Shiitake mushroom compost was tested. Growth and yield performance of the microgreens were enhanced in growing media that consisted of shiitake mushroom compost. The shiitake-based media improved the physicochemical properties of the mixed growing media. In the fourth experiment, the best media from the previous experiment were selected for optimization to improve porosity and drainage by substitution of mushroom compost with PittMoss. Growing medium containing mushroom compost was the most effective growing media to increase microgreens plant growth, yield, and biochemical composition, as compared to the PittMoss based medium. The final experiment (Experiment 5) evaluated the interaction between the best two selected growing media and different ratios of blue: red LED light on growth and quality of microgreens. Growth and yield parameters of microgreens grown in media containing sawdust were enhanced by a larger red fraction of light. Biochemical compositions and antioxidant capacity in those plants grown in media containing PittMoss under a slightly higher proportion of red LED light can be associated with growth-defense trade-off mechanism. Overall, the results of this thesis suggest that the application of natural growing media and the combination of blue and red LEDs have vital role in increasing crop phytonutrients of human health benefit. Based on our findings, we suggest that growing media containing PittMoss and a specific 40blue: 60red ratio can be a suitable alternative to conventional media for growing microgreens to improve productivity and biochemical compounds.

Keywords: LED spectrum, Microgreens, Natural growing media, Phytochemical compounds, preharvest factors.

ABBREVIATIONS AND SYMBOLS

ANOVA	analysis of variance
AMPs	Agricultural management practices
APEX	Ascorbate peroxidase enzyme
B	Blue lights
CRY1	Cryptochrome photoreceptor
C	Carbon
Ca	Calcium
Car	Carotenoid
Chi	Chalcone synthase
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl t	Chlorophyll total
Chs	Chalcone isomerase
CCHS	Canadian Community Health Survey
DPPH	*2,2-diphenyl-1-picrylhydrazyl*
Db	Bulk density
EC	Electrical conductivity
FI	Full irrigation
F3′5′h	Flavonoid 3′4′-hydroxylase
Fls	Flavonol synthase
F3′h	Hydroxylase

FW	Fresh weight
HSPs	Heat shock proteins
HSA32	Heat stress-associated 32-KD protein
GAE	Gallic acid equivalent
G	Mixed growing media
LED	Light emitting diode
LeHY5	Elongated hypocotyl5 gene
Mg	Magnesium
MC1	White oyster mushroom compost
MC2	Shiitake Mushroom compost
NC	Negative control
NPK	Nitrogen (N), phosphorus (P) and potassium (K)
OB	Biochar
PCA	Principal component analysis
PAL	Phenylalanine ammonia-lyase
PC	Positive control
POD	Peroxidase
P	Phosphorus
PSII	Photosystem II
DW	per gram of *dry weight*
PM	PittMoss
PAR	Photosynthetically active radiation

K	Potassium
PUFA	Polyunsaturated fatty acid
G6PDH	Pentose phosphate pathway
K-humate	Potassium (K)-humate
R2R3 MYB	*R2R3-myeloblastosis transcription factors*
ROS	Reactive oxygen species
RI	Restricted irrigation
RA	Rosmarinic acid
SD	Severe drought
T	Treatment
T1DM	Type I Diabetes Mellitus
T2DM	Type II Diabetes Mellitus
TCA	Trichloroacetic acid
TDS	Total dissolved solids
TPC	The total phenolic
US	Ultrasonic treatments
UV	Ultra-violet
UV-B	Ultraviolet B radiation
UV-Vis spectrophotometer	Ultraviolet–visible spectrophotometer

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CHAPTER 1: Introduction

Microgreens are immature greens harvested from tender young plants that are grown for their high health promoting compounds and biological properties (Kyriacou et al., 2019; Bulgari et al., 2021). Previous researchers reported high amounts of phytochemicals such as ascorbic acid, α-tocopherol, β-carotene, phylloquinone, vitamins, and minerals in different species of microgreens (Pinto et al., 2015; Kyriacou et al., 2016). Kale (*Brassica oleracea* L. var. acephala), Swiss chard (*Beta vulgaris* var. cicla), and arugula (*Eruca vesicaria* ssp. sativa) microgreens possess high levels of vitamins A, C, and K, essential lipids, carotenoids, and mineral nutrients (Di Noia, 2014; Pham et al., 2019). Microgreens are delicate and are prone to various stress factors that can greatly affect the edible quality and bio-functional properties. Plant growth, development, phytochemical composition, and subsequent biological properties are all affected by preharvest factors such as genetics, growing media, atmospheric conditions, and agricultural management practices (Aftab, 2019). Preharvest factors refer to plant genotype, growing medium properties, climatic factors, and cultural practices or treatment methods applied to plants cultivated in indoor production systems or the farm before harvesting time that can influence quality and quantity of plant production. Regulating these preharvest parameters can be a practical strategy to increase the bioactive composition of high-quality crops for human food and nutrition benefits (Nguyen et al., 2019). Improving the yield of phytochemicals and their bio-functional such as antioxidant and antidiabetic activities by controlling preharvest factors under greenhouse conditions have widely been reported by many researchers in the last decade (Ullah et al., 2019).

Growing media are materials used to grow plants, and are categorized as soil (i.e., silty, sandy, clayey, and loamy soils) or soilless (i.e., various combinations of pumice, calcine clay, perlite, peat moss, organic amendments, and wood-based substrates) (Gruda, 2011). Variations in

the growing medium characteristics affect plant morphology, productivity, and phytochemical composition (Turhan et al., 2007). Natural amendments are organic substrates added to growing media to improve their bio-physicochemical properties, which will ultimately lead to increases in plant productivity and harvest quality (Zhang et al., 2017; Abbott et al., 2018). These amendments include compost, vermicast, humates, manures, and sawdust. They supply macro- and micro-nutrients, support beneficial microbes, improve water-holding capacity and gas exchange, and promote nutrient availability required for plant growth and development. Multiple scientific studies have presented the effects of different amendments on yield, phytochemicals accumulation and various biological activities like antioxidant properties (Lazcano and Domínguez 2011; Abbey et al., 2018).

Climatic conditions including light, temperature, humidity, salinity, drought, and other environmental factors have either stimulatory or inhibitory effects on plant growth and development and their biosynthesis of chemical compounds **(**Schreiner et al., 2012). Light quality, intensity, and duration have potential effects on seed germination, plant growth, photosynthesis, flowering, and the accumulation of secondary metabolites (Montgomery, 2016). It is well known that wavelength range of 400-700 nm defined as photosynthetically active radiation (PAR) perceived by plant photoreceptors like phytochromes, cryptochromes, and phototropins is involved in regulating gene expression; and consequently, specific physiological and developmental responses (Liu et al., 2018; Ahmed et al., 2020). Interestingly, specific wavelengths of light have a precise impact on plant performance. For instance, the importance of red (610–710 nm) and blue (455–490 nm) wavelengths are widely cited in several studies due to the maximum absorption by photosynthetic pigments, which can provide targeted energy involved in photosynthesis and plant metabolism and thereby, stimulating plant morphophysiological responses (Naznin et al., 2019;

Brazaitytė et al., 2021). The synergetic effect of combined blue and red LEDs on growth characteristics and the accumulation of antioxidant phenolic compounds, flavonoids, anthocyanin, and carotenoid in a variety of microgreens species have been widely reported (Lobiuc et al., 2017; Naznin et al., 2019; Ahmed et al., 2020; Brazaitytė et al., 2021).

Many studies on the effects of individual amendments on plants have been carried out, but not on microgreen plant growth and chemical composition. Additionally, despite the promising studies on the effects of individual amendments and LED light spectrum on various plant species, there is limited information about the interactions of LED light spectrum and growing media or potential synergistic effects of these preharvest factors on microgreen plant growth and biochemical compounds. Therefore, the key goal for this research was to develop an effective, soilless growing medium in combination with a specific LED light spectrum to enhance growth and quality of microgreens in indoor production system. The specific objectives of this research were to: 1) formulate different proportions of mixed media to enhance the physicochemical properties and plant morpho-physiological responses of five different microgreens, *viz*., kale (*Brassica oleracea* L. var. *acephala*), Swiss chard (*Beta vulgaris* var. *cicla*), arugula (*Eruca vesicaria* ssp. *sativa*), pak choi (*Brasica rapa var. chinensis*), and amaranth (*Amaranthus tricolour* L.) under greenhouse conditions; 2) evaluate the effects of the recommended growing media formulation from Experiment 1 on seed germination, seedlings growth and targeted biochemical components of kale, Swiss chard, arugula and pak choi microgreens; and 3) assess the interaction effects of various LED light spectrum and growing media on plant growth and yield responses, and biochemical composition of microgreens under greenhouse condition.

To meet these objectives, five individual experiments were completed to find the optimum proportion of mixed growing media for the subsequent development and testing. The first

experiment (Study objective 1) assessed the effects of different proportions of vermicast and sawdust on growth factors, potential photosynthetic capacity, anthocyanin, and chlorophyll contents in kale and Mexican mint (*Plectranthus amboinicus*). The ratio of 40% vermicast + 60% sawdust was the most effective mixed growing media for the enhancement of agronomic performance in both plant species. This was followed with a second and third experiments (Study objective 1) to assess the physicochemical properties of different growing media *vis-a-vis* plant growth performance for optimization of the mixed growing media using different sources of mushroom compost. Growing media T2.2 (containing 30% vermicast + 30% sawdust + 10% perlite + 30% shiitake (*Lentinula edodes*) mushroom compost) and T4.2 (containing 30% vermicast + 20% sawdust + 20% perlite + 30% shiitake mushroom compost) had the best properties for growth of kale Swiss chard arugula, and pak choi, and amaranth. Therefore, the fourth experiment (Study objective 2) assessed the effects of mushroom compost versus PittMoss (PM) on agronomic performance, biochemical compounds, and antioxidant enzymes activities. Treatment T2.2 (30% vermicast + 20% sawdust + 20% perlite + 30% mushroom compost) improved growth, yield and quality traits compared to the PittMoss based growing media. The final experiment (Study objective 3) assessed the effects of red: blue LED light ratio and growing media interaction on plant growth and quality. The treatment T4.G2 (blue 40: red 60 × growing media containing PittMoss) maximized biochemical traits and antioxidant enzymes activities, as confirmed through multivariate 2-dimensional principal component analysis (PCA) analysis.

The present PhD thesis is structured in a publication format. Chapter 1 gives an overall overview, and the aim and structure of the thesis. Chapter 2 is a literature review conducted to document recent studies that reported on functional plants with health-promoting properties. In this context, several important preharvest factors that can potentially affect the synthesis of

phytoconstituents, which possess biological properties are reviewed. Study objectives 1, 2, and 3 are presented under chapters 3, 4, and 5, respectively. Chapter 6 presents overall conclusions from the preceding chapters and discusses the experimental limitations and contributions of the thesis.

CHAPTER 2: Effects of preharvest factors on antidiabetic potential of some foods and herbal plants

This chapter presents a version of the manuscript titled '**Effects of preharvest factors on antidiabetic potential of some foods and herbal plants**' that has been published in the *Brazilian Journal of Biology* on December 6, 2022. This was a multi-authored publication, in which the PhD Candidate contributed to the research design, data collection, data analysis, and writing. The citation is:

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2.1 Abstract

Diabetes is a metabolic disorder with no definite treatment, but it can be controlled by changing lifestyle and diet. Consumption of high-fiber and nutrient-rich foods including vegetables have been shown to reduce risks of obesity and Type II Diabetes Mellitus (T2DM). Also, many herbal plants have been associated with reduced risks of T2DM because of their composition of secondary metabolites. Antioxidant activities of some secondary metabolites have potent inhibitory effects against inflammation linked with insulin resistance and oxidative stress. More than 800 known medicinal plants are used to control diabetes and its relevant complications. However, variations in preharvest factors including plant genotype, growing medium properties, climatic factors, and management practices can influence plant growth and their accumulation of phytochemicals with health-promoting properties. However, the effects of these preharvest factors on the antidiabetic properties of plant secondary metabolites are neither explicit nor easily accessible in the literature. Therefore, this review aims to document recent studies that reported on under-exploited medicinal plants with antidiabetic properties. We reviewed several important preharvest factors that can potentially affect the synthesis of phytoconstituents which possess antidiabetic properties. This review will help identify gaps for future research in phytomedicine and functional foods.

Keywords: antidiabetic, secondary metabolites, medicinal plants, natural amendment, LED light.

2.2 Introduction

Plant growth, development, phytochemical composition, and subsequent biological properties are determined by preharvest factors such as genotypic characteristics, growing media, atmospheric conditions, and agricultural management practices (Aftab, 2019). Preharvest factors refer to many cultural practices or treatment methods applied to plants cultivated in indoor production systems or the farm before harvesting time that can influence quality and quantity of plant production. Regulating these preharvest parameters can be a practical strategy to increase the bioactive composition of high-quality crops as the growing population demands (Nguyen et al., 2019). In the last decade, previous researchers evaluated the impacts of preharvest factors on plant morphophysiological responses, phytochemicals, and pharmaceutical properties, particularly antioxidant and antidiabetic activities (Ullah et al., 2019).

Secondary metabolites are organic non-nutritional compounds synthesized by plants. They provide long-term advantages to plants such as defense against biotic and abiotic stress agents (Rosenthal and Berenbaum, 2012). Also, secondary metabolites are exploited for various purposes in medicinal, nutritional, and cosmetic companies (Jensen et al., 2014). Secondary metabolites exert an extensive range of bioactive and physiological functions such as antioxidants, anti-inflammatory, antimicrobial, anticancer activities, and many others (Kholkhal et al., 2013). In the last decade, the use of alternative medicines and herbal plants (about 800 species) to treat diabetes has dramatically increased due to availability and low side effects (Zhang et al., 2017a; Arumugam et al., 2013).

Diabetes is a disorder of metabolism divided into three classes: Type I Diabetes Mellitus (T1DM), Type II Diabetes Mellitus (T2DM), and gestational diabetes mellitus. The causes and complications of T2DM can be effectively managed, compared to T1DM and gestational diabetes

mellitus. Obesity is one of the main causes of T2DM because excess fat contributes significantly to insulin resistance by lipid accumulation in the liver and releases increased amounts of pro-inflammatory cytokines, free fatty acids, glycerol, and hormones that have a vital role in developing insulin resistance. Then, insulin resistance, linked to pancreatic β-cells dysfunction, causes an increase in blood sugar concentration (Saad et al., 2017; Hardy et al., 2012). From the research of Bahmani et al. (2014), T2DM can be controlled by healthy lifestyles and diet choices. For instance, high-fiber and nutrient-rich foods (e.g., vegetables, legumes, and fruits) have been proved by research to help reduce risks of obesity and diabetes. Also, the use of medicinal plants is another effective approach to control T2DM because of their phytochemical composites including phenolics, flavonoids, anthocyanins, carotenoids, terpenoids, and many more (Bahmani et al., 2014). Antioxidant activities of some of these secondary metabolites illustrate potent inhibitory effects against inflammation that leads to insulin resistance and oxidative stress correlated with diabetes (Jung et al., 2014). Therefore, the purpose of this review is to document recent studies on under-exploited medicinal plants with antidiabetic properties. We reviewed how changing preharvest factors affects the synthesis of plant secondary metabolites that possess antidiabetic properties.

2.3 Preharvest Factors

Managing preharvest parameters is an effective and practical strategy to provide the health needs of the global population through producing high-quality food crops with enhanced bioactive compounds (Nguyen et al., 2019). Preharvest factors include: 1) genotypic characteristics of the plant; 2) growing medium factors and amendments; 3) environmental factors like light quality, intensity, humidity, and temperature; 4) management practices such as planting and harvest time, irrigation, and fertilization. These factors influence plant growth and development, the

composition, and the functional properties of phytochemicals (Aftab, 2019; Nguyen et al., 2019). Improving the yield of phytochemicals and their functional properties such as antioxidant and antidiabetic activities by controlling preharvest factors under greenhouse conditions have widely been reported by many researchers in the last decade (Ullah et al., 2019). Kaur et al. (2021) demonstrated that preharvest factors including growth stage and different plant parts significantly influence antioxidant and antidiabetic function in Swertia chirata Buch. For instance, it was shown that the DPPH activity was considerably higher in the leaves of Swertia chirata harvested at the bud stage compared to leaves harvested at the flowering stage by 4% at a concentration of 80 μg mL−1 plant extraction. A similar observation was obtained for antidiabetic activity. The highest in-vitro α-amylase inhibitory activity in leaves harvested at the bud stage was higher by 3% compared to the flowering stage. Description of some of these important preharvest factors is further detailed in this review.

2.3.1 Growing media

Growing media are materials used to grow plants, and are categorized as soil (i.e., silty, sandy, clayey, and loamy soils) or soilless (i.e., pumice, calcine clay, perlite, peat moss, organic amendments, and wood-based substrates) (Gruda, 2011). Variations in the growing medium characteristics affect plant morphology, productivity, and phytochemical composition (Turhan et al., 2007). For example, Tabatabaei (2008) study reported that photosynthesis rate, growth, development, and contents of bioactive compounds were associated with optimum levels of growing medium aeration and balanced nutrients. The effects of natural amendments and types of soils on plant morpho-physiology and development, bioactive compounds, and medicinal activities are explained further below.

2.3.1.1 Soil bio-physicochemical properties

Soil is a dynamic substance consisting of mineral particles, water, gases, and organic matter. Texture, structure, and porosity are known as the physical properties of soil. These physical properties play a key role in soil quality and soil condition (Maddela et al., 2017). Soil texture contains the relative quantities of three mineral particles including sand, silt, and clay, which have a profound impact on many other properties such as the transpiration and exchange of gases in distinct soil layers. The soil texture classification to clay, loam, and sandy loam is based on particle size (Malique et al., 2019). Phogat et al. (2015) explained that soil types and structure affect plant growth components, improving aeration, nutrients and water availability, root penetration, and microbial activity. Different soil types possess various properties including different pH, moisture levels, and organic carbon percentage which were shown to have noticeable effects on growth parameters and accumulation of antidiabetic compounds such as polyphenols and vitamin E in *Calotropis gigantea* (Kumari et al., 2018). Soil structures define a soil's density, which has a subsequent effect on morpho-physiological response such as seedling emergence, root penetration, oxygen supply, and respiration (Stack, 2016). Tomato (*Solanum Lycopersicon*) growth parameters were significantly promoted with sandy soil modified with vermicompost (VC), in comparison with clay and silt loam soils amended with VC (Zucco et al., 2015). In addition, physicochemical properties of soils not only affect microbial diversity and their action but also influence microbial mass levels (Hassan and El-Kamali, 2015). Muscolo et al. (2019) illustrated that soil biochemical properties directly affect the biosynthesis of carotenoids and glucosinolates, and the antioxidant potential of *Brassica rupestris* Raf. Soil microbes including bacteria and fungi are involved in the biochemical processes within soils, and they are imperative to retain soil productivity and fertility. According to Radušienė et al. (2019), plant growth and development, and phytochemical

concentrations are significantly influenced by soil fertility. Reduction in a diversity of soil microbes has been stated to have adverse outcomes on soil health and soil quality (Giller et al., 1997), which can considerably influence the biosynthesis of plant-based chemical compounds as reported in *Stevia rebaudiana* by Pal et al. (2015). Ortíz-Castro et al. (2009) explained that some soil microbes produce phytohormones and volatile compounds like auxins, cytokinins, gibberellins, and antibiotics that directly or indirectly influence plant growth and development. Besides, soil microbes also have a vital role in the recycling of major soil mineral elements such as carbon, nitrogen, phosphorus, and other elements that help maintain soil health and productivity (Aislabie et al., 2013). Soil microbes significantly contribute to decaying organic matter and transforming organic nutrients into their plant-available inorganic forms, a process known as mineralization. For instance, soil microbes were shown to have an essential role in the nitrogen cycle, providing inorganic forms of nitrogen like ammonium and nitrate for plants (Aislabie et al., 2013). Montoya-Garcia et al. (2018) also mentioned that mineral constituents have a major effect on the primary metabolism and biosynthesis of bioactive compounds such as alkaloids, terpenoids, and phenolic compounds, which in turn affect plant growth and development. Pathak et al. (2008) clarified that nitrogen is vital in primary metabolism (i.e., the biosynthesis of nucleic acids, different amino acids, lipids, and enzymes). A study involving three medicinal plants (i.e., *Eucomos autumnalis, Tulbaghia ludwigiana,* and *Tulbaghia violaces*) showed that application of nitrogen, phosphorus, and potassium fertilizers or their deficiencies affected plant growth parameters, phytochemical production, and antioxidant activities (Aremu et al., 2014).

2.3.1.2 Natural amendments

Natural amendments consist of organic and inorganic but natural materials added to soil indirectly contribute to plant growth and development by enhancing soil fertility and/or soil

structure and conditions (Abbott et al., 2018). Natural growing amendments including compost, compost derivatives, vermicast, potassium humate, and manures provide potential benefits for the environment including 1) adding nutrients into the soil; 2) attracting earthworms; 3) supporting and protecting beneficial microbes; 4) promoting water retention capacity and release; and 5) enhancing nutrient absorption capacity and availability (Duong et al., 2012). Liu et al. (2016) observed that the application of carbon-rich natural fertilizers positively affected the biodiversity of soil, leading to the soil that is more resistant to pathogenic infections and environmental stress. Also, Lazcano and Domínguez (2011) earlier confirmed that the growth, yield, and phytochemical concentrations of different plant species can potentially be influenced and accelerated by applying various kinds of natural amendments. The positive results of growing medium amendments on plant growth are most likely connected to the optimum supply of essential macro and micronutrients required for growth and development, as well as the enhancement of soil functional activities. Celestina et al. (2019) claimed that the physicochemical features of soil have a vital impact on crop yield and these factors can be altered by applying various organic amendments.

Additionally, Lal (2006) confirmed that the administration of natural amendments effectively promotes crop production due to the amelioration of soil properties. Multiple scientific studies presented the effects of different amendments on phytochemicals accumulation and various biological activities like antioxidant properties. In the work by Antonious et al. (2014), they noticed that the use of natural amendments like chicken manure did not only affect the growth of two species of kale (*Brassica oleracea* cv. Winterbor) and collard (*Brassica oleracea* cv. Top Bunch) but significantly boosted the total content of phenols and ascorbic acid. Moreover, growth factors and accumulation of total phenolics, carotenoid, and carvacrol compounds in *Plectranthus* spp. were promoted by natural amendments. However, various organic amendments including

vermicast, K-humate, and NPK indicated different effects on plant growth and phytochemicals in *Plectranthus* spp (Zhang et al., 2017a). Moreover, soil fertility and the production of essential oils in basil (*Ocimum basilicum*) were promoted in soils amended with vermicompost (Trivedi et al., 2017). Also, growth parameters, phenolic compounds' content, and kale's antioxidant properties (*Brassica oleracea* var. acephala) were differentially affected by different natural amendments such as dry vermicast, K-humate, and volcanic minerals (Iheshiulo et al., 2017).

Another important preharvest factor affecting plants' phytochemistry is growing media chemical composition. In the last decade, the use of alternative medicines and herbal plants has dramatically increased in synchrony with consumers' demand for organic produce. As a result, current researchers are focusing on applying natural amendments to enhance phytomedicines and the development of functional plants (Nguyen et al., 2019; Abbey et al., 2018). Research by Abbey et al. (2018) revealed that essential fatty acids, and mineral nutrients as well as antioxidant activities in kale (*Brassica oleracea* var. acephala) were differentially altered by different natural growing medium amendments like dry vermicast, potassium (K)-humate, and volcanic minerals. Their results illustrated that dry vermicast had stimulatory effects on polyunsaturated fatty acid (PUFA) biosynthesis and monounsaturated glycolipids phosphatidylglycerol by regulating the monounsaturated molecular species metabolism. Also, it was shown that vermicast had a remarkable influence on the accumulation of oleic acid and omega-3 fatty acid compared to potassium (K)-humate, and volcanic minerals, which as a result increases the overall nutritional value and therapeutic properties of kale. In the findings obtained by Vidal et al. (2018), where the accumulation of essential lipids in kale (*Brassica oleracea* var. acephala) including total C18:1n9, C16:3n3, and C18:3n3 fatty acids were enhanced under the application of dry vermicast. It appeared that the positive effect of vermicast on functional lipids was closely associated with the

enhanced potential activity of delta 13 desaturase enzyme that accelerated desaturation of C16:2n6 into C16:3n3. Additionally, the high N and other essential elements in vermicast contributed to the improved biosynthesis of C18:1n9 and C16:3n3 fatty acids in the kale. Collectively, the results obtained from both Abbey et al. (2018) and Vidal et al. (2018) presented promising and cost-effective approaches to enhancing functional lipids accumulation by applying natural growing media, in particular vermicast. The health benefits of these essential lipids on human physiology and reduced susceptibility and debilitating disease risks such as obesity, diabetes, and cancer has been widely reported (Nguyen et al., 2019).

2.3.2 Climatic factors

Climatic conditions including light, temperature, humidity, salinity, drought, and other environmental factors have either stimulatory or inhibitory effects on plant growth and development and their biosynthesis of chemical compounds **(**Schreiner et al., 2012). Ghasemi et al. (2011) acknowledged that climatic factors cause major differences in the accumulation of plant secondary metabolites and biological activities, as further discussed in this review. As Dong et al. (2011) confirmed, temperature and light regimes significantly influenced the accumulation of phytonutrients in *Eucommia ulmoides.* Nevertheless, it should be highlighted that majority of the literature available on how climatic factors impact the antidiabetic potential of food crops and medicinal plants are very scanty, compared to their antioxidative effects.

2.3.2.1 LED light treatments

Light quality, intensity, and duration have potential effects on seed germination, plant growth, photosynthesis, flowering, and the accumulation of secondary metabolites (Montgomery,

2016). According to Metallo et al. (2018), various metabolic pathways can be influenced by lighting. Transcription factors and photoreceptors can significantly control cellular division, endoreplication, and cell growth which are directly affected by different qualities, durations, and intensities of light (Okello et al., 2016). Huché-Thélier et al. (2016) indicated that ultra-violet (UV) and blue (B) lights have considerable roles in controlling and regulating an extensive range of metabolic processes in pepper (*Capsicum annuum)*, lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), *Arabidopsis*, and tomato (*Solanum Lycopersicon*). Thus, the manipulative use of blue and UV-B lights can help improve plant growth and development, and resistance versus pests and pathogenic diseases for increased nutritional and phytochemicals values (Abidi et al., 2013). Ullah et al. (2019) observed secondary metabolites and activating defense mechanisms similarly. Hou et al. (2010) investigated the relationship between low light intensity and growth indices as well as phytochemical compounds in *Glycyrrhiza uralensis* Fisch. According to these researchers, although low light intensity negatively impacted leaf thickness, photosynthesis, plant growth, and productivity, it noticeably promoted chlorophyll and phytochemical contents such as glycyrrhizic acid and liquiritin that can be associated with the stimulatory effects of low light intensity on phytochemical biosynthesis and reduced plant biomass production. These findings disagreed with the work by Neugart et al. (2016) who found that flavonol concentration including quercetin glycosides, a caffeic acid monoacylated kaempferol triglycoside, and disinapoyl-gentiobiose of kale leaf tissue was higher in plants exposed to higher light intensity (400 μmol m−2 s−1) compared to lower light intensity (100 μmol m−2 s−1). It was found that the differences flavonoid content in plants treated under different light intensity was associated with the effect of light intensity on R2R3 MYB transcription factors required in the phenylpropanoid pathway as well as the expression of genes involved in coding protein degradation, transport processes, amino acid

biosynthesis, and different secondary pathways. Moreover, the regulatory effect of light intensity in the expression of genes involved in the flavonoid biosynthetic pathway that enhance flavonoids and hydroxycinnamic acid derivatives accumulation can be linked to anti-photo-oxidative and ROS scavenging mechanisms caused by excess light intensity, had been reported in previous studies. In another study, phytochemical biosynthesis such as phenolics, tocopherols, flavonoids, and glucosinolates in *Ananas comosus* L. was significantly enhanced by the application of UV radiation between 190-280 nm (Freitas et al., 2015). Arakawa et al. (2017) illustrated a potent relationship between different blue light wavelengths (430 to 490 nm) and the accumulation of anthocyanin compounds in *Prunus avium* L. From their work, the wavelength 450 nm (blue light) was found to be more effective at stimulating anthocyanin synthesis as compared to other wavelengths. In agreement with the findings of Eckstein et al. (2012), plants exposed to higher ratio of blue light had an elevated level of soluble carbohydrates such as sucrose, glucose, and fructose. Such as this enhanced plant metabolism and detoxification pathways as well as amino acids, and lipids biosynthesis in response to ROS induced by stressful blue LED treatments. Nishimura et al. (2007) reported that the plant growth parameters and chemical composition of Hypericum perforatum L. can be changed by applying various light qualities including blue, white, and red lights at different intensities. They found the highest rate of growth under white and red-light treatments with 500 μmol m−2 s−1 intensity while the highest content of phytochemicals such as hypericin and pseudohypericin are anthraquinone derivatives, which possess anti-cancer and anti-inflammatory properties, was obtained by the used of red-light with 250 μmol m−2 s−1 intensity. In agreement with this study is the results of Metallo et al. (2018), where the combination of blue and red as well as white LED light treatments significantly influenced yield, morphological characteristics, beneficial nutrients, and phytochemicals in *B oleracea*. Based on the results, the

highest total concentrations of carotenoid and glucosinolates observed with 37 days of white LED treatment and 5% blue/95% red LED may be related to the differences in growth and development stage and cultivar. Moreover, Ali and Abbasi (2014) reported notable effects of light treatments including 24, 27, 30, and 37 days with 40 μmol m−2 s−1 intensity on plant morphology and phytochemical production as well as antioxidant potential in *Artemisia absinthium*. Based on their results, the light showed a stimulatory and positive effect on phenolic compound accumulation and antioxidant activities. The highest level of total phenolic compounds (i.e., 42.96 mg/L) was obtained by applying continuous light treatment for 27 days. Liu (2013) stated that a purposeful manipulation of light quality and intensity to improve the accumulation of secondary metabolites and their bioactive properties enhanced the nutritional value of plants and pharmaceutical activities. Moreover, Bantis et al. (2016) demonstrated that variable LEDs meaningfully influence growth parameters and the total phenolic content of two *Ocimum basilicum* cultivars. An elevated level of phenolic compounds was observed under the combination of 1% UV + 20% blue + 39% green + 35% red + 5% far-red LED light spectra at 200 μmol m−2 s−1 intensity. It may connect to the stimulatory effect of blue light on the function of phenylalanine ammonia-lyase (an important enzyme in the phenylpropanoid biosynthetic pathway) which resulted in improving phenolic production. The effect of monochromatic B LED light, as physical stress elicitation, on the enhanced expression of a gene involved in phenylalanine ammonia-lyase activity and antioxidant potential in red leaf lettuce (*Lactuca sativa*) was already reported by Son and Oh (2015). Based on the results obtained by Hunaefi et al. (2018), combined ultraviolet (UV) and ultrasonic treatments (US) showed a positive potential effect on stimulation of targeted phenolic compounds like rosmarinic acid (RA) and enhanced antidiabetic activity of *Orthosiphon aristatus*. It was shown that the US and UV treatment increased the activities of the pentose phosphate

pathway (G6PDH) and the phenylpropanoid pathway (PAL) enzymes; thereby providing precursors for the phenolic compounds' synthesis. The authors also reported that the combination of UV and US effectively increased the potential activities of α-glucosidase and α-amylase enzymes. These enzymes have prominent effects on the control of hyperglycemia related to type II diabetes. Consequently, this can be connected to the presence of higher phytochemical concentration, which led to the highest antioxidant function in vitro shoot cultures of *Orthosiphon aristatus*. However, more investigation should be conducted regarding interactions between supplemental light treatments and other growing conditions including temperature and various organic amendments towards the enhancement of plant secondary metabolites and their antioxidant and antidiabetic properties.

2.3.2.2 Temperature

Temperature is known as one of the main abiotic stresses that control morpho-physiological components, productivity, and accumulation of phytochemicals in plant species. The optimum range of temperature (20o-30°C) stimulates and increases several important chemical antioxidants and enzymatic antioxidants (i.e., superoxide dismutase, and catalase) (Zobayed et al., 2005). Ncube et al. (2012) explained that several plant physiological, biochemical, and molecular alterations are closely linked to temperature stress and as a result, these changes can influence phytochemical production. For instance, high temperatures (>33 °C) were shown to have an inhibitory effect on growth response, development, productivity, phytochemical content, and physiological activities (i.e., germination and photosynthesis) in *Phaseolus vulgaris* and *Vitis vinifera* (Hasanuzzaman et al., 2013). The molecular assessment results showed a strong correlation between heat stress and increment in ion transporters and signaling molecules. Nitric

oxide and calcium ion, proteins such as cytosolic heat shock proteins (HSPs) and heat stress-associated 32-KD protein (HSA32), osmo-protectants, and antioxidants as well as other factors involving in signaling cascades and transcriptional regulation mitigated the adverse effects of high temperature in plant morphophysiological which caused enhanced phytochemical responses (Hasanuzzaman et al., 2013; Krasensky and Jonak, 2012).

Implementing various ranges of temperatures i.e., 20/12 °C, 25/17 °C, 30/22 °C, 35/27 °C, and 40/32 °C daytime and nighttime respectively, significantly affected seed germination, seedling emergence, growth, and developmental responses in Cotton (*Gossypium hirsutum* L.) (Reddy et al., 2017). Reddy et al. (2017) found a linear and positive relationship between increasing temperatures and improvement of physiological and morphological parameters, with the optimum treatment being a 35/27 °C (day/night) temperature regime. Similarly, temperature variations affected the composition of phytochemical compounds in *Brassica oleracea* as observed by Neugart et al. (2016). Their findings demonstrated that the hydroxycinnamic acid derivative content like disinapoyl-gentiobiose was enhanced at higher temperature treatment (1.65 mg/ g-1 of dry weight at 15 °C), while sinapic acid acylated flavonol tetraglycosides like kaempferol-3-O-sinapoyl-sophoroside-7-O-diglucoside was promoted at lower temperature (2.34 mg/g-1 of dry weight at 5°C). The findings obtained from the molecular-level evaluation showed that temperature factors differentially affected the overall expression of genes involved in phenylpropanoid secondary metabolism. the biosynthesis of aromatic amino acids is a prerequisite of phenolic compounds production and phytohormone synthesis. Also, the results indicated that few more genes were expressed at low temperatures in contrast to high temperatures that may induce genes that are linked to the enrichment of jasmonate hormone metabolism to acclimate lower temperature and reduce cold stress. Furthermore, the highest antioxidant function was

observed in kale species exposed to lower temperatures (i.e., 3.8°C compared to 9.7°C) due to the presence of higher levels of flavonoid glycosides derivatives including quercetin-3-O-hydroxyferuloyl-sophoroside-7-O-D-glucoside (2.54 mmol GAE g-1 of dry matter) and quercetin-3-O-disnapoyl-triglucoside-7-O-D-glucoside (4.19 mmol GAE g-1 of dry matter), which enhance ROS–scavenging activity. Also, biosynthesis of phenylalanine ammonia-lyase and chalcone synthase enzymes involved in the biosynthesis of flavonoid pathway were noticeably increased under lower temperature, resulting in enhanced phenolic compounds production, as reported by Zietz et al. (2010). Odabas et al. (2010) examined the interaction effects of temperature variations on the metabolic profile of *Hypericum perforatum* L. They observed a strong correlation between temperature and light intensity on the biosynthesis of phenolic and polyphenolic accumulations including amentoflavone, apigenin-7-glucoside, cholorogenic acid, hyperoside, kaempferol, quercetin and quercitrin. The significance of temperature (from 24°C to 32°C) and light intensity (803.4 μmol m−2 s−1 to 1618.6 μmol m−2 s−1) increments on enhancement of phenolic compounds may be explained by alterations in photosynthetic activity, resulting in increased carbon availability unusually used for phytochemical synthesis in response during stress. Also, these physiological changes induced by these physical stress elicitations can be linked to increases in secondary metabolites to strengthen defensive systems. Zhang et al. (2009d) also observed the highest level of the terpenoid geosmin in *Lyngbya kuetzingii* at low temperature (10 °C) and low light intensity (10 μmol m^{-2} s^{-1}) for 14 days. However, the content of geosmin production remarkably declined when the plant was subjected to high temperatures (25 and 35 °C) and high light intensities (20 and 75 μmol m^{-2} s−1). The effects of these external environmental stimuli on molecular mechanisms involved in geosmin production have been attributed to the biosynthesis pathway of geosmin. However, Khan et al. (2011) explained that these changes in environmental

factors led to the production of phenolics, flavonoids, and alkaloids compounds, which have a key role in the defense mechanisms of plants exposed to temperature stress. According to Sarıkamış and Çakır (2012), the application of low-temperature treatments (0 °C at two-time durations (1 h) and (2 h) showed an inhibitory effect on the production of glucosinolate constituents and biological activities in broccoli (*Brassica oleracea* var. *Italica* L.). Glucosinolates are accumulated in plant cell vacuoles that lie adjacent to myrosin cells full of myrosinase enzyme, which is responsible for the hydrolysis of glucosinolates. The reduction in glucosinolate production may relate to the adverse effect of lower temperature on cellular integrity and subsequent interaction between myrosinase and glucosinolates, resulting in hydrolyzing and breaking down of glucosinolates. However, Pennycooke et al. (2005) revealed that low-temperature treatments (-5 °C) considerably increased the accumulation of anthocyanin compounds in Petunia (Petunia × hybrida) that may connect to the activation of antioxidant defense systems due to the presence of oxidative damage and lipid peroxidation induced by cold treatments. Nevertheless, the productivity, quality of bioactive compounds, essential oils, and antioxidative capacity in three medicinal plants (i.e., *Nepeta cataria* L*., Melissa officinalis* L., and *Salvia officinalis* L.) were significantly influenced under the amplitudes of 15 °C - 20 °C -25 °C temperatures. Although the maximum essential oils yield in *Melissa officinalis* was obtained at 25 °C, the highest amount and quality of essential oil and yield in *Nepeta cataria* and *Salvia officinalis* were observed at 15 and 25 °C, respectively (Manukyan and Schnitzler, 2006).

Since the effects of various temperature treatments on medicinal properties, specifically, antidiabetic activity is understudied, more research projects should be carried out to clarify temperature effects on phytochemicals and their bioactive properties. Additionally, there is still a

need to consider the interaction between temperature regimes and other environmental factors towards enhancing plant secondary metabolites and their pharmacological activities.

2.3.2.3 Humidity

Humidity is known as one of the important climatic agents that improve germination rate, growth, development, and photosynthesis by increasing stomatal conductance in plant species (Suzuki et al., 2015). As Deng et al. (2016) confirmed, there is a strong correlation between humidity and plant productivity, biomass, and growth factors in many plant species. In one study, the morphological and physiological characteristics of *Rosmarinus officinalis* were highly influenced by the manipulation of humidity under greenhouse conditions (Sánchez-Blanco et al., 2004). According to the results obtained by Fu et al. (2018), the total flavonoids, polyphenols content, and antioxidant properties were promoted in *Pericarpium Citri* Reticulata (*Citrus reticulata* 'Chachi') under low humidity (50%) compared to high humidity (80%). The authors reported positive relationship between lower humidity and enhanced plant phytochemicals through control and improved internal chemical reactions. Water unavailability at lower humidity led to regulating enzymatic and chemical reactions plus decreasing non-enzymatic browning reactivity. It was explained by Zhang et al. (2015c) that non-enzymatic browning link to phenolic decomposition or changes of their chemical structure, which in turn increase decarboxylation and polymerization of phenolics, resulting in a reduction in antioxidant potential. In agreement are the results Kim et al. (2015b) obtained, where ascorbic acid accumulation enhanced from 10 to 84 ppm, and its antioxidant potential was significantly promoted in corn (*Zea mays*) at low humidity. Likewise, Kim et al. (2015a) confirmed that the accumulation of α-tocopherol and antioxidant potential was reduced in corn by increasing relative humidity. In a similar work carried out by

Shin et al. (2007), although phenolic concentrations were significantly enhanced in strawberry (Fragaria × ananassa Duch.) at 75 and 85%, ascorbic acid concentration was decreased due to increased oxidation at lower humidity. While total flavonoids were increased at 95% humidity, anthocyanin concentrations and antioxidant activities were relatively unchanged at different relative humidity.

The effects of various relative humidity on growth factors, phytochemicals, and medicinal properties, particularly antidiabetic activity, has not been extensively studied. Future research should be conducted to elucidate humidity effects on different aspects of plant morpho-physiological components, phytochemical content, and pharmaceutical properties.

2.3.3 Agricultural management practices

Agricultural management practices (AMPs) are beneficial and cost-effective activities associated with the application of production, economic, and management fundamentals (Bai et al., 2018). For example, AMPs present practical guidance regarding the management of fertilizers or manures to effectively restrain or decrease the movement of pollutants into the surface and groundwater as well as air (Sith et al., 2019; Merrill et al., 2011). Proper management practices considerably impact soil texture, fertility, nutrient concentration, moisture, and health status, which directly influence growth response, development, and plant biomass production (Rathore et al., 2011). Thus, contributing to sustainable plant production for food security via the supply of food crops enriched with high-value bioactive compounds (Mózner et al., 2012; García-Mier et al., 2013).

In this review, several important agricultural management practices including irrigation and fertilizer on plant growth aspects, the biosynthesis and concentration of phytochemicals, and medicinal activities are considered in more detail.

2.3.3.1 Irrigation

Irrigation is one of the important agricultural management practices that have a major effect on soil properties, and consequently, affect soil moisture and nutrient transport for plant growth and developmental characteristics (Ascough II et al., 2008). In plants, water availability meaningfully affects all physiological processes which contribute to plant morpho-physiological response, primary, and secondary metabolism (Kleinwächter and Selmar, 2015). Xue et al. (2018) examined the effects of different irrigation regimes on plant growth indicators and chemical composition of *Cassia obtusifolia* L. seed, which is known to possess antihypercholesterolemic and antihyperglycemic properties. The authors observed that a reduction in protein content (from 39.48 to 34.84mg/g) and plant growth factors except seed yield, but an increase in anthraquinone content (from 2.873 to 6.321 mg/g) at lower water availability (70% field capacity). They concluded that increased anthraquinones content may connect to the seed yield that was unaffected by water stress. Furthermore, a study done by Huot et al. (2014) showed that there is growth–defense trade-offs under stressful environmental factors that led to producing predominantly secondary metabolites in response to water deficit that could explain enhanced anthraquinone content at a mild irrigation treatment in *Cassia obtusifolia* L. species. Based on previous work highlighted, water deficit is stated as another important abiotic factor that substantially influences primary and secondary metabolites concentrations by changes in P5CS gene expression linked to carbohydrate metabolism and genes involved in polyphenol production, thereby affecting

subsequent pharmaceutical properties (Elhani et al., 2019). However, Marino et al. (2019) reported that although the concentration of phytochemicals in *Mentha spicata* was not influenced by different irrigation regimes, the yield of essential oils was significantly altered under the application of different regimes of irrigation. Based on their results, the lowest photosynthetic activity, growth traits, and the accumulation of secondary metabolites were observed under strong water stress due to a considerable reduction in total biomass, leaf area, and fresh weight. Herrera et al. (2019), investigated the effects of irrigation treatments including severe drought (SD), restricted irrigation (RI), and full irrigation (FI) on the biosynthesis of several phytochemicals in the common bean (*Phaseolus vulgaris* L.). According to their results, optimal irrigation application (mild hydric stress RI) effectively induced biosynthesis of secondary metabolites including phenolics, flavonoids, glycosides, and terpenoids, as further confirmed by Kusvuran and Dasgan (2017). The authors found that under irrigation deficit, the stomatal closure in the leaves is regulated by abscisic acid signaling mediator, resulting in a drastic reduction in carbon fixation. Irrigation deficit also reduces the amount of energy needed to decrease CO_2 and non-structural carbohydrates; thus, it has significant increase ROS accumulation in the photosynthetic electron transport chain. To detoxify ROS molecules, the synthesis of non-enzymatic antioxidants (i.e., carotenoids, flavonoids, ascorbic acid, and α- tocopherol) and enzymatic antioxidants (i.e., catalase and peroxidase) are encoded by the genes involved in synthesis of phenylalanine ammonia lyase (Pal2), chalcone synthase (Chi), chalcone isomerase (Chs), flavonoid 3′-hydroxylase (F3′h), flavonoid 3′4′-hydroxylase (F3′5′h), and flavonol synthase (Fls) to inhibit cell damage and oxidative damage (Herrera et al., 2019; Gharibi et al., 2019). In agreement with these results is the work of Zhang et al. (2017b), where water stress in *Stellaria dichotoma* L., showed adverse effects on growth characteristics and yield, whereas moderate water stress effectively increased

phytochemicals such as flavonoids and saponins. In addition, Vosoughi et al. (2018) observed that the level of essential oils, phenolic, and flavonoid compounds, as well as the antioxidant potential in *Salvia officinalis* L., were influenced by different irrigation frequencies. Their results demonstrated that under decreased irrigation regimes the antioxidant properties and production of total phenolics, flavonoid compounds, and essential oils were promoted by applying a chitosan elicitor that can relate to stimulate metabolic pathways of bioactive compounds. Moreover, Rodrigues et al. (2019) demonstrated that irrigation with different salinities (ranging from 0, as control, to 600 mM) had different effects on growth factors, plant production, and phytochemical content as well as *in vitro* biological antioxidant and anti-inflammatory properties in *Polygonum maritimum* L. The results demonstrated that irrigation with fresh water and mild salinity significantly had the highest effects on growth, productivity, total phenolic compounds (300 mM salinity: 107 mg GAE/g DW) total flavonoids (200 mM salinity: 26.1 mg GAE/g DW), and *in vitro* anti-inflammatory action (fresh water:79.7% nitric oxide reduction at 100 μg/mL) and *in vitro* antioxidant capacity (fresh water: 96.2% radical-scavenging activity of DPPH at 1 mg/mL) by affecting the activity of oxidases/dehydrogenases, redox status and relevant genes expression linked to synthesis of biochemical compounds under different irrigation treatments.

As irrigation has a significant effect on the biosynthesis of phytochemicals and pharmaceutical activities, more studies should be carried out to gain the optimal degree of irrigation correlated with the increasing yield of bioactive compounds and their subsequent biological potential.

2.3.3.2 Fertilizers

Implementing appropriate chemical fertilizers and/ or organic manures at the right time can supply plants with the required nutrients necessary for optimal growth and production of phytochemicals (Khalid et al., 2017). Ibrahim et al. (2013) clarified that organic fertilizers significantly influence the improvement of soil physicochemical properties and health, which sequentially affect growth responses and nutritional value. In the related study carried out by Yin et al. (2018), nitrogen (N), phosphorus (P), and potassium (K) fertilizers application effectively promoted plant growth indices and yield in *Vigna radiata* L. However, applying different ratios of N, P and K indicated different effects on the plant production and growth parameters of *Vigna radiata* L. From this study, it can be postulated that NPK fertilizer ameliorated growth traits by (1) inducing the biosynthesis of primary metabolites (e.g., proline, sugar, and chlorophyll), (2) increasing pathogen resistance by inducing the phytonutrient biosynthetic pathways (Mondal et al., 2017). Ibrahim et al. (2013) stated that applying organic fertilizer like chicken manure significantly increased the yield of phytochemicals such as total phenolic compounds (1.32 mg/g gallic acid dry weight), flavonoids (0.81 mg/g rutin dry weight), glutathione (632.16 nmol/g dry weight), and saponin (38.16 mg/g) concentrations and antioxidant potential in *Labisia pumila* Benth, in comparison with inorganic fertilizer like NPK. They also found a higher level of soluble sugar in plants treated with the organic fertilizer that may explain enhanced secondary metabolites production. In previous studies conducted by Jaafar et al. (2012), there was a positive correlation between carbohydrate content and biosynthesis of flavonoid and phenolic compounds of *Labisia pumila* Benth. Additionally, higher micronutrient levels were found in plants treated with organic fertilizers that properly supplied required elements for cellular chemical reactions, resulting in increased phytochemical production and relevant biological activities (Ibrahim et al., 2013).

Several studies demonstrated that gallic acid and rutin have potent antidiabetic properties due to their higher potency in scavenging ROS and superior hydroxylation degree (Saravanan and Parimelazhagan, 2014). Therefore, applying organic fertilizers to improve targeted phytochemicals used in treating diabetes is a practical strategy in indoor sustainable agricultural systems. In the related study done by Khalid et al. (2017), the use of organic fertilizer and biofertilizer such as fungus had favorable effects on growth characteristics, yield, nutritional values, and phytochemical compositions including phenolics, flavonoids, and phenolic acid in *Brassica campestris* ssp. *chinensis* L. Their findings showed that mixture of organic fertilizer and biochar (OB) was the most effective growing media in boosting total flavonoids and phenolic acid. This mixture may relate to the stimulatory effects of OB on early (CHS, CHI, and F3H) and late (FLS and ANS) gene expressions. Encoding enzymes involved in the conversion of 4-coumaroyl-CoA precursor to other intermediate compounds used in the biosynthesis pathway of flavonoids. Also, the results indicated the higher antiradical and anti-inflammatory properties of profiled phytochemicals in the inoculated plants by OB which were associated with the inhibition of enzymes involved in the inflammatory process (Khalid et al., 2017). Al-Kharusi et al. (2009) revealed that organic fertilizers were more effective in enhancing secondary metabolites production in date fruit (*Phoenix dactylifera*) and antioxidant activities in cabbage (*Brassica oleracea*) compared with their mineral fertilizer counterparts.

Since limited research has been reported on how chemical fertilizers and/or organic manures influence plant bioactive compounds and their medicinal activities, more studies should be conducted on this subject matter. Such data will provide detailed information on the relationship between various fertilizers, the yield of plant phytochemicals, and their final bioactive properties (i.e., antioxidant and antidiabetic properties).

2.4 Plant Phytochemicals and Their Role in Diabetes

According to Arumugam et al. (2013), a variety of herbal plants that are successfully able to prevent/control diabetes and its complications have been reported in the literature. Based on the literature, there are approximately 800 plants with antidiabetic properties, some of which have been assessed by several experimental techniques (Arumugam et al., 2016; Arunachalam and Parimelazhagan, 2012). Table 2. 1 demonstrates examples of 44 medicinal plants with potent antidiabetic properties. Several important traits connected to these plants including the family of plant species, the type of extracts gained from different parts of the plant (i.e., root, leaves, and shoot), their secondary metabolites, biological activities, as well as brief anti-diabetic or anti-hyperglycemic activities of these plants' extracts are detailed in the table.

Table 2. 1 Antidiabetic properties of some selected medicinal plants.

No.	Plant name	Family	Type of extract	Secondary metabolites	Bioactive activity	Outcome (effects)	References
1	Sage-leaved alangium (*Alangium lamarckii* Thwaites)	Alangiaceae	Alcoholic leaves extract	Alkaloids, Terpenoids, Steroids, Tannins, Phenols	Antidiabetic antihyperglycemic	Balancing blood glucose levels, restoring liver glycogen, and enhancing the activity of antioxidant enzymes	Kumar et al. (2011c)
2	Black Siris (*Albizia odoratissima* Benth)	Mimosaceae	Methanolic bark extract	Steroids, Tannins, Phenolics, Saponins	Hypoglycemic	Reducing the level of sugar, total cholesterol, and triglycerides in the bloodstream	Kumar et al. (2011a)
3	Cashew (*Anacardium occidentalen* L)	Anacardiaceae	Ethanol leaves extract	Glucosides, Flavonoids, Phenolic compounds	Antidiabetic	Reduction in the blood sugar levels, serum insulin, glycated hemoglobin levels, serum lipid parameters	Abdullahi and Olatunji (2010)
4	Kale (*Brassica oleracea* var. acephala)	Brassicaceae	Methanolic extract	Glucosinolates, Anthocyanins Phenylpropanoids Carotenoids	Antihyperglycemic	Reduction in hyperglycemia & CHCl3 fraction acts as an insulin sensitizer	Yoshida et al. (2007)

5	Java Tea (*Orthosiphon aristatus* Boldingh))	Lamiaceae	Methanol shoot extract	Phenolic compounds, Phenylpropanoids, Glucosinolates	Antidiabetic	Preventing the generation of lipid peroxidation products	Hunaefi et al. (2018)
6	Chinese mustard (*Brassica juncea* L.)	Cruciferae	Aqueous seed extract	Glycosides, Flavonoids, Phenolic, Sterols, Triterpene, Glucosinolates	Hypoglycemic	Improved state of availability of insulin to regulate the level of the blood sugar	Thirumalai et al. (2011); Parikh and Khanna (2014)
7	European Barberry (*Berberis vulgaris*)	Berberidaceae	Aqueous root extract	Tannins, Alkaloids, Saponins	Hypoglycaemic	Regulation glucose, cholesterol, and triglycerides levels in the bloodstream as well as an increase in insulin release and restoration of metabolic activities.	Meliani et al. (2011)
8	Goldthread (*Coptis trifolia*)	Ranunculaceae	Not published	Alkaloids compounds	Hypoglycemic	Not published	Elias and Dykeman (1982)
9	Teri pods	Fabaceae	Methanol	Flavonoids,	Antidiabetic	Regulate blood glucose concentration	Narkhede et al. (2011)

	(*Caesalpinia digyna* Rottler.)		root extract	Tannins, Steroids, Triterpenoids, Glycosides		by controlling amylase and glucosidase enzymes actions	
10	Ivy Gourd (*Coccinia grandis* L.)	Cucurbitaceae	Aqueous leaves extract	Flavonoids, Phenolic compounds Alkaloids, Glycosides	Anti-hyperglycemic Antihyperlipidemic	β-cell regenerative by regulation of intracellular glucose homeostasis and reduction in the concentration of serum cholesterol and triglycerides	Attanayake et al. (2016); Kondhare and Lade (2017)
11	Bwerebodeli, Epakun (*Curculigo Pilosa*)	Hypoxidaceae	Corn steep liquor extract	Phenolic compounds like Saponin, Terpenoid, coumarin, Steroid.	Antidiabetic	Decrease in blood glucose concentration	Karigidi and Olaiya (2020)
12	Silk-cotton or kapok tree (*Ceiba pentandra*)	Malvaceae	Methanol extract stem bark	Terpenoids, Flavonoids, Tannins, Saponins, Glycosides.	Antidiabetic	Reduction in blood glucose level and restoring reduced hematological parameters	Odoh et al. (2016)

13	Tanner's Cassia, Mature Tea (*Cassia auriculata* L.)	Caesalpiniaceae	Aqueous leaves extract	Flavonoids, Alkaloids, Phenolics, Saponins, Tannins, Glycosides.	Antihyperglycemic	Increased levels of free radical-scavenging and/or decreased level of lipid peroxidation.	Gupta et al. (2011)
14	East Indian Satinwood or Yellowwood (*Chloroxylon swietenia* DC)	Rutaceae	Aqueous and methanolic root extracts	Alkaloids, Coumarins, Flavonoids, Steroids	Antidiabetic	Regulating plasma insulin levels. Adjusting plasma insulin concentration. Regeneration and regulate the carbohydrate metabolic enzymes and glycogen actions in the liver tissue.	Jayaprasad et al. (2016)
15	Sweet Dattock (*Detarium microcarpum*)	Fabaceae	Methanol root extract	Terpenoids, Saponins, Resins, Glycosides, Flavonoids	Hypoglycemic Antidiabetic	Increased insulin secretion	Okolo et al. (2012)
16	Elephant Apple (*Dillenia Indica* L.)	Dilleniaceae	Methanolic leaves	Phenolic compounds, Tannins,	Antidiabetic	Reduction in the concentration of blood glucose, cholesterol, and	Kumar et al. (2011b)

			extract	Carotenoids, Saponins, Terpenoids	Hypolipidemic	triglycerides or/and prevention of endogenous glucose production and regulation of the bodyweight	
17	Chota-kirayata (*Enicostemma littorale.* Blume)	Gentianaceae	Aqueous extract the whole plant	Alkaloids, Triterpenoids, Sterols, Saponins, Steroids, essential oil	Antidiabetic	Reduction in blood glucose level, urea, creatinine, lipid, cholesterol, and triglycerides	Sonawane et al. (2010)
18	Shan-Zhi-Ma (*Helicteres angustifolia* L.)	Sterculiaceae	Ethanol root extract	Phenolic compounds, Flavonoids, Triterpenoids, Quinines, Lignans	Antidiabetic	Increased in glucose uptake and reduction in the insulin resistance	Hu et al. (2016)
19	Wilayati tulsi (*Hyptis suaveolens*. L)	Lamiaceae	Methanolic extract	Alkaloids, Tannins, Saponins, Flavonoid, Terpenoids	Antidiabetic	Modulate blood glucose concentration through reduction of serum triglycerides and total cholesterol and other biochemical parameters.	Poonkodi et al. (2017)

20	Spade flower (*Hybanthus enneaspermus* L.)	Violaceae	Alcoholic extract of the whole plant	Phenolic compounds, Flavonoids, Alkaloids, Terpenes, Glycosides, Saponins, Tannins	Hypoglycemic Antidiabetic	Decrease glucose level and total cholesterol	Patel et al. (2011)
21	Lippia (*Lippia nodiflora* L.)	Verbenaceae	Methanol extract of the Whole plant	Saponins, Sterols, Tannins, Flavonoids, Coumarins, Quinones	Antidiabetic Hypolipidemic	Decrease blood sugar level and restoration of pancreatic β-cells	Balamurugan and Ignacimuthu (2011
22	Sweet tea (*Lithocarpus polystachyus* Rehd)	Fagaceae	Ethanol & Aqueous leaves extract	Flavonoids, Polyphenols, Sterols	Hypoglycemic	Decrease glucose level, total cholesterol, triglyceride, lipid as well as urea, nitrogen, creatinine in the blood. Improve antioxidant potential.	Wang et al. (2016); Hou et al. (2011)
23	Giant yellow mulberry (*Myrianthus arboreus* P.)	Moraceae	Ethanol stem bark extract	Flavonoids, Alkaloids, Phenolics, Triterpenoids, Tannins, Sterols, Saponins, Glycoside compounds	Hypoglycaemic Antihyperlipidemic	Decreased urea and blood creatinine concentration. Adjusted glucose, cholesterol, triglycerides, lipids levels in blood serum	Dickson et al. (2016); Boudjelal et al. (2012)

24	White Horehound (*Marrubium vulgare* L.)	Lamiaceae	Methanolic leaves extract	Alkaloids, Phenylpropanoids Esters, Phenolic compounds	Antidiabetic	Increasing insulin secretion from pancreatic β-cells and preventing the process of insulin degradation	Elberry et al. (2015); Bouterfas et al. (2014)
25	Savulikodi (*Merremia tridentate* L.)	Convolvulaceae	Aqueous root extract	Phenolic compounds, Flavonoids, Diosmetin, Luteolin, Glucosides	Antidiabetic	Decrease of glucose and lipid profile of STZ in blood serum	Arunachalam and Parimelazhagan (2012)
26	Tulsi, holy Or basil (*Ocimum sanctum* L.)	Lamiaceae	Methanolic extract	Triterpenoids, Flavonoids, Phenolic compounds	Antidiabetic Hypoglycemic	Decrease the blood glucose cholesterol, triglycerides levels. & The presence of Zing element in plant	Patil et al. (2011); Pattanayak et al. (2010)
27	Mexican mint (*plectranthus amboinicus* mints)	Lamiaceae	Ethanol extract	Flavonoids, Phenolics, Terpenoids	Antidiabetic Hypoglycemic	Reduced level of blood sugar by regulation of carbohydrate metabolizing enzyme	Koti et al. (2011); Zhang et al. (2017a)
28	Purslane (*Portulaca oleracea* L.)	Portulacaceae	Aqueous leaves extracts	Flavonoids like quercetin, kaempferol, luteolin, apigenin	Hypoglycemic Hypolipidemic activities	Reduced blood glucose, triglycerides, LDL-cholesterol concentration but enhanced HDL-cholesterol level	Moukette et al. (2017)

29	Honey mesquite (*Prosopis glandulosa* Torr.)	Fabaceae	Gelatine/Jelly of the whole plant	Flavonoids, Steroids, Alkaloids, Terpenoids	Antidiabetic	Stimulation of insulin release and improvement of insulin sensitivity	George et al. (2011); Kumar et al. (2011d)
30	Foxtail millet (*Setaria italic*)	Poaceae	Aqueous seed extract	Steroids, Alkaloids, Carbohydrates, Glycosides	Antihyperglycemic	Control blood glucose, triglycerides, total cholesterol such as LDL and VLDL cholesterols (bad cholesterols)	Sireesha et al. (2011)
31	Toothache plant (*Spilanthes Africana* Murr)	Asteraceae	Aqueous leaves extracts	Phenolic compounds, Flavonoids, Tannins, Polyphenols Alkaloids, Terpenoids, Coumarins, acetylenes,	Hypoglycemic Hypolipidemic	Reduced blood glucose, triglycerides, LDL-cholesterol concentration, and improved HDL-cholesterol (good cholesterols)	Moukette et al. (2017)
32	Asoka Tree (*Saraca asoca* Roxb)	Caesalpiniaceae	Methanol extract	Lactones Tannin, Flavonoid, Saponin, Glycosides, Steroids	Antihyperglycemic	Controlling glucose concentration in blood	Kumar et al. (2012); Saha et al. (2012)

33	Turkey berry (*Solanum torvum* Swartz)	Solanaceae	Methanol Fruit extract	Methyl caffeate (a phenol constituent), Flavonoid sulfate, Steroidal glycosides	Antihyperglycemic	Decreased glucose level and regeneration pancreatic β-cells	Gandhi et al. (2011)
34	Parala, Pachotti, (*Symplocos cochinchinensis* L.)	Symplocacea	Hexane leaves extract	Steroids, Triterpenoids, Phenolic compounds	Antidiabetic	Improved insulin sensitivity and reduced the level of cholesterol, triglycerides, and lipids (free fatty acids) in blood plasma	Sunil et al. (2011)
35	Arrow-leaf sida (*Sida rhombifolia*)	Malvaceae	Aqueous leaves extracts	Flavonoids, Tannins, Polyphenols, Mucilages, Triterpenoids	Hypoglycemic Hypolipidemic	Decreased in blood sugar, triglycerides, LDL-cholesterol level and promoted the HDL-cholesterol level.	Moukette et al. (2017)
36	Yellow-berried Nightshade (*Solanum xanthocarpum*)	Solanaceae	Methanolic leaves extract	Flavonoids, Alkaloids, Saponin, Sterols, Glycosides	Antihyperglycemic	Decreased lipid peroxidation and improved antioxidant enzymes potential	Poongothai et al. (2011)
37	Stinging Nettle (*Urtica dioica* L.)	Urticaceae	Hydroalcoholic leaves extract	Flavonoids, Tannins, Scopoletin, Sterols, fatty acids	Hypoglycemic	Regulating glucose concentration and	Ahangarpour et al. (2012); Asgarpanah and Mohajerani (2012)

						insulin resistance in blood	
38	Saudi mistletoe (*Viscum schimperi* Engl)	Viscaceae	Methanolic aerial parts extract	Polysaccharides, Oligosaccharides, Alkaloids	Antihyperglycemic Hypolipidemic	Decreased the level of sugar, triglycerides, LDL-cholesterol in blood as well as stimulation and potentiation of insulin release	Abdel-Sattar et al. (2011)
39	Chinese chaste tree (*Vitex negundo* L.)	Lamiaceae	Methanolic leaves extract	Volatile oils, Flavonoids, Iridoid glucoside	Antihyperglycemic	Improved insulin release from β-cells in pancreatic tissue by iridoid glucoside, increased glucose uptake and metabolism	Sundaram et al. (2012)
40	Tetraena alba (*Zygophyllum album*)	Zygophyllaceae	Ethanol extract of Whole plant	Phenolic compounds, Flavonoids, Carbohydrates, tannins	Antidiabetic	Decreased blood glucose concentration, lipid peroxidation. Reinforced defence systems by increasing potential enzymatic and nonenzymatic antioxidants	Ghoul et al. (2011)

2.5 Secondary Metabolites

Secondary metabolites are non-nutritive organic compounds biosynthesized by plants, bacteria, and fungi. Though they are not directly associated with primary metabolic activities such as growth, development, or reproduction of an organism, they are vital for the plants to survive and persist in their environment (Bartwal et al., 2013). Based on the origin of their biosynthesis, plant phytochemicals are classified into three main categories: namely phenolics, terpenoids, and sulfur- and nitrogen-containing alkaloids compounds (Crozier et al., 2008). They can provide long-term advantages to the plants such as protection against environmental stress (Rosenthal and Berenbaum, 2012). In addition, secondary metabolites give plants their characteristic features such as color and smell that attract potential pollinators. The amounts of secondary metabolites in plants were increased when exposed to herbivores or pathogens (Rosenthal and Berenbaum, 2012).

Apart from the importance of these compounds for adaptation to environmental stressors, they also exhibit practical applications in the medicinal, nutritional, and cosmetic companies (Jensen et al., 2014). Secondary metabolites have been proven to exert an extensive range of bioactive actions including antidiabetic, antioxidant, antimicrobial, anti-inflammatory, antiviral, anticancer, and antifungal activities (Kholkhal et al., 2013; Atanasova- Penichon et al., 2016). Antioxidant activities of plant-based chemical compounds illustrate potent inhibitory effects against inflammation responsible for insulin resistance and oxidative stress correlated with diabetes and cardiovascular diseases (Bajaj and Khan, 2012; Jung et al., 2014). As antioxidants, secondary metabolites have various therapeutic strategies including inhibiting free radical formation, eliminating free radicals, and enhancing the capabilities of endogenous antioxidant enzymes (Hamilton et al., 2007).

2.5.1 Phenolic compounds

Phenolic compounds are one of the major groups of bioactive compounds. Several physical characteristics of plants are connected to these compounds. They are directly involved in plants' taste, smell, and color. Not only are these compounds play a vital role in the growth, development, and defense mechanism in plants, ample research has shown their remarkable impacts on human health (Sun et al., 2008). 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 disease (Lin et al., 2016). The high antioxidant capacities of phenolic compounds have an important role in managing and controlling diabetes progression and its relevant complications via modulating starch and lipid digestion, reducing hyperglycemia and insulin resistance, improving β-cells' ability to produce insulin, and preventing oxidative stress (Asgar, 2013; Lin et al., 2016).

2.5.2 Anthocyanins

Anthocyanins are phenolic compounds sub-grouped under flavonoids. Anthocyanins are known as water-soluble pigments that depend on environmental pH, they appear red, purple, or blue (Ghosh and Konishi, 2007). Anthocyanins have an antioxidant function in plants against reactive oxygen species caused by biotic and abiotic stresses (Qiu et al., 2016). Furthermore, they are known to serve as attractants for pollination and seed dispersal (Saito and Harborne, 1992). In human health, they have been proven to have a significant role in vision health by eliminating retinal inflammation (Miyake et al., 2012). In addition, anti-mutagenic, anti-carcinogenic, and anti-microbial properties have been attributed to anthocyanin-rich foods/plants. The antidiabetic activities of anthocyanins are primarily correlated to their antioxidant capacities (Sancho and

Pastore, 2012). The antioxidant properties of anthocyanin are closely connected to the number of hydroxyl groups present in their ring B (Guo and Xia, 2018; Sancho and Pastore, 2012). Anthocyanins control diabetes in two different ways; namely, prevention of oxidative stress and stimulation of β-cells to secret insulin (Li et al., 2013). Thus, anthocyanin-rich foods/plants have a high potential to protect against diabetes and cardiovascular diseases.

2.5.3 Carotenoids

Carotenoids are plant pigments categorized under tetraterpenoids. They have a vital role in fruit and vegetable colors (Sluijs et al., 2015). Various physiological properties including antidiabetic, antioxidant, anti-inflammation, and anti-obesity activities have been ascribed to carotenoids (Roohbakhsh et al., 2017; Sanjeevi et al., 2019). Carotenoids' high antioxidant capacity considerably affects the management and reduction of T1DM, T2DM, and associated complications like obesity and heart and blood vessel disease (Sanjeevi et al., 2019). Roohbakhsh et al. (2017) stated that oxidative stress and inflammation are two main components associated with the development of T2DM due to impaired insulin secretion and enhanced insulin resistance. Carotenoids can restrain oxidative stress and inflammation as well as regulate immune system activity by reducing chemokine and cytokine secretion which are the main factors in insulin resistance. Additionally, carotenoids adjust lipid metabolism in adipose tissues, thus, acting as an anti-obesity factor (Voutilainen et al., 2006; Maeda et al., 2008).

2.5.4 Glucosinolates

Glucosinolates are another plant phytochemicals mainly discovered in the Brassicaceae family (Ma et al., 2018). They are present as salts of sulfate synthesized from various amino acids. There are more than 120 diverse glucosinolates based on the type of amino acid from which they

are synthesized (Soledade et al., 2010). Recent studies showed glucosinolates as antimicrobial, anti-inflammation, and antioxidant compounds (Bischoff, 2016). Like other secondary metabolites mentioned in this review, glucosinolates can reduce the risks of T2DM by their capacity to limit oxidative stress and inflammation (Jeon et al., 2018).

Overall, there are many health benefits of plant secondary metabolites on human health. Having properties like antioxidative, α-amylase, and α-glucosidase properties makes these molecules a great potential in reducing chronic diseases related to obesity and diabetes.

2.6 Diabetes

Diabetes is a metabolic disorder related to impaired insulin secretion or insulin insensitivity of the body cells to insulin (Chiang et al., 2014). Diabetes is divided into three classes including Type I Diabetes Mellitus (T1DM), Type II Diabetes Mellitus (T2DM), and gestational diabetes mellitus (Choudhury et al., 2018). The pancreas of a person with T1DM does not produce adequate insulin, with infected persons completely dependent on the use of external insulin (Arumugam et al., 2013). In contrast, a person with T2DM has insulin resistance leading to a decline in insulin sensitivity. Another category of diabetes called gestational diabetes mellitus can be found in pregnant women with no previous diagnosis of diabetes (Choudhury et al., 2018). Diabetes is known as one of the major widespread diseases and is the fourth leading cause of death (Bahmani et al., 2014). According to the World Health Organization report, that approximately 425 million people were diagnosed with diabetes globally in 2017, which may enhance to 629 million by 2045. The Canadian Community Health Survey (CCHS) reported that around 2.27 million Canadians were diagnosed with diabetes in 2017.

In comparison with T1DM, the causes and complications of T2DM can be effectively managed or controlled through healthy lifestyles and dietary choices. Some important factors

associated with the steady rise in diabetes include obesity, physical inactivity, and aging (Choudhury et al., 2018). Research has shown a strong connection between T2DM and obesity. Obesity is one of the main causes of T2DM because excess fat makes a significant contribution to insulin resistance, causing an increase in blood glucose concentration (Saad et al., 2017). Having a healthy diet such as adjusting carbohydrates intake is one of the most effective ways of losing weight and balancing blood sugars. Consuming nutritious high-fiber foods and vegetables can provide the essential vitamins and minerals needed to help decrease risks of obesity and T2DM (Arumugam et al., 2013; Saad et al., 2017). Additionally, the use of medicinal plants is another effective approach to avoid or manage T2DM (Hahn et al., 2020).

2.6.1 Evaluation of anti-diabetic potential

Regulation of α-amylase and α-glucosidase enzymes actions is an effective and practical way to control hyperglycemia (Sekhon-Loodu and Rupasinghe, 2019). Although synthetic drugs including acarbose and miglitol are used to restrain α-amylase and α-glucosidase potential, research has associated their use with negative side effects such as dizziness, headache, flatulence, and diarrhea. Thus, medicinal plants which possess potential antidiabetic benefits are safer alternatives (Patel et al., 2011). Previous researchers have confirmed that the composition of the secondary metabolites of medicinal plants has potent inhibitory actions against α-amylase and α-glucosidase (Patel et al., 2012).

2.7 Conclusion

Considering the high global demand for natural foods and functional plants for the prevention or management of diabetes, there is a need for sustainable production systems.

However, variations in preharvest factors can significantly influence plant growth, development, and biosynthesis of phytochemicals with positive health benefits. Nevertheless, the effects of preharvest factors on the antidiabetic properties of food crops and medicinal plants are not explicit nor easily accessible in the literature. Findings of this review showed that the biosynthesis of secondary metabolites responsible for the antidiabetic potential of food crops and medicinal plants are largely influenced by preharvest factors. For future perspectives, optimum preharvest parameters should be investigated to provide in-depth data for developing new functional foods with top-notch antidiabetic properties.

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CHAPTER 3: Growth and Yield of Kale, Swiss chard, Amaranth, and Arugula microgreens in response to different growing medium substrates

This chapter presents a version of the manuscript titled '**Growth and Yield of Kale, Swiss chard, Amaranth, and Arugula microgreens in response to different growing medium substrates**' that has been published in the *Horticulture International Journal* on October 21, 2022. This was a multi-authored publication, in which the PhD Candidate contributed to the research design, data collection, data analysis, and writing. The citation is:

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3.1 Abstract

Microgreens are relatively novel food with high nutrition and dietary benefits that can be influenced by the growing medium. Two experiments were performed to develop an optimum media for microgreens from kale (*Brassica oleracea* L. var. acephala), Swiss chard (*Betavulgaris* var. cicla), arugula (*Eruca vesicaria* ssp. sativa), and amaranth (*Amaranthus tricolour* L.). Experiment 1 was screening of media T1 = 30% vermicast + 40% sawdust + 30% perlite; T2 = 30% vermicast + 50% sawdust + 20% perlite; T3 = 50% vermicast + 30% sawdust + 20% perlite; T4 = 30% vermicast + 40% sawdust + 30% mushroom compost; T5 = 30% vermicast + 20% sawdust + 20% perlite + 30% mushroom compost; and a negative control (NC) = 50% sawdust + 50% mushroom compost. The positive control was Promix BX™ potting mix alone. Experiment 2 was to test the efficacy of two different sources of mushroom compost (White oyster mushroom compost (MC1) and Shiitake mushroom compost (MC2)) added to media T1 to T5 above. The results showed that the media physicochemical properties varied across treatments. Higher chemical parameters were obtained for T4, T5, and media containing MC1. Porosity and water retention were increased in media containing MC2 compared to the other. Seed germination, plant height, and microgreen yield were statistically ($P>0.05$) enhanced by T2 and T4 that contained MC2 compared to the rest. Microgreens yield was approximately three times higher in T2 and T4 with added MC2, except the yield of arugula which was two times higher in these media compared to the control. Overall, T5 alone, and T2 and T4 with added MC2 were the most effective media for microgreen production. Future studies will assess microgreens' nutrients in different media.

Keywords: leafy vegetable, natural amendment, organic food, sustainable farming, healthy food

3.2 Introduction

Preharvest factors refer to management practices applied before the final harvest of crops that affect postharvest productivity and quality of the crop. Preharvest factors include 1) plant genotypic traits; 2) growing medium factors and amendments; 3) environmental factors (light quality, duration, intensity); 4) management practices (planting and harvest time, irrigation, and fertilization) (Nguyen et al. 2019) Natural amendments (organic natural material) are added to soil or growing media to enhance the fertility and/or the structure of the soil, thereby helping plant growth and development (Abbott et al., 2018; Aftab 2019). Various natural amendments application was shown to differentially alter the physicochemical properties of growing media and provided higher nutrients that consequently improved the yield index of different crops (Sarma and Gogoi, 2015; Celestina et al., 2019). Previous studies corroborated that adding vermicast into growing media promoted aeration, porosity, and capacity of holding water, and supported microbial activity and antipathogenic response (Singh et al., 2016; Iheshiulo et al., 2017).

A study by Zhang et al. (2017) indicated that fresh weight and leaf area were higher in two Plectranthus spp treated with vermicast compared to K-humate and NPK amendments, which can be associated with more balanced nutrients in vermicast. As confirmed by Iheshiulo et al. (2017) kale (*Brassica oleracea* L. var. *acephala*) growth rate, leaf elongation, and fresh weight yield were improved by the application of natural amendments compared to Pro-mix BX alone as the control. The authors found that vermicast was the most effective amendment in enhancing the growth rate of kale which may relate to the presence of higher N content. Another popular natural amendment is sawdust produced from industrial wood waste and forestry with high carbon content. It provides substantial advantages for the environment including 1) promoting water holding capacity; 2) increasing soil porosity and aeration; and 3) providing good drainage (Maharani et al., 2010).

According to Singh et al. (2020) sawdust and vermicompost are locally available materials and environmentally friendlier alternatives compared to traditional strategies for microgreens production. There are limited studies regarding the effects of sawdust on plant production, in particular microgreens. A study by Agboola et al.(2018) revealed a delay in the initial growth response of tomato (*Solanum lycopersicum*) to sawdust but there was an increase in response after 7 days. The authors concluded that sawdust was an economical medium substrate and an effective alternative to peat.

A research study done by Cheng (1987) confirmed that adding 30% sawdust into total soil treated with NPK (nitrogen, phosphorus, and potassium) compound fertilizer led to a significant increase in tomato yield in comparison with using sawdust alone. Mahboub Khomami et al. (2019) demonstrated that the application of vermicompost-sawdust extract caused a significant increase in the yield and mineral nutrients of *Syngonium podophyllum*. A recent study by Lin et al. (2020) indicated that a combination of 60% vermicast + 40% sawdust mixed growing media noticeably enhanced growth factors including fresh/ dry weight, plant height, and leaf number in Swiss chard, pak choi, and kale microgreens. It was shown that the enhanced growth factors were linked to the positive effects of vermicast-vermicast mixed media on growing media physicochemical factors including enhanced microbial activities and nutrient mineralization. Mushroom compost is a mixture of different natural compost like chopped straw, gypsum, manure, and water used to grow mushrooms. It is known to possess the potential benefits for plant growth and growing media properties including 1) supplying readily available macro and micronutrients; 2) increasing water retention capacity and drainage; 3) supporting beneficial micro-organisms. Renaldo et al. (2014) reported higher germination rate and growth parameters including root/ shoot ratio, shoot/ root day mass cucumber (*Cucumis sativus*) treated with mushroom compost and biochar compared to corn

stalks. It was demonstrated that the higher decomposition rate in mushroom compost, hence an increased nutrient availability may be connected to enhanced growth indices. In the work done by Hernández et al. (2021) enhanced germination percentage, fresh shoot weight, and yield in red baby leaf lettuce by mushroom compost application. Interestingly, the results obtained from previous studies present obvious affirmation regarding the potential effects of mushroom compost on growth attributes and plant productivity.

Microgreens are immature seedlings of edible vegetables and herbs, which are known to possess high nutritional values and biological functions (Vidal et al., 2018; Kyriacou et al., 2019) According to literature, microgreens possess higher levels of phytonutrients such as ascorbic acid, b-carotene, a-tocopherol, and phylloquinone, vitamins, and minerals compared to their mature leaf counterparts (Pinto et al., 2015; Kyriacou et al. 2016). Kale, Swiss chard, and pak choi have been shown to have high vitamins A, C, and K, functional lipids, carotenoids, and mineral nutrients content (Di Noia, 2014; Pham et al., 2019). However, microgreens response to variations in growing media has not been well studied. Therefore, the objective of the current study was to determine properties exhibited by different mixed proportions of growing media and their effects on plant growth components and yield of different plant species of microgreens.

3.3 Materials and Methods

Two separate greenhouse experiments were carried out between July and December 2020 to formulate and optimize mixed growing media. Each experiment was repeated, and the data was merged due to a small coefficient of variation of less than 7%[1]. Seeds of kale (*Brassica oleracea*

[1] https://www.formpl.us/blog/coefficient-variation

L. var. *acephala*), Swiss chard (*Beta vulgaris* var. *cicla*), arugula (*Eruca vesicaria* ssp. *sativa*), pak choi (*Brasica rapa var chinensis*), and amaranth (*Amaranthus tricolour* L.); and perlite, Pro-mix BX and vermicast were purchased from Halifax Seeds, NS, Canada. Sawdust was obtained from Thermal Woods Inc., NB; and Shiitake (*Lentinula edodes*) and white oyster (*Pleurotus ostreatus*) mushroom compost from Maritime Gourmet Mushroom, Great Village, NS.

3.3.1 Formulation and testing of media

Table 3. 1 shows the mixture for each growing media treatment.

Table 3. 1 Proportions of mixed growing media

Treatment	**Formulation**
T1	30% vermicast + 40% sawdust + 30% perlite
T2	30% vermicast + 50% sawdust + 20% perlite
T3	50% vermicast + 30% sawdust + 20% perlite
T4	30% vermicast + 40% sawdust + 30% mushroom compost (MC)
T5	30% vermicast + 20% sawdust + 20% perlite + 30% MC
Positive control	Promix BX potting medium alone
Negative control	50% sawdust + 50% MC

Physical characteristics of growing media in terms of bulk density, porosity, and field capacity were determined in triplicate as suggested by Peterson (1999) with slight modifications. Bulk density (Db) was obtained from the weight (M) and volume (V1) of the soil using a graduated glass cylinder after continuous tapping until there was not any visible change in soil volume and calculated as:

$$\text{Bulk density} = {}^{M}/_{V_1} \text{---------- (1)}$$

The soil was air-dried under room temperature (*ca*. 22oC) after which 15.24-cm plastic pots with drainage holes were filled with known mass of the soil (Ms) and weighed (Msp). The potting soil placed in a saucer was saturated with distilled water. After 48hr, the saturated soil weight (Msat) was recorded. To drain the free water, the saucer was removed, and the drained soil (Mdrained) was weighed after 72hr under atmospheric pressure. Then, the drained soil was spread uniformly in a tray to dry at ambient temperature for 72hr and weighed (Mdried).

$$\text{Porosity} = {}^{Ms}/_{V_2} \text{----------(2)}$$

$$\text{Field capacity } (F_c) = \frac{M_{drained} - M_{sp}}{M_s} \text{ x } 100\text{----------(3)}$$

The chemical characteristics of growing media including pH, salinity, electrical conductivity (EC), and total dissolved solids (TDS) were determined by the mixture of 500g of each media and 400mL of deionized water. These chemical properties were recorded by an ExStik® II EC500 waterproof pH/conductivity meter (Extech ITM Instruments Inc., Canada).

3.3.2 Seeding

Seeds of kale, Swiss chard, arugula, pak choi, and amaranth were sown by broadcasting uniformly on a flat plastic tray (19cm length x 12cm width x 2.5cm deep) containing the different mixed media (200g) under high pressure sodium lamp. The temperature cycle in the growth room from seedling stage to time of harvest was 24o/22°C and 16/8hrs day/night light regime. The seedlings were irrigated every two days. No fertilizer was used.

3.3.3 Seed germination, plant growth and yield

Germination data were collected after six days of sowing. Plant height was measured by using a ruler at six days interval. The microgreens were harvested and weighed as the yield (whole shoot area growing above ground) after 14days of sowing using Ohaus Navigator® XT Portable Balance (ITM Instruments Inc., Canada). The optimum proportion of mixed media was selected for the subsequent experiments.

3.3.4 Optimization of mixed media

In the second experiment, new formulations of different mixed growing media were made from two different mushroom composts sources i.e., White oyster mushroom compost (MC1) and Shiitake mushroom compost (MC2) as presented in Table 3. 2.

Table 3. 2 Proportions of mixed growing media

Treatment	**Formulation**
T1.1	30% vermicast + 30% sawdust + 40% White oyster mushroom compost (MC1)
T1.2	30% vermicast + 30% sawdust + 40% Shiitake mushroom compost (MC2)
T2.1	30% vermicast + 30% sawdust + 10% perlite + 30% MC1
T2.2	30% vermicast + 30% sawdust + 10% perlite + 30% MC2
T3.1	30% vermicast + 40% sawdust + 30% MC1
T3.2	30% vermicast + 40% sawdust + 30% MC2
T4.1	30% vermicast + 20% sawdust + 20% perlite + 30% MC1
T4.2	30% vermicast + 20% sawdust + 20% perlite + 30% MC2
Negative control 1	50% sawdust + 50% MC1
Negative control 2	50% sawdust + 50% MC2
Positive control	Promix BX™ potting medium alone

3.3.5 Experimental Design and Statistical Analysis

The experiment was arranged in a completely randomized design with three replications. Plastic trays were rearranged every two days to minimize variations in microclimate of the greenhouse. Data was analyzed by 2-way ANOVA using Minitab (version 18.3), and the Fisher method was applied to compare treatment means at $\alpha = 0.05$ when ANOVA showed $P \leq 0.05$. Correlation analysis was performed to identify the relationship between media quality components and plant data. Multivariate analysis by principal component analysis (PCA) was performed using GenStat. Graphs were plotted using Microsoft Excel.

3.4 Results

3.4.1Experiment 1

3.4.1.1 Growing media properties

Physicochemical properties of the different growing media were significantly ($P<0.05$) different (Table 3. 3). It was found that NC and T4 had a significantly ($P<0.05$) higher bulk density of an average of 0.184 g/cm3 compared to an average of 0.150g/cm3 for the other treatments. The highest porosity and field capacity were observed by T4 followed by NC and T1 but T3 recorded the least.

Table 3. 3 Physicochemical properties affected by different proportion of mixed growing media

Treatment	Bulk density (g/cm^3)	Porosity (%)	Field capacity (%)	pH	Salinity (mg/L)	Electrical conductivity (µS/cm)	Total dissolved solid (mg/L)
T1	0.139de	42.9b	34.1bc	5.8d	375.4d	1091.4d	629.5d
T2	0.161c	41.2bc	33.4bc	5.9d	481.4d	885.5d	745.0c
T3	0.165bc	34.5d	26.6d	6.3bc	355.5d	601.9e	512.8e
T4	0.181ab	45.8a	36.6a	7.2a	1938.0a	3243.3a	2365.7a
T5	0.158cd	38.0cd	29.3cd	7.3a	1369.8b	2445.9b	2295.7a
PC	0.128e	41.3bc	33.3c	6.0cd	798.0c	1521.1c	1217.5b

NC	0.187a	43.3b	34.6b	6.6ab	2039.6a	1737.6c	2585.0a
P-value	<0.001	<0.001	<0.001	0.000	<0.001	<0.001	<0.001

T1: 30% vermicast + 40% sawdust + 30% perlite; T2: 30% vermicast + 50% sawdust + 20% perlite; T3: 50% vermicast + 30% sawdust + 20% perlite; T4: 30% vermicast + 40% sawdust + 30% mushroom compost; T5: 30% vermicast + 20% sawdust + 20% perlite + 30% mushroom compost; Positive control (PC):Pro-mix BX™ potting medium alone; Negative control (NC):50% sawdust + 50% mushroom compost.

The different growing media had distinct pH ranging from 5.8 to 7.3. The pH for T4 and T5 was significantly ($P<0.05$) higher than the other treatments. Different trends for salinity, electrical conductivity and total dissolve solids were observed among the treatments (Table 3). The salinity, electrical conductivity, and total dissolved solids were higher in T4, T5, and NC compared to other treatments.

3.4.1.2 Plant growth

The effects of the different mixed growing media, plant species, and the interaction of growing media × plant species on seed germination and plant height were significant ($P < 0.05$) (Figures 3. 1A-B). Seed germination of Swiss chard and kale were increased by *ca.* 18% and 13% in T5 respectively, compared to their counterparts in the PC. In amaranth, T4 showed the highest germination percentage that was 25% higher than that of the control. Moreover, the different growing media did not exhibit a positive effect on arugula seed germination as the highest rate was observed in PC (Figure 3. 1A). Among the microgreen plant species, the overall trend for germination percentage was arugula (76.9%) > amaranth (61.5%) = kale (61.3%) > Swiss chard (55.6%) (Figure 3. 1A). Consistently, microgreens plant height was significantly ($P < 0.05$)

increased by T5 and PC in all the plant species (Figure 3. 1B). Overall, the trend for the plant height was arugula (4.9cm) > kale (4.7cm) > Swiss chard (4.1cm) >amaranth (2.6cm) (Figure 3. 1B).

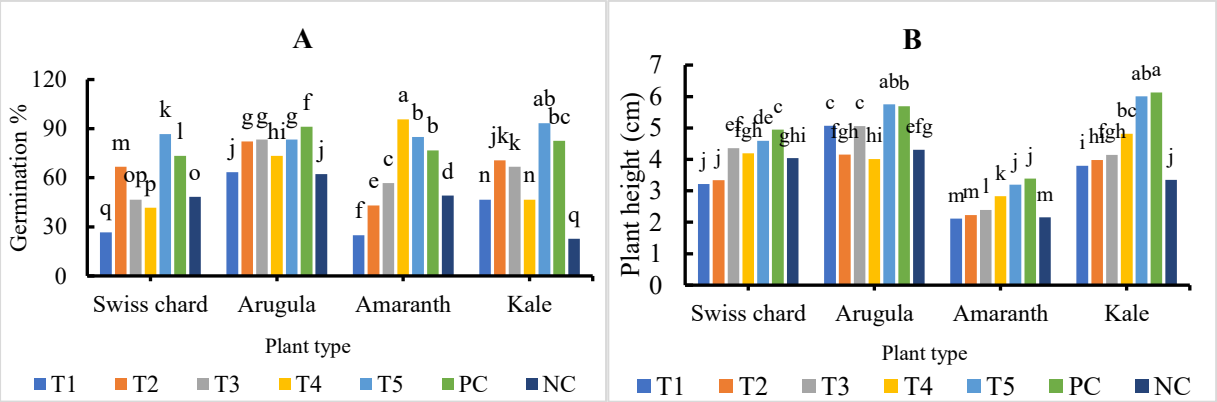


Figure 3. 1. Germination (A) and plant height (B) of Swiss chard (Beta vulgaris var. cicla), arugula (Eruca vesicaria ssp. sativa), amaranth (Amaranthus tricolour L.), and kale (Brassica oleracea L. var. acephala) microgreens affected by different growing media:T1: 30% vermicast + 40% sawdust + 30% perlite; T2: 30% vermicast + 50% sawdust + 20% perlite; T3: 50% vermicast + 30% sawdust + 20% perlite; T4: 30% vermicast + 40% sawdust + 30% mushroom compost; T5: 30% vermicast + 20% sawdust + 20% perlite+ 30% mushroom compost; PC: Pro-mix BX™ potting medium alone; NC: 50% sawdust + 50% mushroom compost. (n = 3); significant at $P < 0.05$.

The correlation analysis between physicochemical attributes of mixed growing media and plant growth factors is presented in Table 3. 4. In general, there was a negative correlation between physical factors of mixed growing media and the plant growth factors in all tested microgreens. However, there was a significant positive correlation between pH, salinity, EC, and TDS of the mixed growing media and seed germination in amaranth.

Table 3. 4 Correlation coefficients between growing media physicochemical factors and plant data

	Physical properties of mixed media	Chemical properties of mixed media

	Porosity	Bulk density	Field capacity	pH	Salinity (mg/L)	EC (µS/cm)	Tds (mg/L)
Swiss chard germination	-0.352	-0.205	-0.383	0.190	0.441	0.315	0.497
Arugula germination	0.532**	-0.395	-0.521**	-0.026	0.350	0.502**	0.460
Amaranth germination	0.099	0.193	-0.003	0.689**	0.544**	0.758**	0.560**
Kale germination	0.574**	-0.566**	-0.642**	0.017	0.426	0.561**	0.661**
Swiss chard height	0.590**	-0.110	-0.324	0.409	0.279	0.326	0.343
Arugula height	0.494	-0.666**	-0.573**	0.019	-0.577**	0.461	0.519**
Amaranth height	0.404	-0.365	-0.199	0.388	0.190	0.498	0.305
Kale height	0.586**	-0.479**	-0.296	0.314	0.047	0.416	0.188

EC: Electrical conductivity; TDS: Total dissolved; ** Significant at $P < 0.05$

3.4.1.3 Association between media and plant data

A Principal component analysis (PCA) followed by a biplot was employed to examine the association between physicochemical characteristics of growing media and plant data affected by the variations in growing media (Figure 3. 2). T5 followed by T4 are close to the origin of the axes suggesting higher stability in these treatments compared to the others that are located at the periphery. Therefore, T5 and T4 can be associated with improved physicochemical properties of the growing media and plant growth components of all the microgreen plant species. Interestingly, the chemical parameters of growing media are strongly influenced by different growing media formulations compared to that of the physical parameters. The overall trend for media chemical parameters was pH > EC > TDS > Salinity. In addition, amaranth germination and seedling height, and Swiss chard seedling height followed by kale seedling height were strongly influenced by the

interaction of growing media × plant species compared to that of the arugula plant. Finally, T5 and T4 were selected as desirable media for microgreen production compared to the others irrespective plant species.

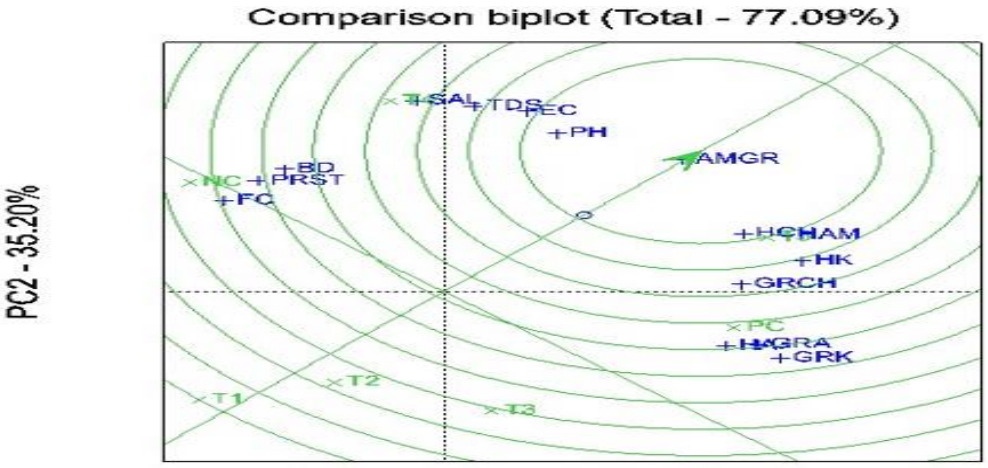


Figure 3. 2. Ranking total × total biplot for comparison of treatment × plant species interaction effects on all growth and physicochemical growing media properties variations in all microgreens. PRST: Porosity; BD: Bulk density; FC: Field capacity; SAL: Salinity; EC: Electrical conductivity; TDS: Total dissolved solid; GRCH: Swiss chard germination; GRA: Arugula germination; GRK: Kale germination; AMGR: Amaranth germination; HCH: Swiss chard height; HA: Arugula height; HK: Kale height; HAM: Amaranth height.T1: 30% vermicast + 40% sawdust + 30% perlite; T2: 30% vermicast + 50% sawdust + 20% perlite; T3: 50% vermicast + 30% sawdust + 20% perlite; T4: 30% vermicast + 40% sawdust + 30% mushroom compost; T5: 30% vermicast + 20% sawdust + 20% perlite+ 30% mushroom compost; PC: Pro-mix BX™ potting medium alone; NC: 50% sawdust + 50% mushroom compost.

3.4.2 Experiment 2

3.4.2.1 Growing media properties

The physicochemical properties of the growing media were significantly ($P<0.05$) different from each other, possibly due to the distinct composition of each growing media (Table 3. 5). It was found that PC had the highest bulk density among treatments. However, treatments formulated with White oyster mushroom compost (MC1) had a higher bulk density of an average of 0.095g/cm3 compared to an average of 0.087g/cm3 for treatments formulated with Shiitake mushroom compost (MC2).

Table 3. 5 Physicochemical properties of growing media affected by different proportion of mixed amended

Treatment	Bulk density (g/cm^3)	Porosity (%)	Field capacity (%)	pH	Salinity (mg/L)	Electrical conductivity (µS/cm)	Total dissolved solid (mg/L)
T1.1	0.099abc	27.0de	20.4b	7.2ab	2135.0ab	4551.5a	2524.7bc
T1.2	0.091abc	30.5bc	22.9ab	6.2b	1714.1bc	2619.5cd	1711.6e
T2.1	0.078bcd	25.5e	15.5d	6.8ab	1499.2cd	3176.4bc	1993.6de
T2.2	0.076d	30.6bc	20.8b	6.4ab	836.8e	1508.4e	1381.3f
T3.1	0.105ab	29.1cd	20.1bc	7.1ab	2132.9ab	4238.2a	2574.4bc
T3.2	0.103abc	30.5bc	24.3a	6.7ab	1881.7bc	2935.7c	2431.5bc
T4.1	0.087abc	27.1de	14.7d	7.4a	1486.6cd	2758.3cd	2286.6bcd
T4.2	0.077cd	32.4b	16.1cd	7.0ab	1328.6d	2325.9d	2191.5cd
PC	0.108a	38.0a	24.4a	6.2b	798.5e	1560.5e	1275.0fg
NC1	0.106a	27.2de	19.9bc	7.4a	2523.2a	3797.7ab	3434.7a
NC2	0.089 abc	28.4cd	20.2b	6.5ab	830.2e	1685.1e	2603.4b
P-value	<0.003	0.000	0.000	0.008	<0.001	<0.001	0.000

T1.1: 30% vermicast + 30% sawdust + 40% MC1; T1.2: 30% vermicast + 30% sawdust + 40% MC2; T2.1: 30% vermicast + 30% sawdust + 10% perlite + 30% MC1; T2.2: 30% vermicast + 30% sawdust + 10% perlite + 30% MC2; T3.1: 30% vermicast + 40% sawdust + 30% MC1; T3.2:

30% vermicast + 40% sawdust + 30% MC2. T4.1: 30% vermicast + 20% sawdust + 20% perlite + 30% MC1; T4.2: 30% vermicast + 20% sawdust + 20% perlite + 30% MC2. NC1: 50% sawdust + 50% MC1; NC2: 50% sawdust + 50% MC2; Positive control: Pro-mix BX™ potting medium alone.

The highest porosity was observed in PC followed by T4.2 and the least were T2.1. Field capacities of media PC, T3.2 and T1.2 were the highest compared to the others. Consistently, field capacity was increased in growing media formulated with MC2 compared to MC1.Moreover, T4.1 and NC1 had higher pH of an average of 7.4 compared to an average of 6.2 for T1.2 and PC. The overall trend for salinity, EC and TDS of the growing media parameters were different among treatments. The highest salinity, EC, and TDS were observed in NC1 followed by T3.1 and T1.1 (Table 3. 5).

3.4.2.2 Plant growth

The ANOVA showed that seed germination, plant height, and yield index in all tested microgreen species were significantly ($P<0.05$) influenced by the different growing media, plant species, and their interaction (Figure 3. 3A-C). Swiss chard, arugula, and pak choi seed germination were increased by *ca.*9% in T2.2 and T4.2, *ca.*59% in T2.2 and T3.2, and *ca.*25% in T2.2, respectively, compared to their counterparts that were grown in the PC. The different mixed growing media did not exhibit positive effect on seed germination of arugula as the highest rate was observed in the PC (Figure 3. 3A).

Similarly, the different growing media did not have a positive effect on seed germination of kale as the highest rate was observed in PC (Figure 3. 3A). Moreover, microgreens plant height was significantly ($P < 0.05$) increased by T2.2, T4.2, PC in all the plant species (Figure 3. 3B). Contrary

to this, T1.1, NC1, NC2 followed by T4.1 reduced plant height of all the microgreen plants. The yield index was significantly ($P < 0.01$) increased by T2.2 and T4.2 in all the plant species (Figure 3. 3C). The yield of Swiss chard, arugula, and kale increased by 31%, 17%, and 43% in T2.2 respectively compared to their PC counterparts. However, pak choi yield was enhanced in T4.2 by 35% compared to PC (Figure 3. 3C).

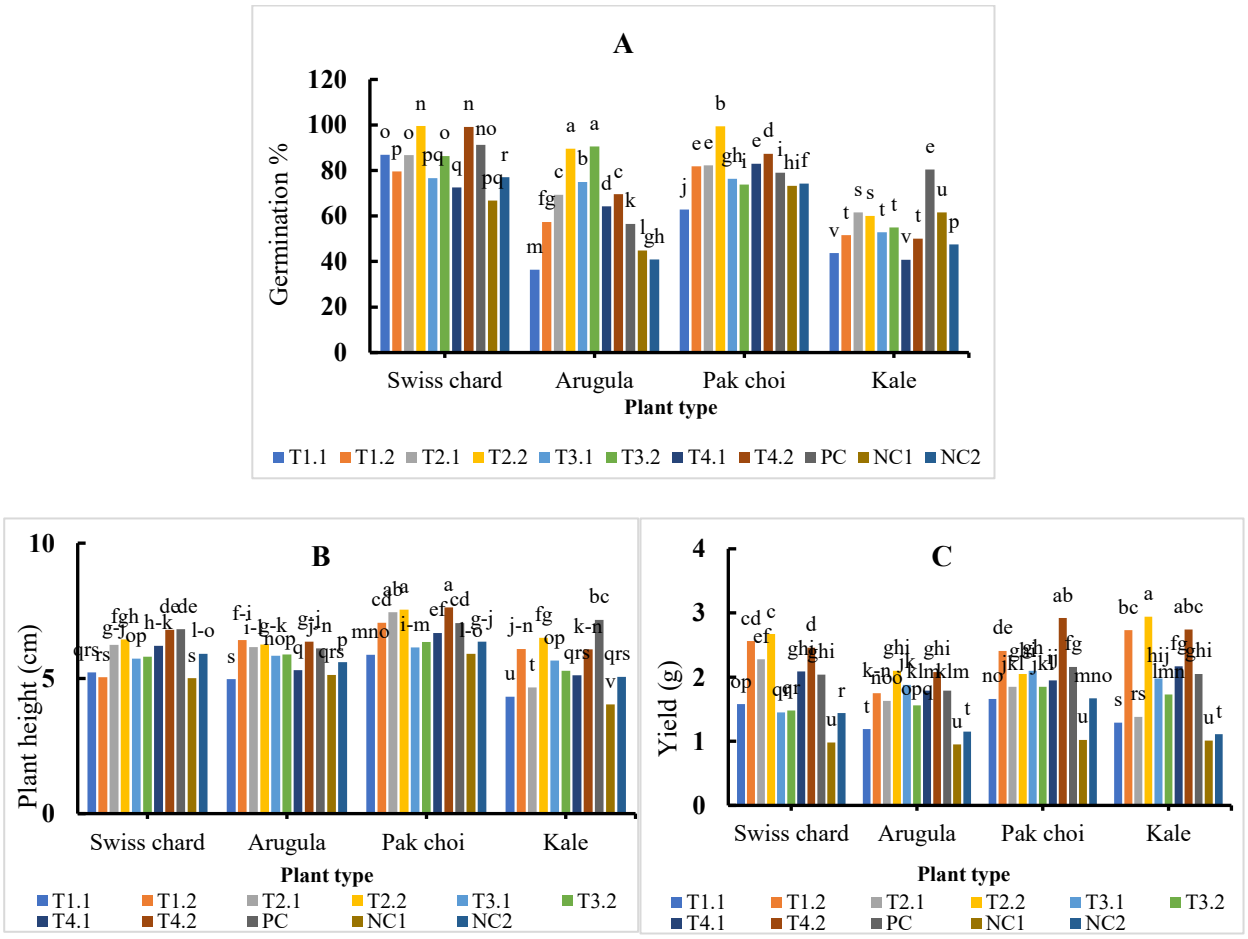


Figure 3. 3. Germination (A); plant height (B); yield (C) of Swiss chard (Beta vulgaris var. cicla), arugula (Eruca vesicaria ssp. sativa), pak choi (Brasica rapa var chinensis.), and kale (Brassica oleracea L. var. acephala) microgreens affected by different growing media including; T1.1: 30% vermicast + 30% sawdust + 40% MC1; T1.2: 30% vermicast + 30% sawdust + 40% MC2; T2.1: 30% vermicast + 30% sawdust + 10% perlite + 30% MC1; T2.2: 30% vermicast + 30% sawdust

+ 10% perlite + 30% MC2; T3.1: 30% vermicast + 40% sawdust + 30% MC1; T3.2: 30% vermicast + 40% sawdust + 30% MC2. T4.1: 30% vermicast + 20% sawdust + 20% perlite + 30% MC1; T4.2: 30% vermicast + 20% sawdust +20% perlite + 30% MC2. NC1: 50% sawdust + 50% MC1; NC2: 50% sawdust + 50% MC2; Positive control: Pro-mix BX™ potting medium alone. ***, significant at P<0.001

There was a positive correlation between porosity and yield of most microgreen species, while there was significant negative correlation between bulk density and field capacity and the measured growth parameters of the microgreens. Furthermore, there was significant (P<0.05) negative correlation between pH, salinity, EC, and germination of pak choi and kale. There was no significant (P>0.05) correlation between TDS and the measured traits in all the microgreens (Table 3. 6).

Table 3. 6 Correlation coefficients between media physicochemical parameters and plant characteristics

	Physical properties of mixed media			**Chemical properties of mixed media**			
	Porosity	**Bulk density**	**Field capacity**	**pH**	**Salinity (ppm)**	**EC (µs)**	**Tds (mg/L)**
Swiss chard GR	0.318	-0.577**	-0.242	-0.191	-0.299	-0.267	0.001
Arugula GR	0.298	-0.361	-0.209	-0.191	-0.227	-0.270	-0.276
Pak choi GR	0.324	-0.681**	-0.519**	-0.591**	-0.531**	-0.614**	-0.457
Kale GR	0.048	-0.158	0.236	-0.589**	-0.646**	-0.592**	-0.219
Swiss chard height	0.489	-0.601**	-0.578**	0.116	-0.330	-0.319	0.200
Arugula height	0.329	-0.553**	-0.216	-0.414	-0.236	-0.360	-0.172
Pak choi height	0.418	-0.858**	-0.605**	-0.261	-0.437	-0.488	-0.279

Kale height	0.534**	-0.406	-0.096	-0.306	-0.330	-0.425	0.023
Swiss chard Yield	0.688**	-0.823**	-0.617**	-0.188	-0.309	-0.331	-0.304
Arugula Yield	0.537**	-0.676**	-0.314	-0.067	-0.279	-0.269	-0.168
Pak choi Yield	0.506**	-0.574**	-0.440	0.029	-0.011	-0.073	0.086
Kale Yield	0.618**	-0.575**	-0.348	-0.262	-0.333	-0.407	-0.334

EC: Electrical conductivity; Tds: Total dissolved solids; GR: Germination; ** Significant at P < 0.05

3.4.2.3 Association among media and plant components

The PCA demonstrated the association among physicochemical parameters of growing media and seed germination and growth parameters affected by the variations in growing media formulations (Figure 3. 4). T4.2 showed a high association and stability compared to the other treatments located at the periphery of the axes. Thereby, the enhanced growth traits in all the microgreens can be attributed to T4.2. Seed germination, plant height and yield were strongly influenced by the interaction between growing media × microgreen plant species.

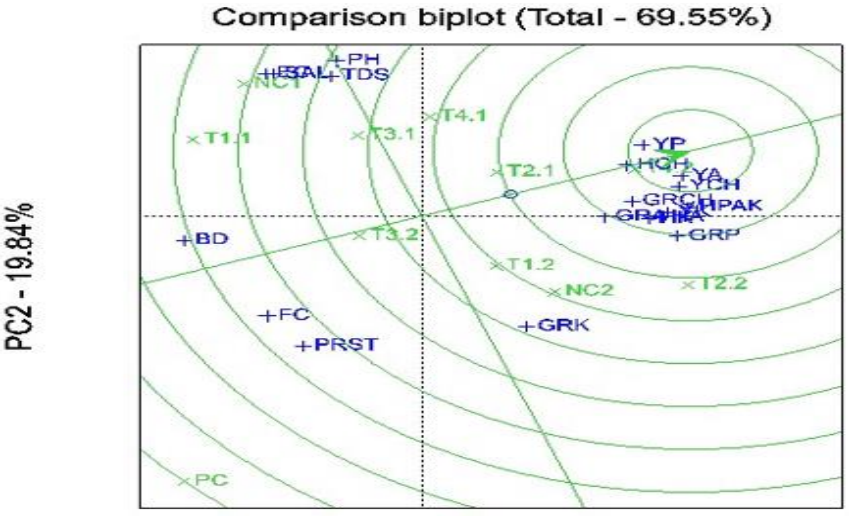


Figure 3. 4. Ranking total × total biplot for comparison of treatment × plant species interaction effects on all growth and physicochemical growing media properties variations in all microgreens.

PRST: Porosity; BD: Bulk density; FC: Field capacity; SAL: Salinity; EC: Electrical conductivity; TDS: Total dissolved solid; GRCH: Swiss chard germination; GRA: Arugula germination; GRP: Pak choi germination; GRK: Kale germination. HCH: Swiss chard height; HA: Arugula height; HPAK: Pak choi height; HK: Kale height. YCH; Swiss chard yield; YA: Arugula yield; YP: Pak choi yield; YK: Kale yield. T1.1: 30% vermicast + 30% sawdust + 40% MC1; T1.2: 30% vermicast + 30% sawdust + 40% MC2; T2.1: 30% vermicast + 30% sawdust + 10% perlite + 30% MC1; T2.2: 30% vermicast + 30% sawdust + 10% perlite + 30% MC2; T3.1: 30% vermicast + 40% sawdust + 30% MC1; T3.2: 30% vermicast + 40% sawdust + 30% MC2. T4.1: 30% vermicast + 20% sawdust + 20% perlite + 30% MC1; T4.2: 30% vermicast + 20% sawdust +20% perlite + 30% MC2. NC1: 50% sawdust + 50% MC1; NC2: 50% sawdust + 50% MC2; Positive control: Pro-mix BX™ potting medium alone.

3.5 Discussion

In the present work, the effects of different natural amendments on the physicochemical attributes of formulated growing media and the response of different microgreens plant species were studied in indoor cultivation system. The results indicated that T4 and T5 growing media had considerable effect on seed germination while T5 and PC growing media had the greatest effect on plant height of all the microgreen species (Figure 3. 1A). Physicochemical properties of the growing media play a significant role in seed establishment and consequently, plant growth.

Amendments are considered one of the major strategies to improve the physical features of growing media such as drainage, water retention capacity, and porosity, which in turn affect plant growth factors ((Rawls et al., 2013; Karthikeyan et al., 2014). In this experiment, it seems that the structure and physicochemical traits of media T4 and T5 were ameliorated, mostly by the

presence of the mushroom compost and as a result, germination and plant height of all microgreens were increased.

In agreement with current results, Vahid Afagh et al. (2019) attributed improved crop productivity to increased aeration and water holding capacity by adding 15% mushroom compost into a growing medium. It has been shown that high bulk density reduces root growth and yield of lettuce (Chen et al., 2019). However, the bulk density of T5 was about 0.158 g/ cm3, which was below root-restriction threshold bulk density (1.66g/ cm3). This results, together with the average value of porosity in T5, could be responsible for the improved plant height in this media. In contrast, decreased plant height of arugula in T4 can be ascribed to high level of salinity (Figure 3. 1B), as confirmed by significant negative correlation between arugula plant height and media salinity (Table 3. 4). Similar to our results, Warrence et al. (2002) explained that root penetration and root growth can be negatively influenced by the higher level of salinity and EC. Addition of perlite and wood-based substrates into growing media can diminish the negative effects of high EC and salinity levels (Zhang et al., 2009; Lee et al., 2011) Accordingly, the more positive effects of T5 on plant height compared to T4 can be explained by the presence of high portions of perlite in the former compared to the latter. Moreover, the presence of mushroom compost in T4 and T5 may supply more nutrients for plants that may explain the observed higher germination and plant height in these media (Kumar et al., 2022). PCA analysis results validated T4 and T5 enhancement of microgreens plant performance compared to other treatments (Figure 3. 2). As a result, T4 and T5 were selected for further investigation in the Experiment 2.

Mixed media with added White oyster mushroom compost (MC1) had higher bulk density and lower porosity, field capacity, and recorded low plant growth (Table 3. 5).Given that the optimum range of pH for leafy greens is 5.5 to 6.5, (Gillespie et al. , 2020) the lower growth rate

in the mixed media made from MC1 can be attributed to the high pH, which negatively affects plant nutrients availability (Arnon and Johnson 1942). It is well known that salinity adversely affect water and nutrient uptake by reducing osmotic potential and leading to nutrient imbalance in plants (Kaymakanova and Stoeva, 2008; Corwin and Yemoto, 2020), in addition to a negative relationship between EC and TDS and soil nutrients availability (Qados, 2011; Lvova and Nadporozhskaya, 2017). In the present study, a high salinity and EC levels were observed in growing media made from MC1 (Table 3. 5). Therefore, a reduction in plant growth in T1.1 and NC1 can be attributed to high EC and salinity levels in these media. Like our results, Zhang et al. (2009) reported negative effects of high EC levels on plant growth and productivity. These results were further confirmed by correlation data analysis in which there was a negative relationship between germination, plant height, yield factors on one hand, and high salinity, EC, and TDS levels on the other hand (Table 3. 6).

Consequently, there was high germination rate, plant height, and yield of microgreens grown in mixed media added with Shiitake mushroom compost (MC2), particularly T2.2 and T4.2 (Figure 3. 3).These results could be due to the improved physical factors such as porosity and field capacity in these media. Moreover, the presence of MC2 in the media may provide more readily available nutrients for the plants compared to MC1. In support of this, higher levels of nutrients including N, C, P, Ca have been reported in Shiitake mushroom compost compared to White oyster mushroom compost by Hernández et al.(2021) and Kumar et al.(2022) In addition, PCA results suggested that growing media made from MC2 were better for the improvement of plant growth and yield performance in all tested microgreens (Figure 3. 4). In agreement with our results, it previously shown that application of mushroom compost enhanced germination rate and seedling growth in cucumber and lettuce plants (Renaldo et al., 2014; Hernández et al., 2021). Furthermore,

Lin et al (2020) demonstrated that growth parameters including plant height, leaf number, fresh and dry mass of Swiss chard, pak choi, and kale were drastically enhanced when grown in the mixed media formulated with 60% vermicast + 40% sawdust.

3.6 Conclusion and recommendation

The results of this study demonstrated variations in physicochemical parameters and the effectiveness of different proportions of mixed media and their impact on the growth and yield of microgreens. The media containing Shiitake mushroom compost substantially promoted plant growth and yield in all microgreen plant species due to improved physicochemical parameters of the growing media and possibly superior nutrient status. In contrast, the reduced plant growth in media containing White oyster mushroom compost may be attributed to the higher salinity, EC, and TDS levels. Overall, it was found that T2.2 and T4.2 were the most effective treatments in improving germination rate, plant height, and yield in all microgreens. We concluded that adding Shiitake mushroom compost and perlite into a growing media will enhancemedia physical features and make nutrients more available to microgreen plants. Future studies will evaluate the effect of different mixed growing media on the chemical composition of microgreens.

CHAPTER 4: Growth and Biochemical Composition of Microgreens Grown in Different Formulated Soilless Media

This chapter presents a version of the manuscript titled '**Growth and Biochemical Composition of Microgreens Grown in Different Formulated Soilless Media**' that has been published in the *Plants* Journal on 15 December 2022. This was a multi-authored publication, in which the PhD Candidate contributed to the research design, data collection, data analysis, and writing. The citation is:

Saleh, R., Gunupuru, L. R., Lada, R., Nams, V., Thomas, R. H., & Abbey, L. (2022). Growth and Biochemical Composition of Microgreens Grown in Different Formulated Soilless Media. *Plants*, *11*(24), 3546.

4.1 Abstract

Microgreens are immature young plants grown for their health benefits. A study was performed to evaluate the different mixed growing media on growth, chemical composition, and antioxidant activities of four microgreen species: namely, kale (Brassica oleracea L. var. acephala), Swiss chard (Beta vulgaris var. cicla), arugula (Eruca vesicaria ssp. sativa), and pak choi (Brassica rapa var. chinensis). The growing media were T1.1 (30% vermicast + 30% sawdust + 10% perlite + 30% PittMoss (PM)); T2.1 (30% vermicast + 20% sawdust + 20% perlite + 30% PM); PM was replaced with mushroom compost in the respective media to form T1.2 and T2.2. Positive control (PC) was Pro-mix BX™ potting medium alone. Root length was the highest in T1.1 while the shoot length, root volume, and yield were highest in T2.2. Chlorophyll and carotenoid contents of Swiss chard grown in T1.1 was the highest, followed by T2.2 and T1.1. Pak choi and kale had the highest sugar and protein contents in T2.2, respectively. Consistently, total phenolics and flavonoids of the microgreens were increased by 1.5-fold in T1.1 and T2.2 compared to PC. Antioxidant enzyme activities were increased in all the four microgreens grown in T1.1 and T2.2. Overall, T2.2 was the most effective growing media to increase microgreens plant growth, yield, and biochemical composition.

Keywords: microgreens; natural amendment; soil health; phytochemicals; healthy food

4.2 Introduction

Microgreens are immature greens harvested from tender young plants that are grown for their high health promoting compounds and biological properties (Kyriacou et al., 2019; Bulgari et al., 2021). Previous researchers reported high amounts of phytochemicals such as ascorbic acid, α-tocopherol, β-carotene, phylloquinone, vitamins, and minerals in different species of

microgreens (Pinto et al., 2015; Kyriacou et al., 2016). Kale (*Brassica oleracea* L. var. *acephala*), Swiss chard (*Beta vulgaris* var. *cicla*), and arugula (*Eruca vesicaria* ssp. *sativa*), as microgreens, possess high levels of vitamins A, C, and K, essential lipids, carotenoids, and mineral nutrients (Di Noia, 2014; Pham et al., 2019). Microgreens are delicate and are prone to various stress factors that can adversely affect the edible quality and bio-functional properties. Like all plants, the key preharvest factors that can affect a microgreen's edible quality are genotypic characteristics, growing media, climate, and management practices (Zietz et al., 2010; Aftab, 2019; Nguyen et al., 2019). Hence, the presented study focuses on the impact of various growing media amendments on the quality of different microgreens. Natural amendments are organic substrates added to a growing medium to improve plant productivity and harvest quality, through enhancement of the physicochemical properties and functional activities of the media (Zhang et al., 2017; Abbott et al., 2018). These amendments include compost, vermicast, humates, manures, and sawdust. They supply macro- and micro-nutrients, support beneficial microbes, improve water-holding capacity and gas exchange, and promote nutrient availability required for plant growth and development (Abbey et al., 2018; da Costa Jaeggi et al., 2019).

Vermicast (worm casting) is humus-like material from the excreta of earthworms. It is rich in beneficial microbiome, and humic and non-humic substances such as mineral elements, amino acids, plant hormones, and other macromolecules, that contribute to plant growth and development (Abbey et al., 2017; Lin, 2020). According to Karthikeyan et al. (2014), vermicast enhanced seed germination rate and plant growth parameters, leaf pigmentation, root nodulation and yield of Lantana (*Lantana camara*) and cluster bean (*Cyamopsis tetragonoloba*) compared to inorganic fertilizer. In addition, adding vermicast to a growing media ameliorated soil physicochemical properties, leading to improved aeration, media porosity, field capacity, and

microbial activity (Singh et al., 2016; Zhang et al., 2017). Similarly, Abbey et al. (2018) showed that morphological indices of kale and postharvest essential fatty acids, mineral nutrients, phenolic compounds, and antioxidant capacity were increased by the application of dry vermicast, potassium humate and volcanic minerals.

Sawdust is another potential growing medium substrate that is a waste from the forestry and wood industries. Currently, sawdust is burned or taken to landfills. There is a growing concern over the mining and use of Sphagnum peat moss. Therefore, sawdust can be used as an environmentally friendlier alternative or supplement to traditional substrates like peat moss or in combination with other substrates. Maharani, et al. (2010) showed that sawdust can improve the porosity and drainage of a growing medium. A study showed that sawdust delayed the initial growth of seedlings of tomato (*Solanum lycopersicum*), but plant growth soared seven weeks after planting when the seedlings were established, and yield was higher than that of the control (Agboola et al., 2018). This delay can be attributed to toxic compounds from the wood such as lignin, cellulose, hemicellulose, and terpenes, which probably leached out, decomposed, or diluted by reaction with other amendments after seven weeks of planting (Lin, 2020; Mohan et al., 2021).

Cheng (1987) showed that the combination of sawdust with 30% soil, plus nitrogen (N), phosphorus (P), and potassium (K) compound fertilizers gave rise to higher productivity of tomato plant compared to sawdust alone. Plant growth components, yield index, and nutritional values of *Syngonium podophyllum* were drastically increased following the application of vermicompost-sawdust extract (Mahboub-Khomami et al., 2019). A recent study showed that the combination of different proportions of vermicast and sawdust improved plant growth and biochemical compounds in Swiss chard, pak choi, and kale microgreen (Lin, 2020). The authors found that 40% vermicast + 60% sawdust or 60% vermicast + 40% sawdust improved the physicochemical

properties of the growing media and enhanced active microbial activity and nutrient mineralization necessary to meet potential plant growth requirements.

Therefore, amendments such as vermicast and compost can be added to sawdust to improve nutrient status and functionality of the growing medium. A study by Hernández et al. (2021) showed that application of spent mushroom compost increased seed germination percentage, fresh shoot weight, and yield of red baby leaf lettuce (*Lactuca sativa* L.) by up to 7-fold as compared to peat alone. Few studies on the effects of individual amendments on plants have been reported but not on their combining effect on microgreen plant growth and chemical composition. Therefore, the objective of the present study was to evaluate the physicochemical properties of different proportions of mixed media and their effects on the growth and biochemical composition of four different plant species (kale, Swiss chard, arugula and pak choi) that can be grown and harvested as microgreens.

4.3 Materials and Methods

4.3.1 Plant Material and Growing Condition

The experiment was carried out in July 2020 and repeated in December 2020 in the Department of Plant, Food, and Environmental Sciences greenhouse (45°23′ N, 63°14′ W), Dalhousie University, Truro, NS, Canada. The microgreens were kale (*Brassica oleracea* L. var. *acephala*), Swiss chard (*Beta vulgaris* var. *cicla*), arugula (*Eruca vesicaria* ssp. *sativa*), and pak choi (*Brasica rapa var. chinensis*), purchased from Halifax Seed Co., Halifax, NS, Canada. The growing media were PittMoss, vermicast, sawdust, mushroom compost, perlite, and Pro-mix BX™. PittMoss® is a soilless potting mix made from recycled paper (Ambridge soil company, PA, USA). It is expected that the PittMoss will improve aeration and water retaining potential, resulting

in the better delivery of nutrients to the root-zone environment. Vermicast, sawdust, and shiitake (Lentinula edodes) mushroom compost were obtained from Modgarden Company, Toronto, ON, Canada. Perlite and Pro-mix BX™ potting medium were purchased from Halifax Seed Company, NS, Canada. Kale, Swiss chard, arugula, and pak choi seeds were sown in flat plastic cell trays, measuring 19 cm length x 12 cm width x 2.5 cm deep, each containing a different mixed medium. The trays were kept in the greenhouse under a 16/8-hr day/night light regime (from high pressure sodium lamp) at a 24°/22 °C day/night temperature cycle with a 71% mean relative humidity. A 600 W HS2000 high-pressure sodium lamp with NAH600.579 ballast (P.L. Light Systems, Beamsville, ON, Canada) supplied the supplementary lighting. Air distribution in the greenhouse was distributed by a horizontal air-flow ventilation system. Watering was carried out every two days with 200 mL of tap water for each pot until the final harvest 15 days after sowing. No additional fertilizer was applied.

4.3.2 Experimental Treatment and Design

The 2-factor experiment (i.e., plant species x growing media) was arranged in a completely randomized design with three replications. Seeds were sown in six different proportions of mixed media (Table 4.1). Pots were rearranged weekly on the growth shelf to offset microclimate variations in the greenhouse. The entire study was repeated twice. The data from the two studies were merged because the coefficient of variation was less than 5%. Seed germination, plant growth, yield, and various biochemical characteristics were measured.

Table 4. 1 Proportions of mixed growing media

Treatment	**Formulation**
T1.1	30% vermicast + 30% sawdust + 10% perlite + 30% PittMoss (PM)
T1.2	30% vermicast + 30% sawdust + 10% perlite + 30% mushroom compost (MC)
T2.1	30% vermicast + 20% sawdust + 20% perlite + 30% PittMoss (PM)

T2.2	30% vermicast + 20% sawdust + 20% perlite + 30% mushroom compost (MC)
NC	60% sawdust + 40% PittMoss
PC	Pro-mix BX™ potting medium alone

NC and PC are negative and positive control, respectively.

4.3.3 Growing Media Physicochemical Properties

To evaluate chemical properties of the growing media, 50 g of each media was added to 50 mL of deionized water and was thoroughly mixed before the determination of chemical properties. pH, salinity, electrical conductivity, and total dissolved solids were measured using an ExStik® II EC500 waterproof pH/conductivity meter (Extech ITM Instruments Inc., Canada). The growing media physical properties and water retention characteristics were determined in triplicate as described by Armah (2021), with slight modifications. Bulk density (D_b) was determined from the weight (M) and volume (V_1) of the soil core, using a graduated glass cylinder after continuous tapping, until there was no observable change in soil volume.

$$\text{Bulk density} = {}^{M}/_{V_1} \text{---------- (1)}$$

$$\text{Porosity} = {}^{Ms}/_{V_2} \text{----------(2)}$$

Water saturation, field capacity, and wilting point were determined after the soil was air-dried under ambient conditions (ca. 22 _C). A known mass of the fresh soil sample (Ms) was placed in a 15.24 cm plastic pot with drainage holes and was weighed (Msp). The potted soil was placed in a saucer and was saturated with distilled water, and the saturated soil weight (Msat) was recorded after 48 h. Then, the saucer was removed so that the free water could drain out under atmospheric pressure for 72 h and was then weighed (Mdrained). The drained soil was spread evenly in a flat aluminum tray and air-dried under ambient conditions for 72 h and then weighed (Mdried).

Field capacity (F_c) = $\frac{M_{drained} - M_{sp}}{M_s}$ x 100----------(3)

4.3.4 Plant Growth and Yield Components

Data on seedling growth indices were collected 14 days after sowing the seeds. Plant samples (n = 15) were randomly and gently uprooted from the middle section of the growing trays for each treatment per replicate using a spatula. The seedlings were placed on tissue paper before carefully removing chunks of loosely attached media from the roots. The roots were then thoroughly washed under a gentle running deionized with minimum root loss (i.e., ca. < 2%). After drying with a blotting paper, the total lengths of roots and shoots and root volume were determined using a Perfection V800 Photo Color Scanner Digital ICE® Technologies (Epson America Inc., Los Alamitos, CA, USA). The shoots of the remaining microgreens were cut with a pair of scissors at the growing media surface after 14 days of sowing, and the fresh weights were recorded as the estimated yield per treatment. At the final harvest, there was no seed residue on the shoots that we had to worry about.

4.3.5 Microgreen Quality and Phytochemical Analysis

4.3.5.1 Chlorophylls a and b, Total Chlorophyll, and Total Carotenoid

Samples of the microgreens per treatment from the final harvest in Section 4.3.4 above were immediately frozen in liquid N to avoid changes in the biochemical compounds present in the plants. Pooled samples of the microgreens frozen in liquid N were ground to fine powder and stored in −20 °C until analyzed. Briefly, 0.2 g of each ground microgreen was separately dissolved in 10 mL of 80% acetone. After centrifuging at 12,000 rpm for 15 min, the supernatant was

collected and transferred into 96 micro-well plates to measure the absorbance at 646.8 nm and 663.2 nm wavelength, using a UV-Vis spectrophotometer (Evolution™ Pro, Thermo Fisher scientific, Waltham, MA, USA) against acetone as blank, using the method described by Lightenthaler (1987). Chlorophyll and carotenoid concentrations were obtained by the following formula.

$$\text{Chla}\ (\mu g/mL) = 12.25 * A663.2 - 2.79 * A646.8 \quad (4)$$

$$\text{Chlb}\ (\mu g/mL) = 21.50 * A646.8 - 5.1 * A663.2 \quad (5)$$

$$\text{Chlt}\ (\mu g/mL) = \text{chla} + \text{chlb} \quad (6)$$

$$\text{Car}\ (\mu g/mL) = (1000 * A470 - 1.8 * \text{chla} - 85.02 * \text{chlb})/198 \quad (7)$$

Finally, the calculated value was multiplied by the total volume (10 mL) and then divided by the total fresh weight (0.2 g), which was expressed as µg/g FW.

4.3.5.2 Total Sugar

The total sugar content of the microgreens was measured using the method described by Mohammadkhani and Heidari (2008), with some modifications. Firstly, 0.2 g of powder was dissolved in 10 mL of 90% ethanol and was incubated in a water bath for 60 min. The mixture was topped with up to 25 mL with 90% ethanol and centrifuged at 4000 rpm for 3 min. An amount of 1 mL of the supernatant was transferred into a glass test tube and 1 mL of 5% phenol was added and vortexed. Subsequently, 5 mL of sulfuric acid was added and incubated in the dark for 15 min. The mixture was cooled, and the absorbance was measured at 490 nm using a UV-Vis spectrophotometer against a blank made up of deionized water, phenol, and sulfuric acid. The total sugar was obtained by a standard sugar curve prepared by dissolving sucrose in distilled water at

different concentrations, from 0 to 300 µg. Then, 1 mL of 5% phenol and 5 mL of sulfuric acid was added to the mixture and the absorbance was recorded at 490 nm. The sugar content was expressed as µg glucose/g FW.

4.3.5.3 Total Protein

The total protein content was measured using the Bradford assay, as described by Hammond and Kruger (1988). In brief, 0.2 g of the ground microgreen tissue samples was transferred into a test tube, added with 5 mL ice-cold extraction buffer (i.e., 50 mM potassium phosphate buffer at pH 7.0) and 0.1 mM EDTA. The mixture was vortexed for 30 s before centrifugation at 15,000 rpm for 20 min. The supernatant was collected and kept on ice. Subsequently, the supernatant was mixed with 100 µL of enzyme extract and 1 mL of Bradford reagent, before recording the absorbance against a blank (Bradford reagent) at 595 nm after a 5 min incubation. The protein concentration was determined by the regression equation obtained from a Bovine serum albumin at different concentrations (200–900 µg mL^{-1}) and was expressed as µg Bovine/g.

4.3.5.4 Total Phenolics

The total phenolic (TPC) was measured using the Folin–Ciocalteu method, as described by Alothman et al. (2009). Briefly, 0.2 g of the ground microgreens was dissolved in ice-cold 80% methanol and incubated at an ambient temperature (approximately, 22 °C) for 48 h in the dark. The mixture was then centrifuged at 13,000 rpm for 5 min. A 100 µL sample of the supernatant, the standard at different concentrations (i.e., 0, 5, 10, 15, 20, 25 mg/L), and a methanol blank were added into distinct tubes before adding 200 µL Folin-Ciocalteu reagent and 800 µL of Na_2CO_3 and

then incubating it for 2 h in the dark. Eventually, 200 µL of the mixture, the standard, and the blank were individually transferred into a microplate to measure the absorbance at 765 nm by UV-vis spectrophotometer. TPC concentration was determined by the standard curve obtained from Gallic acid equivalents and expressed as mM Gallic acid per g of fresh sample (mg GAE/g).

4.3.5.5 Total Flavonoids

The total flavonoid was measured using the method described by Chang et al. (2002). Ground samples of each microgreen (0.2 g) and 2.5 mL of 95% methanol was mixed and vortexed before centrifugation at 13,000 rpm for 10 min. The supernatant (500 µL) standard (1 mg quercetin dissolved in 95% methanol at 5, 10, 15, 25, 50, 100, 150, 200 µg/mL concentrations), and 95% methanol were transferred into separate tubes. Then, 1.5 mL 95% methanol, 0.1 mL 10% $AlCl_3$, 0.1 mL 1 M potassium acetate, and 2.8 mL distilled water were added to each tube. Afterward, the mixture was incubated at an ambient temperature for 30 min, and the absorbance was recorded at 415 nm against a blank using a UV-Vis spectrophotometer. The flavonoids content was measured by the standard curve obtained from the quercetin standard curve. The total flavonoids content was expressed as µg quercetin/g of plant fresh weight.

$$\text{Total flavonoid} = \frac{([\textbf{flavonoids}](\mu\textbf{g}/\boldsymbol{mL}) \times \textbf{total volume of methanolic extract (mL)})}{\textbf{mass of extract (g)}} \quad \ldots\ldots\ldots\ldots (8)$$

4.3.5.6 Total Ascorbate

The total ascorbate was measured using the method described by Ma et al. (2008). In brief, 0.2 g of the ground microgreens was mixed with 1.5 mL ice-cold 5% trichloroacetic acid (TCA) and centrifuged for 15 min at 4 °C. Then, 100 µL of the supernatant was collected and added to

400 µL phosphate buffer (150 mM KH_2PO_4), 5 mM EDTA, and 100 µL10 mM dithiothreitol and vortexed. Following the incubation of the mixture, 0.5% N-ethylmaleimide was added to the mixture and vortexed. To obtain color, 400 µL 10% TCA, 400 µL 44% orthophosphoric acid, 400 µL4% dipyridyl and 200 µL 30 g/L $FeCl_3$ was added to the mixture and incubated at 40 °C for 1 h before recording the absorbance at 525 nm using a UV-Vis spectrophotometer against a blank. The standard was prepared from L-ascorbic acid in 5% TCA (0–5 mM). Total ascorbate content was expressed as µmol/g FW.

4.3.5.7 Antioxidant Enzyme Activity

The peroxidase (POD) and ascorbate peroxidase enzyme activities (APEX) were measured using the method described by Patterson et al. (1984). Briefly, 0.2 g of the ground microgreens was mixed with 5 mL ice-cold extraction buffer and centrifuged at 15,000 rpm for 20 min. The extraction buffer contained mM potassium–phosphate buffer (pH 7.0), 1% polyvinylpyrrolidone, and 0.1 mM EDTA. The supernatant (i.e., enzyme extract) was collected for POD and APEX assays. For POD, the reaction mixture was prepared from the combination of 100 mM potassium-phosphate buffer (pH 7.0), 0.1 mM pyrogallol, and 5 mM H_2O_2. Then, 10 µL of the supernatant was added to the mixture and incubated for 5 min at room temperature. To stop any enzyme reaction in the mixture, 0.1 mL of NH_2SO_4 was added. Finally, the absorbance was recorded at 420 nm using a UV-Vis spectrophotometer against a blank (Milli-Q water). The enzyme activity was calculated by the following formula and expressed as unit/mg FW.

$$POD = A420 \times 3/(12 \times 0.1))/0.2 \quad (9)$$

To assay APEX, 100 µL of the supernatant was added to the reaction mixture, i.e., 1372 µL of 50 mM potassium–phosphate buffer (pH 7.0), 75 µL of 10 mM ascorbate, and 3 µL of 100 mM H_2O_2. The mixture was incubated for 1 min before reading the absorbance at 290 nm using a UV-Vis spectrophotometer against a blank. The enzyme activity in unit/mg FW was obtained by:

$$APEX = (A\ 290 \times 1/(2.8 \times 0.1))/0.2 \quad (10)$$

4.3.5.8 Statistical Analysis

All the data were subjected to a two-way analysis of variance (ANOVA) using Minitab version 18.3. Fisher method was used to separate treatment means when the ANOVA showed a significant difference at $p < 0.05$. Furthermore, a multivariate analysis using a two-dimensional principal component analysis (PCA) was carried out using GenStat software.

4.4. Results

4.4.1 Growing Media Properties

The different additives in the growing media significantly affected the physicochemical properties (Table 4. 2). It was found that T1.1 and T2.1 had a significantly ($p < 0.05$) low bulk density of an average of 0.07 g/cm^3 compared to an average of 0.10 g/cm^3 for T1.2, T2.2, PC, and NC. The highest porosity was observed in PC, followed by T1.2, and T2.2 compared to the other treatments. Porosity and field capacity of media T1.1, T2.1, and NC were significantly ($p < 0.05$) lower than the other media.

Table 4. 2 Physicochemical properties of growing media affected by different proportions of mixed amended.

Treatment	Bulk Density (g/cm^3)	Porosity (%)	Field Capacity (%)	pH	Salinity (mg/L)	Electric Conductivity (µS/cm)	Total Dissolved Solids (mg/L)
T1.1	0.07 b	31.3 c	29.2 c	5.7 b	1299.7 c	2260.0 c	1719.6 c
T1.2	0.12 a	35.0 b	34.2 a	6.4 a	1689.7 a	2570.0 b	2139.7 b
T2.1	0.07 b	26.6 d	25.5 d	5.8 ab	1319.7 c	1233.0 e	1709.6 c
T2.2	0.10 ab	35.7 b	33.2 ab	6.3 ab	1494.9 b	2205.5 c	2028.4 b
PC	0.10 ab	37.8 a	30.2 bc	6.1 ab	802.9 d	1486.0 d	1233.5 d
NC	0.09 ab	27.6 d	24.5 d	5.9 ab	1861.2 a	3412.5 a	2479.7 a
p-value	0.015	0.000	0.000	0.029	0.000	0.001	0.001

T1.1: 30% vermicast + 30% sawdust + 10% perlite + 30% PittMoss (PM); T1.2: 30% vermicast + 30% sawdust + 10% perlite + 30% mushroom compost (MC); T2.1: 30% vermicast + 20% sawdust + 20% perlite + 30% PM; T2.2: 30% vermicast + 20% sawdust + 20% perlite + 30% MC; negative control (NC): 60% sawdust + 40% PittMoss; and positive control (PC): Pro-mix BX™ potting medium alone; significant at $p < 0.05$. Treatment means followed by a common letter are not significantly different.

The different growing media had pH values ranging from 5.7 to 6.4. The pH for T1.1 was significantly ($p < 0.05$) lower than that of T1.2. The overall trend for salinity, electrical conductivity, and total dissolved solids of the growing media was similar among the treatments (Table 4. 2). NC had the highest salinity, electrical conductivity, and total dissolved solids followed by T1.2, then T1.1, and T2.1, and the least by NC.

4.4.2. Plant Growth and Yield

The growing media, plant species, and the interaction of growing media × plant species influenced plant growth components significantly ($p < 0.01$). Total root lengths of arugula, pak choi, kale, and Swiss chard were increased by ca.79%, 83%, 61%, and 62% in T1.1, respectively, compared to the average for their counterparts grown in the PC and NC (Figure 4. 1A). T1.1, T2.1,

and T2.2 similarly had the highest effect on total root length compared to the others. Total shoot length of arugula, pak choi, kale, and Swiss chard were increased by ca. 99%, 105%, 62%, and 115%, respectively, in T2.2, compared to their counterparts in the PC (Figure 4. 1B).

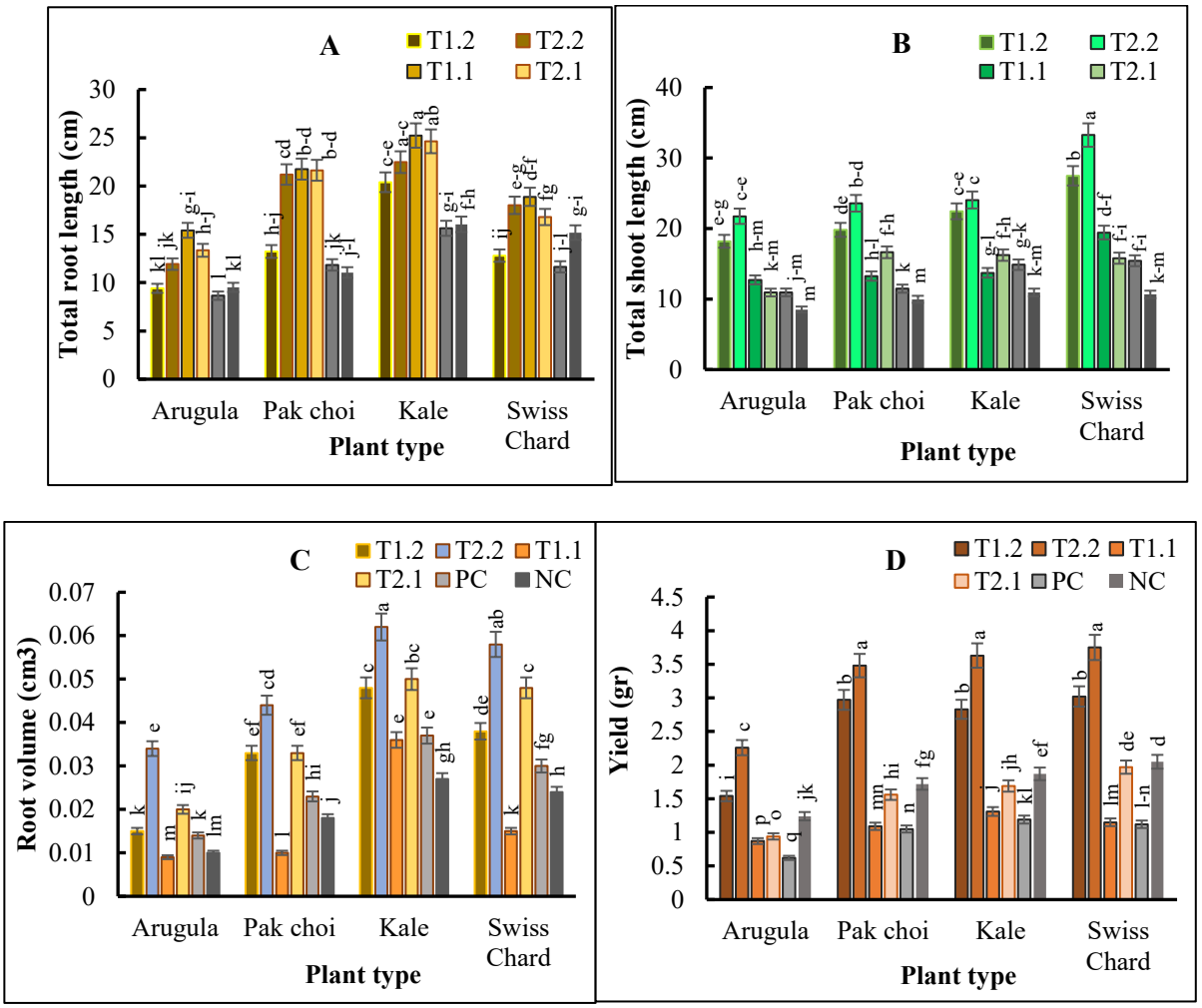


Figure 4. 1. Total root length (A); total shoot length (B); root volume (C); yield (D) of arugula (Eruca vesicaria ssp. sativa), pak choi (Brasica rapa var chinensis), kale (Brassica oleracea L. var. acephala) and Swiss chard (Beta vulgaris var. cicla) microgreens as affected by different growing media comprised of T1.1: 30% vermicast + 30% sawdust + 10% perlite + 30% PM; T1.2: 30% vermicast + 30% sawdust + 10% perlite + 30% MC; T2.1: 30% vermicast + 20% sawdust + 20%

perlite + 30% PM; T2.2: 30% vermicast + 20% sawdust + 20% perlite + 30% MC; NC: 60% sawdust + 40% PittMoss; and PC: Pro-mix BX™ potting medium alone. Vertical bars represent standard errors of the means (N = 3).

Consistently, the PC and the NC significantly ($p < 0.01$) reduced the total length of the roots and shoots of all the microgreen plants. Furthermore, the root volume was increased by ca. 67% to 143% in plants grown in T2.2, compared to those grown in the PC (Figure 4. 1C). Consistently, the root volume of each plant was significantly ($p < 0.01$) reduced in T1.1, followed by NC and then PC (Figure 4. 1C). The plant yield of the microgreens was significantly ($p < 0.01$) increased by ca. 230% in T2.2 and 160% in T1.2, respectively, compared to their PC counterparts (Figure 4. 1D). Consistently, PC and T1.1 significantly ($p < 0.01$) reduced the yield of all the microgreens.

4.4.3. Microgreens Biochemical Composition

The ANOVA demonstrated that variations in the mixed media, plant species, and their interaction, significantly ($p < 0.01$) affected the biochemical compositions of the microgreens (Figure 4. 2A–D). Total carotenoids, Chl a, Chl b, and Chl t of all the microgreens were increased significantly ($p < 0.05$) by T1.1 and T2.2, except Chl b in the pak choi, which was increased by T2.2 (Figure 4. 2B). T1.2 had a similar effect to T1.1 and T2.2 in increasing Chl a, Chl b, Chl t and the total carotenoids in arugula and kale microgreens, but the effect varied for pak choi and Swiss chard (Figure 4. 2A–D). Total chlorophyll and carotenoids were approximately 1.5-fold higher in T1.1 and T2.2 compared to their PC counterparts. Moreover, among the different plant

species, kale and Swiss chard exhibited the highest Chl t by 67% in T1.2 and by 116% in T1.1 compared to PC (Figure 4. 2C).

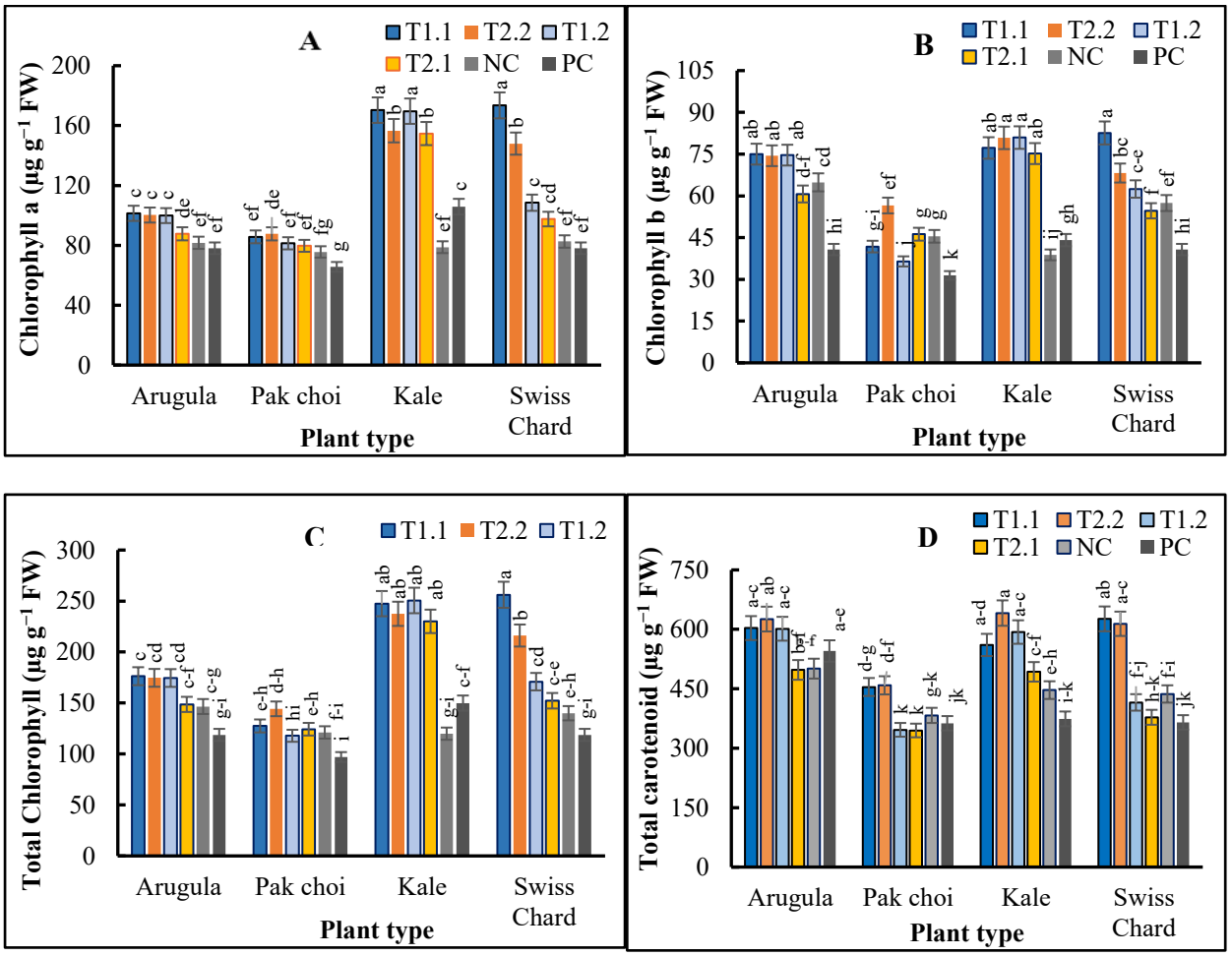


Figure 4. 2. Chlorophyll a (A) and b (B), total chlorophyll (C) and carotenoid (D) contents of arugula (Eruca vesicaria ssp. sativa), pak choi (Brasica rapa var chinensis), kale (Brassica oleracea L. var. acephala) and Swiss chard (Beta vulgaris var. cicla) microgreens as affected by different growing media comprised of T1.1: 30% vermicast + 30% sawdust + 10% perlite + 30% PM; T1.2: 30% vermicast + 30% sawdust + 10% perlite + 30% MC; T2.1: 30% vermicast + 20% sawdust + 20% perlite + 30% PM; T2.2: 30% vermicast + 20% sawdust + 20% perlite + 30% MC; NC: 60%

sawdust + 40% PittMoss; and PC: Pro-mix BX™ potting medium alone. Vertical bars represent standard errors of the means (N = 3); significant at $p < 0.01$.

Likewise, the highest total carotenoid content was about 72% higher for both kale and Swiss chard in T2.2 and T1.1, respectively, compared to their PC counterpart (Figure 4. 2D). The total carotenoid content of arugula and pak choi was increased by ca. 15% and 24% in T2.2, respectively, compared to plants grown in the PC. Consistently, the lowest total carotenoid content was observed in all the microgreens grown in the T2.1, except for kale, which was lowest in the PC (Figure 4. 2D). The overall trend for total carotenoid was arugula (562.35 µg/g FW) > Swiss chard (518.02 µg/g FW) > kale (472.69 µg/g FW) > pak choi (391.68 µg/g FW) (Figure 3D).

The highest sugar content was recorded by arugula microgreens grown in the PC, followed by T2.2 compared to other treatments (Figure 4. 3A). On the contrary, the sugar content of pak choi was increased by 73% in T2.2 while T1.1 increased the sugar content of kale and Swiss chard by ca. 23% and 65%, respectively, compared to the PC (Figure 4. 3A). Consistently, T2.1 significantly ($p < 0.01$) reduced the sugar content of all the four different microgreens. Among the microgreen plant species, the overall trend for the sugar content was arugula (3624.40 µg glucose/g) > kale (3204.99 µg glucose/g) > pak choi (3118.44 µg glucose/g) > Swiss chard (1944.46 µg glucose/g) (Figure 3A). As shown in Figure 4. 3B, T1.1 significantly ($p < 0.01$) increased the protein content in arugula and Swiss chard by ca. 37% and 55%, respectively; while T2.2 significantly ($p < 0.01$) increased the protein content in pak choi and kale by ca. 23% and 105%, respectively, compared to their counterparts grown in the PC. The other media had similar effects on the total protein content of microgreens. Overall, the trend for the protein content was Swiss chard (6372.85 µg Bovine/g) > kale (4941.84 µg Bovine/g) > arugula (4782.70 µg Bovine/g) > pak choi (3901.83 µg Bovine/g) (Figure 4. 3B).

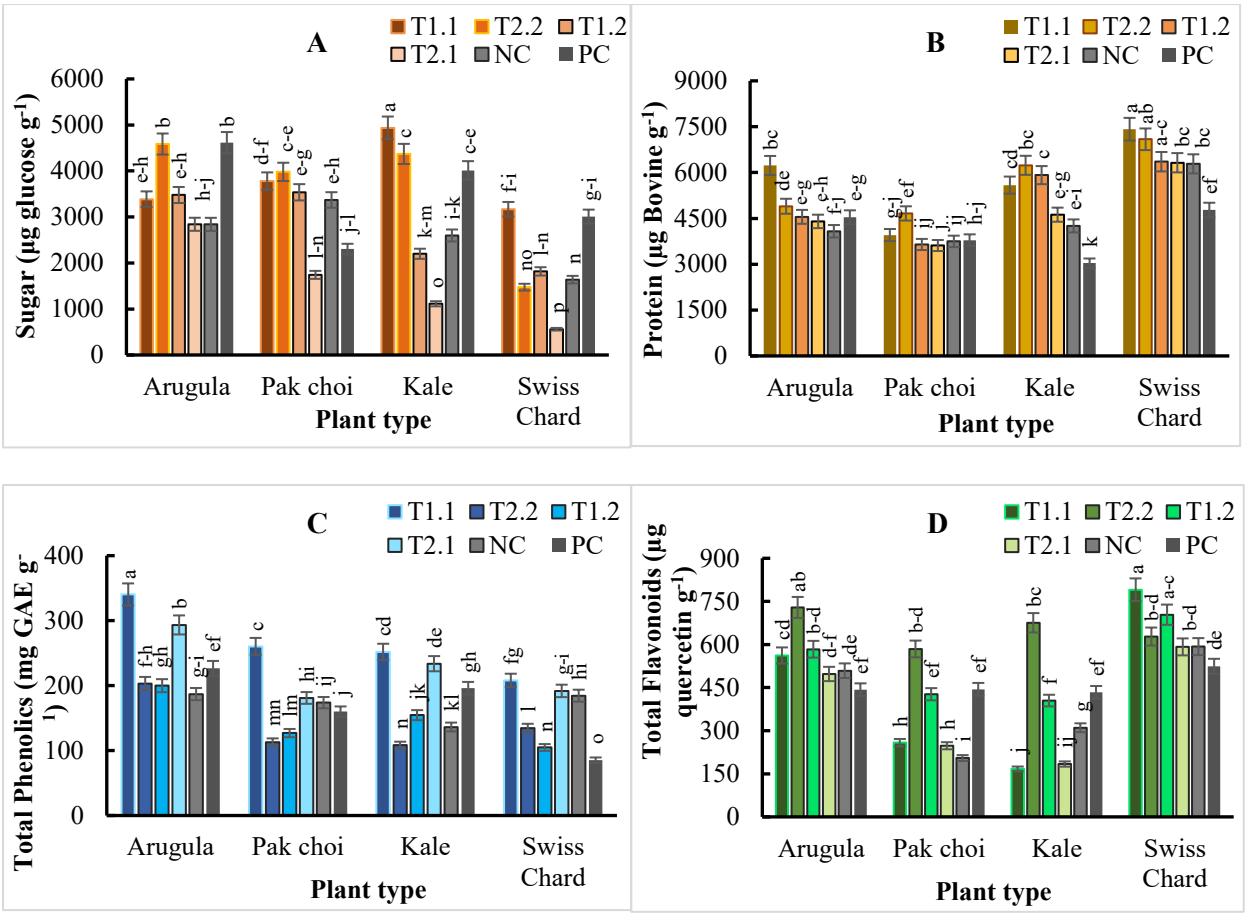


Figure 4. 3. Sugar (A); protein (B); total phenolics (C); total flavonoids (D) contents of arugula (Eruca vesicaria ssp. sativa), pak choi (Brasica rapa var chinensis), kale (Brassica oleracea L. var. acephala) and Swiss chard (Beta vulgaris var. cicla) microgreens as affected by different growing media comprised of T1.1: 30% vermicast + 30% sawdust + 10% perlite + 30% PM; T1.2: 30% vermicast + 30% sawdust + 10% perlite + 30% MC; T2.1: 30% vermicast + 20% sawdust + 20% perlite + 30% PM; T2.2: 30% vermicast + 20% sawdust + 20% perlite + 30% MC; NC: 60% sawdust + 40% PittMoss; and PC: Pro-mix BX™ potting medium alone. Vertical bars represent standard errors of the means (N = 3); significant at $p < 0.01$.

Total phenolics were significantly ($p < 0.01$) increased in all the plants grown in T1.1, followed closely by T2.1, which were not significantly ($p > 0.05$) different for Swiss chard (Figure 4. 3C). The increase in total phenolics in arugula, pak choi, and kale by T1.1 and T2.1 were on

average, 1.5- and 1.2-fold higher than their counterparts that were grown in the PC. Interestingly, Swiss chard, followed by pak choi, and then arugula and kale had phenolics contents of ca. 144%, 63%, 50%, and 29% in T1.1, respectively, compared to their counterparts that were grown in the PC. Comparatively, the trend for the phenolics content in the microgreens was arugula (241.76 mg GAE/g) > kale (180.08 mg GAE/g) > pak choi (169.18 mg GAE/g) > Swiss chard (151.44 mg GAE/g) (Figure 4. 3C). Total flavonoids in all the microgreens grown in T2.2, except for Swiss chard, increased by 1.5-fold compared to the microgreens grown in PC (Figure 4. 3D). Total flavonoids in Swiss chard increased by 51% in T1.1 compared to PC. Total flavonoids in arugula, kale, and pak choi increased by 65%, 56%, and 31%, respectively, in T2.2 compared to PC. Among the microgreen plant species, the overall trend for the flavonoid was Swiss chard (638.34 µg quercetin/g) > arugula (553.84 µg quercetin/g) > kale (362.50 µg quercetin/g) > pak choi (360.96 µg quercetin/g) (Figure 4. 3D).

The total ascorbate was increased by 57%, 64%, and 51% in arugula, pak choi, and kale grown in T1.2, respectively, compared to PC (Table 4. 3). Furthermore, Swiss chard ascorbate content was significantly ($p < 0.01$) increased by 83% and 73% in T2.2 and T1.2, respectively, compared to PC. On the contrary, ascorbate was significantly ($p < 0.01$) reduced in microgreens grown in the T2.1 (Table 4. 3). The overall trend for the microgreens' ascorbate content was kale (25.90 µmol/g FW) > Swiss chard (24.22 µmol/g FW) > arugula (23.41 µmol/g FW) > pak choi (22.40 µmol/g FW) (Table. 2). Peroxidase was significantly ($p < 0.01$) increased in arugula and Swiss chard by T1.1 and T2.2 while T1.2 significantly ($p < 0.01$) increased POD in pak choi and kale.

Table 4. 3. The effects of mixed growing media on total ascorbate, peroxidase activity and ascorbate peroxidase activity

Treatment	Total Ascorbate (µmol g^{-1} FW)				Peroxidase Activity (Unit mg^{-1} FW)				Ascorbate Peroxidase Activity (Unit mg^{-1} FW)			
	Arugula	Pak Choi	Swiss Chard	Kale	Arugula	Pak Choi	Swiss Chard	Kale	Arugula	Pak Choi	Swiss Chard	Kale
T1.1	24.0 de	20.4 fgh	25.4 cd	23.6 de	0.94 bc	0.50 ij	0.96 bc	0.56 gh	0.23 a	0.08 fg	0.07 fg	0.06 g
T2.2	24.2 cde	28.0 bc	32.0 a	29.7 ab	0.67 ef	1.05 b	1.02 b	0.54 hij	0.15 cd	0.07 g	0.19 ab	0.19 ab
T1.2	32.2 a	29.5 ab	30.0 ab	32.0 a	0.60 fgh	1.25 a	0.50 ij	0.64 f	0.11 e	0.11 e	0.16 bcd	0.17 bcd
T2.1	21.5 efg	22.4 ef	21.9 ef	28.9 b	0.50 ij	0.94 bc	0.40 k	0.48 j	0.18 abc	0.04 j	0.11 e	0.05 hi
NC	17.9 hij	16.0 j	18.6 ghij	19.8 fgh	0.29 k	0.75 de	0.51 ij	0.17 m	0.06 g	0.06 gh	0.07 j	0.08 f
PC	20.6 fgh	18.1 hij	17.4ij	21.4efg	0.50ij	0.87cd	0.62fg	0.50ij	0.15d	0.06gh	0.05de	0.07fg
p value												
G	0.001	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
P	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000
G x P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

T1.1: 30% vermicast + 30% sawdust + 10% perlite + 30% PM; T1.2: 30% vermicast + 30% sawdust + 10% perlite + 30% MC; T2.1: 30% vermicast + 20% sawdust + 20% perlite + 30% PM; T2.2: 30% vermicast + 20% sawdust + 20% perlite + 30% MC; negative control (NC): 60% sawdust + 40% PittMoss; and positive control (PC): Pro-mix BX™ potting medium alone.; significant at $p < 0.01$. Treatment means followed by a common letter are not significantly different. G, growing media; P, plant species; G x P, interaction of growing media and plant species (N = 4).

Comparatively, Swiss chard followed by arugula had the highest POD activity and pak choi followed by kale had the lowest. The overall trend for the microgreens POD activity was pak choi (0.88 Unit/mg FW) > Swiss chard (0.66 Unit/mg FW) > arugula (0.58 Unit/mg FW) > kale

(0.48 Unit/mg FW). Furthermore, APEX activity increased by 77% in Swiss chard and by 68% in kale when grown in T2.2, compared to PC. APEX activity of arugula and pak choi were increased by 55% and 54% in T1.1 and T1.2, respectively, compared to those grown PC (Table 4. 3). Among the microgreen plant species, the overall trend for the microgreens APEX activity was arugula (0.146 Unit/mg FW) > Swiss chard (0.103 Unit/mg FW) = kale (0.103 Unit/mg FW) > pak choi (0.066 Unit/mg FW) (Table 2). Consistently, PC and NC significantly ($p < 0.01$) reduced the biochemical composition of the different microgreens (Table 4. 3).

4.4.4. Association among Media, Plants, and Biochemical Composition

A multivariate two-dimensional PCA biplot was used to assess the association between the microgreens plant yield and biochemical parameters, as influenced by variations in growing media formulations (Figure 4. 4). The PCA explained 80% of the total variations in the dataset. Treatments that are close to the origin of the PCA axes show a higher association and stability than those on the periphery. The PCA demonstrated that treatment T2.2 can be associated with an improved plant yield and biochemical composition of microgreens. The interaction of the growing media and plant species can be closely associated with the microgreens' yield and total ascorbates. Furthermore, kale carotenoid content was strongly influenced by the interaction between the growing media × plant species compared to the other plant species. APEX activities were associated with the interaction between the growing media and plant species in all the microgreens except arugula (Figure 4. 4). Overall, the interaction between the growing media and plant species can be associated with the kale yield and its biochemical parameters compared to the other plant species.

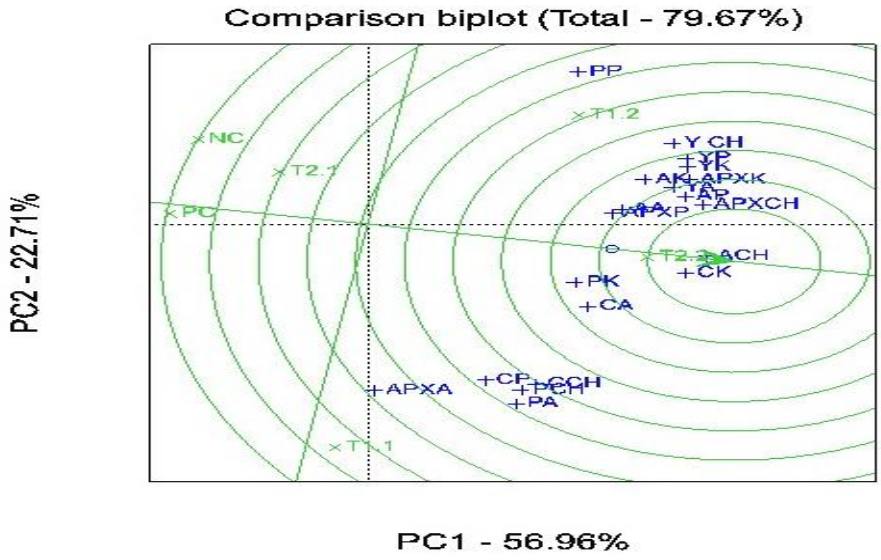


Figure 4. 4. Ranking total × total biplot for comparison of treatment × plant species interaction effects on biochemical variations in all microgreens. Arugula yield (YA), arugula ascorbate (AA), arugula carotenoids (CA), arugula POD Activity (PA), arugula APEX Activity (APXA); Chard yield (YCH), chard ascorbate (ACH), chard carotenoids (CCH), chard POD Activity (PCH), chard APEX Activity (APXCH); kale yield (YK), kale ascorbate (AK), kale carotenoids (CK), kale POD Activity (PK), kale APEX Activity (APXK); pak choi yield (YP), pak choi ascorbate (AP), pak choi carotenoids (CP), pak choi POD Activity (PP), pak choi APEX Activity (APXP). T1.1: 30% vermicast + 30% sawdust + 10% perlite + 30% PM; T1.2: 30% vermicast + 30% sawdust + 10% perlite + 30% MC; T2.1: 30% vermicast + 20% sawdust + 20% perlite + 30% PM; T2.2: 30% vermicast + 20% sawdust + 20% perlite + 30% MC; NC: 60% sawdust + 40% PittMoss; and PC: Pro-mix BX™ potting medium alone.

4.5 Discussion

The effects of different substrates on the physicochemical characteristics of the formulated growing media and the differential response of the four different microgreens plant species were investigated under greenhouse conditions. The growing medium T1.2, followed by T2.2, had the

highest effect on most of the plant growth components, except for root length. The growing media T1.1 and T2.1 contained PittMoss, which was made from mainly shredded cardboard, and T1.2 and T2.2 contained mushroom compost. The results show that mushroom compost was more beneficial than PittMoss. Similarly, Renaldo et al. (2014) reported that mushroom compost increased the shoot and root dry mass in cucumber (*Cucumis sativus*) compared to biochar and corn stalks but had no effect on lettuce (*Lactuca sativa*), probably due to lettuce intolerance of the high salt content in the mushroom compost. Furthermore, Vahid Afagh et al. (2019) reported that a 15% mushroom compost mixed in sandy loam soil increased both the plant growth and yield of German chamomile (*Matricaria recutita* L.) due to the improved medium structure, increased nutrient availability, and beneficial microbial activity (Demir, 2017). Furthermore, the results also suggested that the variations in response of the microgreen plants to the different media were dependent on genotypic differences.

It was obvious that the improved structure and functionality of growing media T1.2 and T2.2 improved plant growth in all the plant species except for pak choi, as previously explained by (Emami and Astaraei, 2012; Vahid Afagh et al., 2019). The root lengths of all the microgreens were significantly increased in T1.1 and T2.1 compared to the other media. According to Vahid Afagh et al. (2019), an addition of 15% mushroom compost to a medium increased aeration and water-holding capacity, leading to an improved crop productivity. The addition of PittMoss in T1.1 and T2.1 reduced the growing media bulk density, which in turn promoted root growth compared to the mushroom compost. A previous study using a high bulk density of (i.e., 1.35 g/cm^3) growing medium led to a reduction in lettuce root growth and yield (Chen et al., 2019). In the present study, the bulk density ranged between 0.07 and 0.12 g/cm^3, which was below the root-restriction threshold bulk density of 1.6 g/cm^3, especially in T1.2 and T2.2. This may be the reason for the

enhanced plant growth and yield of microgreens grown in T1.2 and T2.2. Moreover, Gillespie et al. (2020) stated that the optimum range of pH for leafy greens growth is a 5.5 to 6.5 range, at which more nutrients become available to plants. However, it does not seem that the pH was a limitation in the present study, since all the media pH fell within the sufficiency range for the microgreen plants. Nevertheless, Ur Rahman et al. (2021) reported that pH variation of the medium (from 5 to 9) significantly influenced the yield and biochemical constitutions in wheat (*Triticum aestivum* L.). The highest yield, total chlorophyll, and carotenoid contents were observed in seedlings grown in media with a neutral pH (6.5–7), while the lowest one was obtained in acidic (pH 5) and alkaline (pH 9) media that correspond with the results of this study.

Notably, there was a significant positive association between the yield, salinity, and TDS, suggesting sufficient growing medium fertility levels in particular, T1.2 and T2.2, which were the only media with mushroom compost. Previous studies showed that high electric conductivity and salinity can reduce plant growth (Warrence et al., 2002; Vahid Afagh et al., 2019), which can be managed by adding perlite and wood-based substrates into the growing media to improve texture, structure, and porosity (Zhang et al., 2009; Lee et al., 2011). However, T1.2 and T2.2 had acceptable ranges of salinity thresholds between 640 and 1600 mg/L, as recommended for most vegetable crops (Machado and Serralheir, 2017). Generally, NC recorded the highest salinity and the lowest yield, as previously reported by Shannon et al. (2000), for kale and Swiss chard grown in media with excess salinity levels > 3.0 dS/m. Lin et al. (2020) reported an increase in the plant growth and yield components of Swiss chard, pak choi, and kale in a medium consisted of 60% vermicast and 40% sawdust, with a considerably high electric conductivity of 1450 µS/cm and a pH of 7.3. Furthermore, Hernández et al. (2021) attributed increased germination rate, fresh shoot weight, and yield in red baby leaf lettuce to mushroom compost, with a pH of 7 and an electric

conductivity of > 4000 µS/cm. There was no significant correlation between EC and the measured growth components, but there was a strong relationship between pH and growth plant components in all the plants.

The microgreens' biochemical composition was significantly altered by the different mixed growing media. There are very few documented reports on the effect of different mixed growing media on biochemical quality of microgreens. Previous studies have demonstrated that vermicast and mushroom compost are well known to be rich in macro- and micro-elements including N, which is essential for chlorophyll and carotenoid synthesis as well as photosynthesis (Zietz et al., 2010; Gonani et al., 2011; Zhang et al., 2017). In this study, total flavonoids and ascorbates ranged from 404.1 to 653.7 µg quercetin/g, and 18.1 to 30.9 µmol/g FW, respectively. Media T1.2 and T2.2 impacted the highest amount of microgreen flavonoids and ascorbate contents, respectively, that most likely can be associated with media nutrient availability and a balance in C/N ratio, due to the added mushroom compost as explained by Hernández et al. (2021). Moreover, it was demonstrated that mushroom compost may be chitin-rich, which can be a significant source of plant growth stimulants and elicitors for the biosynthesis of secondary metabolites (Sharp, 2013; Li et al., 2022). Therefore, a significant amount of chitin might be present in T1.2 and T2.2, leading to the high microgreen plants content of total carotenoid, flavonoids, and ascorbate, compared to media without mushroom compost. Treatments T1.1 and T2.1 improved phenolics content in all the microgreens irrespective of plant species. This can be ascribed to the high-carbon input from the thermally treated sawdust and PittMoss. This carbon might have improved the carbon-based phenolic compounds and their precursors involved in plant defense mechanisms and responses to environmental stress (Treutter, 2010). Contrary to this, the total phenolics was lower in T2.2, which suggested that the probably high N content in T1.2 and T2.2, due to the addition of N-rich

vermicast and mushroom compost, might have reduced phenolic content in the microgreens as previously reported (Treutter, 2010; Ibrahim et al., 2011; Abbey et al., 2018). The difference in growing media had a significant effect on POD and APEX enzymes activities in the microgreens. Several studies have reported a strong correlation between bioactive phytochemicals and antioxidant properties (Shiri et al., 2011; Arumugam et al., 2016). Besides the increased ascorbate and flavonoids contents, POD and APEX were highly increased in the microgreens grown in T1.2 and T2.2. Our results are consistent with findings obtained by Shiri et al. (2011), who reported a significant increase in antioxidant capacity with an elevated ascorbic acid content in plants.

4.6 Conclusions

Global warming and climate change have had adverse impacts on plant production and food security. During the last decade, synthetic chemical fertilizers and pesticides have been extensively used in conventional agriculture to meet global food and nutrition demand. However, their application negatively affects the environment and human health. Therefore, the development of an innovative and climate-smart approach to food production is of high importance. In the present study, the effect of different mixed natural growing media on the growth and biochemical properties of different microgreen plant species was investigated. Overall, our results showed that variations in the growing media characteristics had a significant effect on the studied traits of microgreens. Overall, growing media containing mushroom compost, i.e., T2.2, was found to be the most favorable. The efficacy of T2.2 on the assessed growth, yield, and quality traits was further confirmed through the PCA analysis. The ingredients used to make the mixed growing media in this study are reasonably inexpensive and locally available. Therefore, they can be used

as an alternative to conventional media such as Pro-mix BX™ potting medium for growing microgreens to improve productivity and nutrient and non-nutrient bioactive compounds.

CHAPTER 5: Blue and Red LED Lighting Enhance Growth Characteristics, phytochemicals, and Antioxidant enzyme functions in Microgreens Cultivated in Mixed growing media

This chapter presents a version of the manuscript titled '**Blue and Red LED Lighting Enhance Growth Characteristics, phytochemicals, and Antioxidant enzyme functions in Microgreens Cultivated in Mixed growing media**' that has been submitted in the *Horticulture Journal* on March 5, 2022. This was a multi-authored publication, in which the PhD Candidate contributed to the research design, data collection, data analysis, and writing. The citation is:

Saleh, R., Nams, V., Lada, R., Thomas, R. H., & Abbey, L. (2023). **Growth and Biochemical Composition of Microgreen Plants Grown in Optimized Growing Media under different LED light spectrum**'. *Horticulture Journal,* under review

5.1 Abstract

Microgreens are the plantlet stage of various species grown for their health-promoting compounds, which can be influenced by many production factors that are yet to be comprehensively studied. A study was conducted to assess the interaction effects between LED light and growing media on plant growth, phytochemicals and antioxidant enzymes actions of kale (*Brassica oleracea* L. var. *acephala*), Swiss chard (*Beta vulgaris* var. *cicla*), arugula (*Eruca vesicaria* ssp. *sativa*), and pak choi (*Brasica rapa var chinensis*) microgreens. The microgreens were grown in 20% sawdust+20% perlite (G1) and 30% PittMoss+10% perlite (G2) all containing 30% vermicast and 30% mushroom compost under different proportion of blue (B) and red (R) LED light treatments B80:R20 (T1); B20:R80 (T2); B60:R40 (T3); B40:R60 (T4); B50:R50 (T5), and white LED light as a positive control (PC) at 100 µmol m^{-2} s^{-1} light intensity. Seed germination was increased in microgreens grown in G1 under T1 and mostly, growth parameters increased with increasing redlight ratio. . Also, yield of microgreens increased from 7 to 44.5% in T2.G1 compared to PC.G1. Moreover, the highest chlorophyll, carotenoids, and flavonoids in all microgreens was reported in T4.G2 and T4.G2. Treatment T2.G2 increased protein content of the microgreens by 76% compared to PC.G2. Sugar content of arugula, pak choi and kale were enhanced by an average of 56% in T3.G2. The highest total phenolics in all microgreens was reported in T1.G2 compared to the other treatments. The increase in POD and APEX enzymes activities of microgreens grown in T4.G2 was on the average, 1.4- and 4.2-folds higher than those grown in the PC.G2. Overall, this study suggested that T2.G1 and T4.G2 could be potential target treatments to enhance yield and phytochemicals of microgreens. Future studies are needed to assess the effects of other spectra combined with B: R LED ratios on yield and nutrients of microgreens for optimization of the LED spectrum.

Keywords: LED spectrum, microgreens, natural amendment, phytochemicals, preharvest factors.

5.2 Introduction

Microgreens are immature plants of vegetables, herbs, or even wild species grown for their high amount of biochemical compounds, mineral nutrients, and high potential biological functions compared with mature plants (Pinto et al., 2015; Kyriacou et al., 2016). Several studies reported high level of polyunsaturated fatty acids, carotenoids, minerals, and vitamins in kale, Swiss chard, and arugula microgreen species (Zietz et al., 2010; Kyriacou et al., 2016). Agronomic performance and edible quality of different microgreen species can be influenced by manipulating preharvest factors including genotypic variation, growing medium factors, environmental factors, and agricultural management practices (Zietz et al., 2010; Aftab, 2019; Nguyen et al., 2019).

Growing media can be formulated to have different physicochemical properties, which can influence the morpho-physiological characteristics, yield, and phytochemistry of plants (Tabatabaei, 2008; Saleh et al., 2022). Natural amendments such as vermicast, mushroom compost, manures, PittMoss, and sawdust are a subclass of soilless growing media. They are added to different substrates to enhance plant morphophysiological traits and postharvest quality through the improvement of the physicochemical properties and beneficial microbial activities (Abbott et al., 2018). Vermicast is the pure excreta of earthworms, which is an excellent source of macro- and micro-nutrients, useful microorganisms, and humic acid, fulvic acid and non-humic compounds, and promotes plant growth and development (Abbey et al., 2017). Dry vermicast, potassium humate and volcanic minerals affects the morphological indices, essential lipids, minerals, phenolics accumulation, and antioxidant activities in kale (Abbey et al., 2018). Sawdust is made from the forestry industries and wood waste which is good source of micronutrients potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and carbon (C) (Alpert and Maron, 2000; Lin, 2020). Agboola et al. (2018) reported a delay in the initial growth of seedlings of tomato

(*Solanum lycopersicum,* Mill) due to the presence of sawdust toxic compounds such as lignin, cellulose, hemicellulose, and terpenes. However, seven weeks after establishing tomato seedlings, yield was increased compared to the control, which is most likely connected to leaching and dilution of toxic compounds from sawdust (Lin, 2020; Mohan et al., 2021). The positive effects of a combination of sawdust and other growing substrates is related to improved physical features of the growing media like enhancement of porosity and drainage (Maharani et al., 2010). Lin et al. (2020) showed that 40vermicast: 60sawdust or 60vermicast: 40sawdust mixed media ratios remarkably enhanced plant growth factors and mineral nutrients in Swiss chard, pak choi, and kale microgreens, which were associated with improved physicochemical properties, active microbial activities, and nutrient mineralization. Therefore, natural amendments considerably enhanced the functionality of the growing media and nutrient availability, which is crucial in meeting potential plant growth requirements. For instance, adding mushroom compost into growing substrate increased germination, shoot length, and biomass of lettuce (*Lactuca sativa* L.) by 700% in comparison with peat counterparts (Hernández et al., 2021). PittMoss is another potential growing substrate mainly consisting of shredded cardboard with added gypsum. Adding 30% of PittMoss into growing media increased root growth and total phenolic in kale, pak choi, Swiss chard, and arugula (Saleh et al., 2022).

Light quality distribution refers to the wide range of the electromagnetic spectrum ranging from UV (280-400 nm) to far-red (700-800 nm) wavelengths. Wavelength range of 400-700 nm describe photosynthetically active radiation (PAR), which is intercepted by phytochromes, cryptochromes, and phototropins photoreceptors, which are involved in regulating gene expression, and thus, specific physiological and developmental responses (Liu et al., 2018; Ahmed et al., 2020). Specific wavelengths of light have precise impact on plant performance. For instance,

red (610–710 nm) and blue (455–490 nm) wavelengths are important in maximizing absorption by photosynthetic pigments, which can provide targeted energy involved in photosynthesis and plant metabolism, thereby positively affecting plant morphophysiological responses (Naznin et al., 2019; Brazaitytė et al., 2021). Blue light contributes to leaf development, photomorphogenesis, stomatal opening, photosynthesis, chlorophyll accumulation, and biochemical concentrations, while red light contributes to photosynthetic apparatus development, chloroplast movement, shoot elongation, leaf expansion, petiole growth, and flower induction (Lobiuc et al., 2017; Liu et al., 2018). Compared to monochromatic and white LED light, the combination of blue and red LEDs has synergetic effects on many growth characteristics and antioxidant compounds accumulation in lettuce (*Lactuca sativa, L*) (Ahmed et al., 2020), chlorophyll, flavonoids, and antioxidants in basil (*Ocimum basilicum*) (Lobiuc et al., 2017), and yield, anthocyanin, and carotenoid in mustard (*Brassica juncea* 'Red Lace') (Brazaitytė et al., 2021). A study done by Metallo et al. (2018) different blue: red LED ratios differentially altered yield, morphological characteristics, beneficial nutrients, and phytochemicals in kale. The authors attributed the highest total concentrations of carotenoid and glucosinolates in kale treated with 5% blue/95% red LED to differences in growth and development responses and different metabolic pathways. Furthermore, anthocyanin compounds of *Prunus avium* L. grown under monochromatic blue light significantly increased by stimulatory effect of blue light on phenylalanine ammonia-lyase enzyme activity (PAL) (Arakawa et al., 2017). Similarly, monochromatic blue LED light act like a physical stress elicitation which led to increasing gene expression involved in activation of PAL enzyme, thereby increasing antioxidant potential in red leaf lettuce (*Lactuca sativa*) (Son et al. 2015). The elevated soluble carbohydrates concentration in *Arabidopsis thaliana* growing under higher blue: red LED ratio

may connect to improved plant metabolic pathway and detoxification pathway in response to reactive oxygen species (ROS) induced by blue light.

Despite the promising scientific reports on the impacts of various organic amendments and LED light spectrum on various plant species, there is limited information about their interactions on microgreens morphophysiological traits and biochemical compounds. Thus, the aim of the current study was to assess the interaction effect between red: blue LED ratios and mixed growing substrates on plant growth components, biochemical compounds, and antioxidant enzymes functions of arugula, pak choi, kale, and Swiss chard microgreens.

5.3 Materials and Methods

5.3.1 Plant materials and growing condition

The experiment was accomplished in October 2021 and repeated in January 2022 in the Plant, Food, and Environmental Sciences Department, Dalhousie University, Truro, Nova Scotia, Canada (45°23′ N, 63°14′ W). The LED panels were purchased from (OSRAM PHYTOFY® RL Company, Munich, Germany). Soilless potting growing media including Shiitake mushroom compost (*Lentinula edodes*), PittMoss® (Ambridge soil company, PA, USA), sawdust, and vermicast were provided by Modgarden Company, Toronto, ON, Canada. Perlite, Pro-mix BX™, and four microgreens seed i.e., arugula, pak choi, kale, and Swiss chard were bought from Halifax Seed Company, NS, Canada. In flat plastic cell trays (19 × 12 × 2.5 cm) filled with different mixed growing medium and exposed to different combinations of blue: red LED ratios under the greenhouse condition. The light regime and temperature were set for 16/8 hrs day/night at 22°±2°C. Each container was watered by 200 mL of tap water every second day. No fertilizer was applied during the experiment.

5.3.2 Experimental treatment and design

The factorial experiment (LED x growing media) was applied in a completely randomized design with three replications. Seeds were sown in two different mixed growing substrates (Table 5. 1).

Table 5. 1 Proportions of mixed growing media.

Mixed growing media	**Formulation**
G1	30% vermicast + 20% sawdust + 20% perlite + 30% MC
G2	30% vermicast + 30% PittMoss + 10% perlite + 30% MC

Pots were exposed to different red: blue ratios of LED light spectrum (Table 5. 2). The pots were rearranged daily on the growth shelves to reduce microclimate variations in the greenhouse. Seed germination, plant growth, yield, and various biochemical characteristics were measured.

Table 5. 2 LED Spectrum Variations at 100 μmol m^{-2} s^{-1} intensity.

LED Treatments	**Formulation**
T1	Blue (B)80: Red ®20
T2	B20: 80R
T3	B60: R40 % Red
T4	B40: 60R
T5	B50: 50R
Positive Control (PC)	White LED alone

5.3.3 Plant growth and yield components

Germination and growth characteristics in terms of plant height, root and shoot length, root and shoot surface area, root volume, and yield were measured 14 days after sowing. Data on root and shoot parameters were collected using perfection V800 Photo Color Scanner Digital ICE® Technologies (Epson America Inc., Los Alamitos, CA, USA). To sample, the tender plants (n = 15) were randomly and carefully taken out from different parts of the growing trays using a spatula.

Then, the roots were gently cleaned with water to remove the residue media from the roots with minimal damage). Plant height was recorded using a ruler from the apex of the youngest leaf to the line of junction between the root of a plant and its stem at six days intervals. The aboveground tissue of microgreens was harvested using a pair of scissors and the fresh weight wase recorded. At the final harvest, seed residue was not observed on the shoots.

5.3.4 Microgreen quality and biochemical analysis

5.3.4.1 Chlorophylls a and b, total chlorophyll, and total carotenoid

Fresh plant materials were ground into a fine powder using a mortar and pestle in liquid nitrogen and then kept at -80 °C. For extraction, each plant powder (0.2 g) and 80% acetone (10 mL) were mixed and centrifuged at 12,000× g for 15 min. The supernatant was transferred into microplates to quantify the absorbance at 646.8 nm and 663.2 nm wavelengths using a UV-Vis spectrophotometer (Evolution™ Pro, Thermo Fisher Scientific, Waltham, MA, USA) versus acetone as blank. Total chlorophylls (Chl) and carotenoid (Car) content was expressed as μg g^{-1} fresh weight (FW) of the sample (Saleh er al., 2022; Lightenthaler, 1987).

5.3.4.2 Total sugar

First, 0.2 g samples of the powder were homogenized with 90% ethanol (10 mL). After incubating the mixture in a water bath at 60 o C for 60 min, the final volume of the mixture was adjusted to 5 mL with 90% ethanol and centrifuged at 12,000× g for 3 min. An aliquot of 1 mL was transferred into a thick-walled glass tube to be mixed with 1 mL of 5% phenol. Next, the reaction mixture and a volume of 5 mL of concentrated sulfuric acid were mixed, vortexed, and incubated in the dark for 15 min. After cooling the mixture, the absorbance was read at 490 nm

against a blank. Total sugar was calculated using a standard sugar curve and expressed as µg of glucose g^{-1} FW (Mohammadkhani and Heidari 2008).

5.3.4.3 Total protein and antioxidant enzyme activities

To quantify protein content and antioxidant enzyme activities, 0.2 g of ground sample was mixed in 3 mL ice-cold extraction buffer (50 mM potassium phosphate buffer (pH 7.0), 1% polyvinylpyrrolidone, and 0.1 mM EDTA). Following centrifuging the homogenate at 15,000× g for 20 min, the supernatant (crude enzyme extract) was collected in a new microfuge tube on ice. After 5 min incubating enzyme extract with Bradford's reagent, the protein content was read at 490 nm. The protein content expressed as µg Bovine/g was obtained from a standard curve of bovine serum albumin (200–900 µg mL^{-1}). For peroxidase (POD), pyrogallol was used as a substrate. The reaction mixture included 100 mM potassium-phosphate buffer (pH 6.0), 5% pyrogallol, 0.5 % H_2O_2, and 100 µL of crude enzyme extract, which was incubated at 25 °C for 5 min. Then, 1 mL of 2.5 N H_2SO_4 was added to stop the reaction, and the absorbance was read at 420 nm against a blank (ddH_2O). The POD activity expressed as unit/mg FW was one unit of POD forms 1 mg of purpurogallin from pyrogallol in 20 s at pH 6.0 at 20 °C. The reaction mixture used for determining ascorbate enzyme peroxidase (APEX) activity consisted of 1372 µL of 50 mM potassium–phosphate buffer (pH 7.0), 75 µL of 10 mM ascorbate, and 3 µL of 100 mM H_2O_2. The reaction mixture was incubated at room temperature for 1 min, and the absorbance was measured at 290 nm. The APEX activity was expressed as unit/mg FW (Saleh et al., 2022; Hammond and Kruger 1988).

5.3.4.4 Total phenolics

The Folin-Ciocalteu method presented by Alothman et al. (2009) was used to quantify total phenolics (TPC) of microgreens. First, each sample (0.2 g) and ice-cold 80% methanol (10 mL) was mixed and kept at 22°C for 48 h in the dark. Following centrifugation at 13,000× g for 5 min, the supernatant (100 µL), standard (Gallic acid) at different concentrations ranging from 0 to 25 mg/L, and a blank were transferred into separate tubes and mixed with Folin-Ciocalteu reagent (200 µL) and Na_2CO_3 (800 µL). Subsequently, the mixtures were incubated, and the absorbance was measured at 765 nm against a blank. Finally, the amount of TPC expressed as mg gallic acid equivalents per g FW (mg GAE g^{-1} FW) was calculated using a gallic acid standard curve.

5.3.4.5 Total flavonoids

In brief, plant powder (0.2 g) was homogenized in 95% methanol (2.5 mL). Following vertexing and centrifuging at 13,000× g for 10 min the mixture, 500 µL of supernatant was added to a reaction mixture containing 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and 2.8 mL distilled water. The mixture was incubated for 30 min, and the absorbance was read at 415 nm versus a blank lacking $AlCl_3$. The total flavonoids were estimated using quercetin equivalents and expressed as µg quercetin g-1 FW (Saleh et a., 2022; Chang et al., 2002).

5.3.5 Statistical analysis

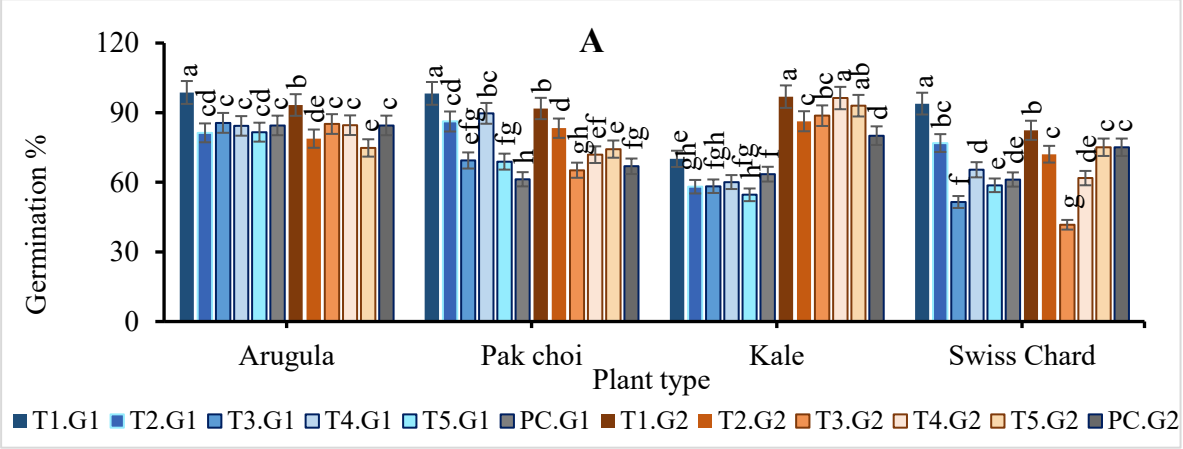
Datasets were analyzed by two-way analysis of variance (ANOVA) using Minitab statistical software version 18.3. Treatment means were compared using Fisher's least significant difference (LSD) post hoc test at $p \leq 0.05$. Furthermore, the principal component analysis (PCA) biplot was performed using GenStat software to indicate the relationships between the measured indices in microgreens under variation of LED spectrum × mixed growing media. Pearson

correlation analysis was carried out to find the relationship between the biochemical components and plant yield data at $p < 0.05$.

5.4 Results

5.4.1 Seed germination, plant growth and yield

The effect of different B: R LED ratios, mixed growing media, and their interaction between different LED spectrum × mixed growing media on seed germination and plant growth parameters were highly significant ($P < 0.01$). Seed germination was enhanced by T1.G1 in almost all microgreen species, except for kale. Kale germination was increased by ca. 20% in both T1.G2 and T4.G2 (Fig 5. 1A). Seed germination of pak choi, Swiss chard, and arugula was significantly increased by ca. 60%, 53%, and 16% in T1.G1 respectively, with comparison with microgreens grown in the PC.G1 (Fig. 1A). However, the maximum plant height was reported in PC.G1 and PC.G2 followed by T2.G1 and T2G2 irrespective of plant species (Fig 5. 1B).



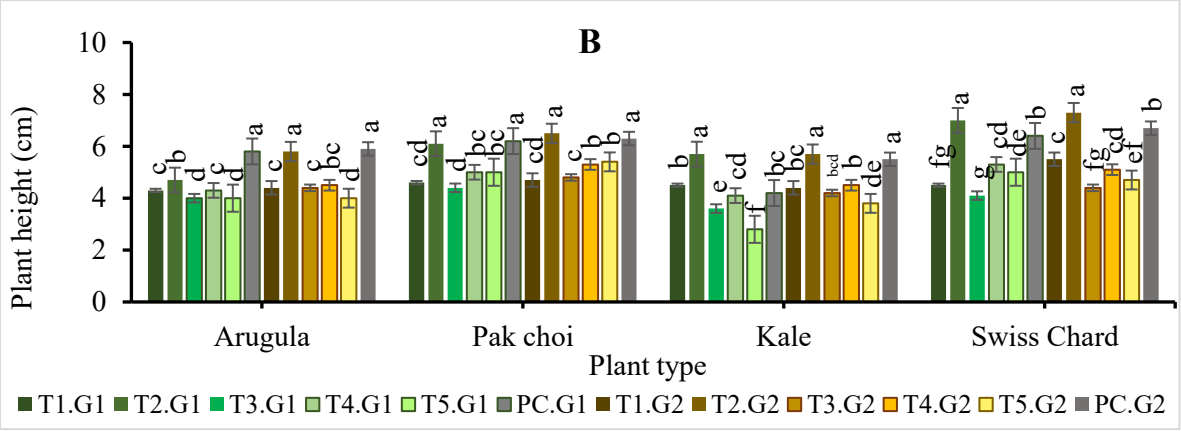
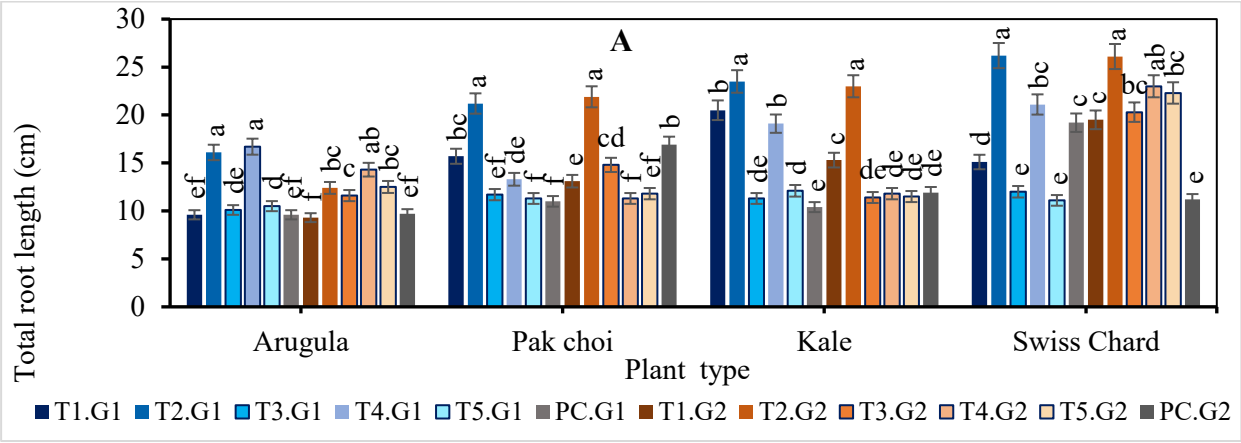
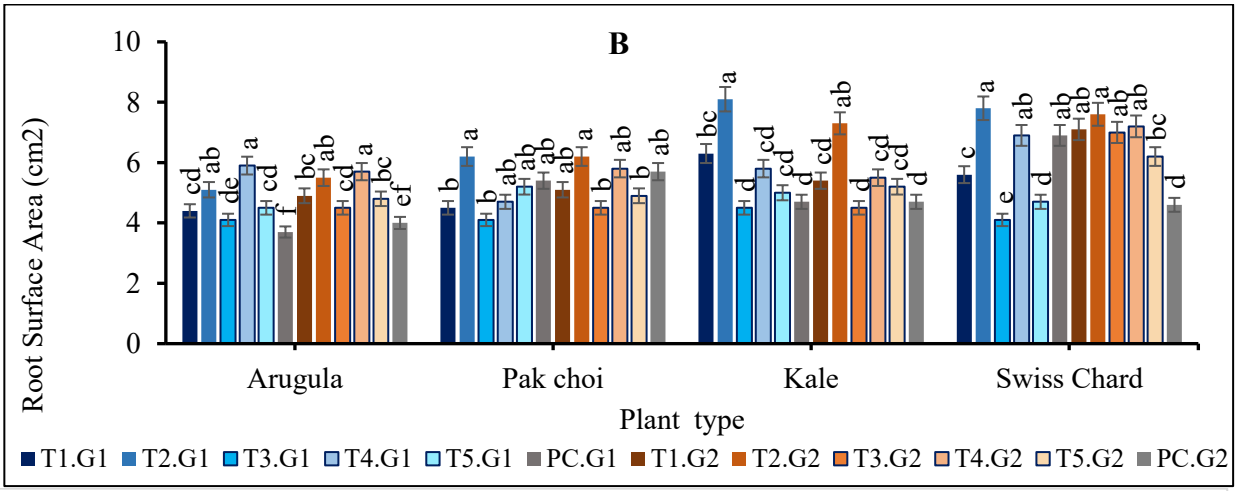


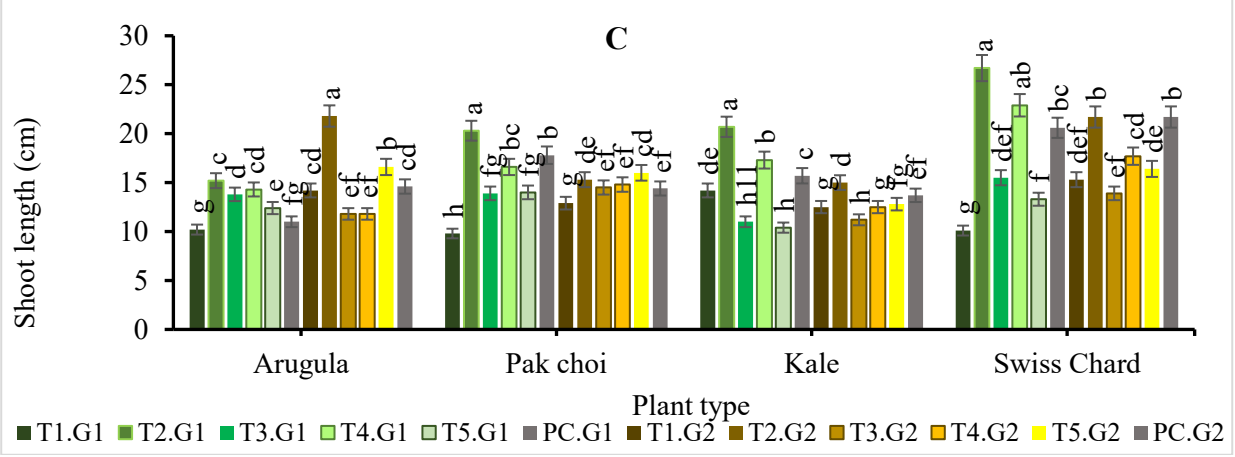
Figure 5. 1. See germination (A) and heights (B) of arugula, pak choi, kale, and Swiss chard plant species in response to LED spectra and different mixed growing media. Error bars indicate standard deviations. Values are the means of three replicates and different alphabetical letters demonstrate significant differences based on Fisher's least significant (LSD) post hoc test within each plant species at $p < 0.05$.

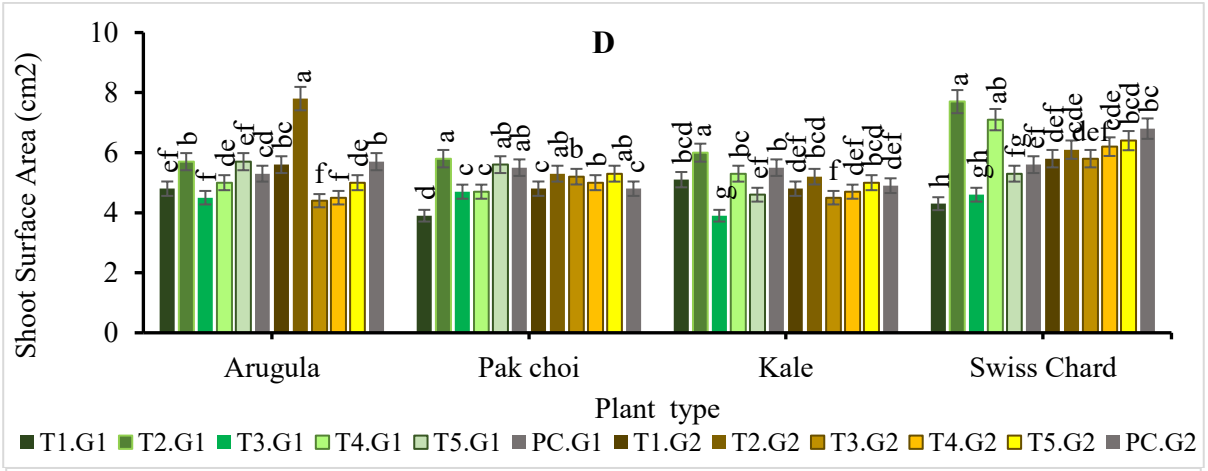
The root length of arugula grown in G1 was improved by 67.7% under B20: R80 and 73.9% under B40: 60R LED ratios compared to those grown in the PC.G1 (Fig 5. 2A). Also, root length of pak choi, kale, and Swiss chard exposed to B20: R80 LED ratio were significantly enhanced by 92.7%, 125.9%, and 36.4% in G1 and 29.5%, 93.2%, and 133% in G2, respectively in comparison with their PC (Fig 5. 2A). Similarly, root surface area of arugula was exposed to B40: R60 LED ratio significantly increased by 59.4% in G1 and 42.5% in G2 in comparison with their counterparts in the PC (Fig 5. 2B). Root surface area of pak choi, kale, and Swiss chard plants exposed to B20: R80 LED ratio were significantly enhanced by 14.8%, 72.3%, and 13.1% in G1 and 8.7%, 55.3%, and 65.2% in G2, respectively in comparison with their PC (Fig 5. 2B). Arugula shoot length exposed to B20: R80 ratio was increased by 49.3% in G2 compared to PC.G2, while pak choi, kale, and Swiss chard shoot length kept under B20: R80 ratio were enhanced by 14.1%,

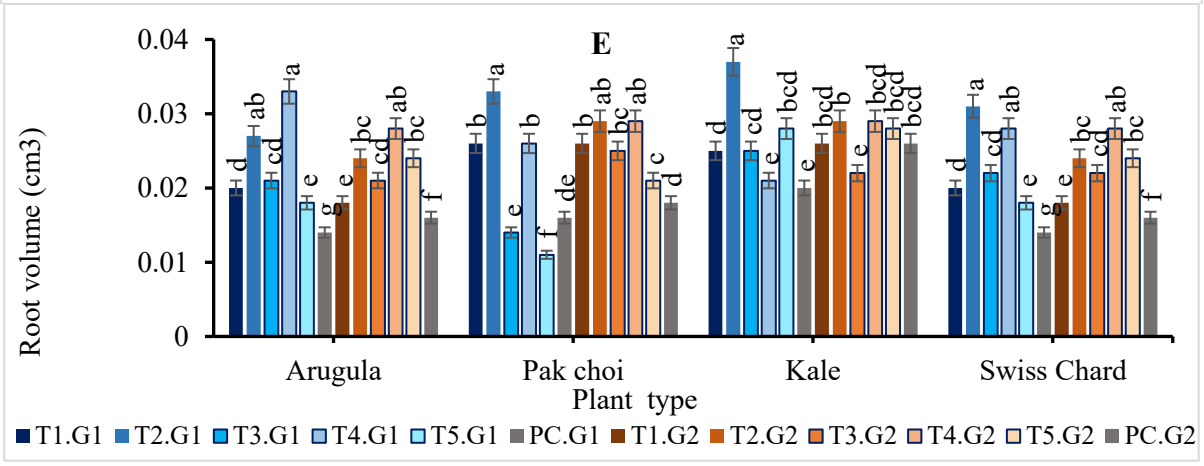
31.8%, and 29.6% in G1 in comparison with PC.G1 (Fig 5. 2C). Similarly, arugula shoot surface area exposed to B20: R80 was increased by 36.8% in G2 while pak choi, kale, and Swiss chard shoot surface area enhanced by 5.4%, 10%, and 37.5% in G1 respectively, compared to those grown in PC (Fig 5. 2D).








D
Shoot Surface Area (cm2)
10
8
6
4
2
0
Arugula
Pak choi
Kale
Swiss Chard
Plant type
T1.G1 T2.G1 T3.G1 T4.G1 T5.G1 PC.G1 T1.G2 T2.G2 T3.G2 T4.G2 T5.G2 PC.G2


E
Root volume (cm3)
0.04
0.03
0.02
0.01
0
Arugula
Pak choi
Kale
Swiss Chard
Plant type
T1.G1 T2.G1 T3.G1 T4.G1 T5.G1 PC.G1 T1.G2 T2.G2 T3.G2 T4.G2 T5.G2 PC.G2

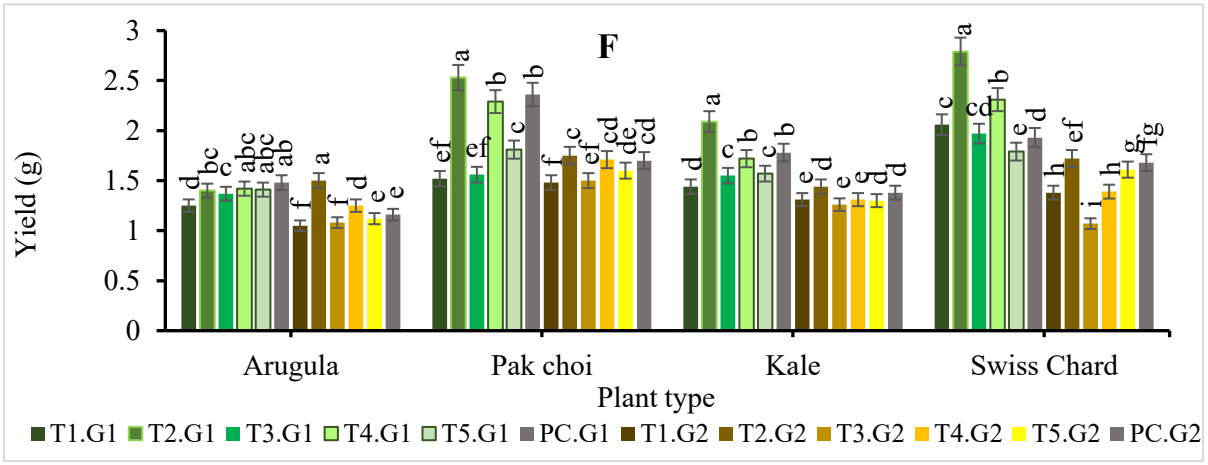

F
Yield (g)
3
2.5
2
1.5
1
0.5
0
Arugula
Pak choi
Kale
Swiss Chard
Plant type
T1.G1 T2.G1 T3.G1 T4.G1 T5.G1 PC.G1 T1.G2 T2.G2 T3.G2 T4.G2 T5.G2 PC.G2

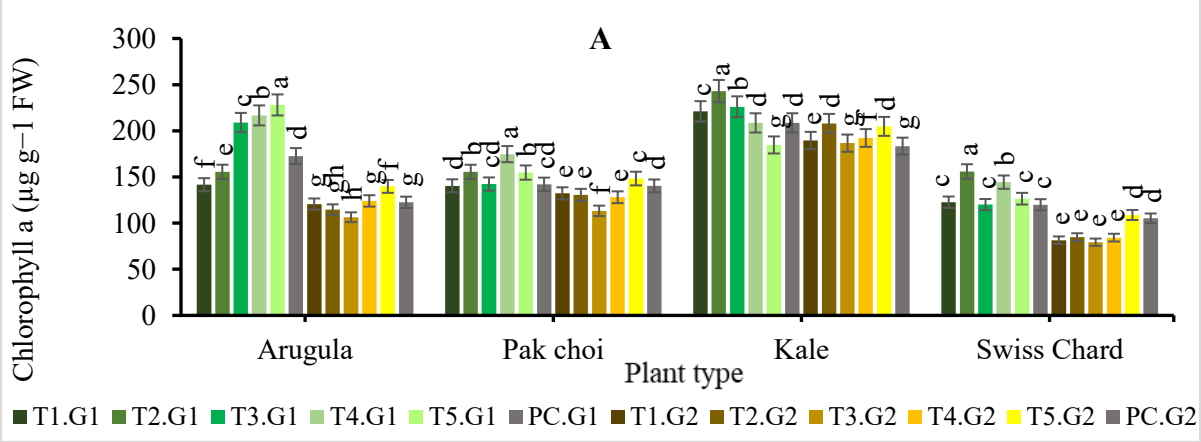
Figure 5. 2. Total root length (A); root surface area (B); total shoot length (C); shoot surface area (D), root volume (E), yield (F) of arugula, pak choi, kale, and Swiss chard plant species in response to LED spectrums and mixed growing media variation. Error bars indicate the standard deviations. Values are the means of three replicates and different alphabetical letters demonstrate significant differences based on Fisher's least significant (LSD) post hoc test within each plant species ($p < 0.05$).

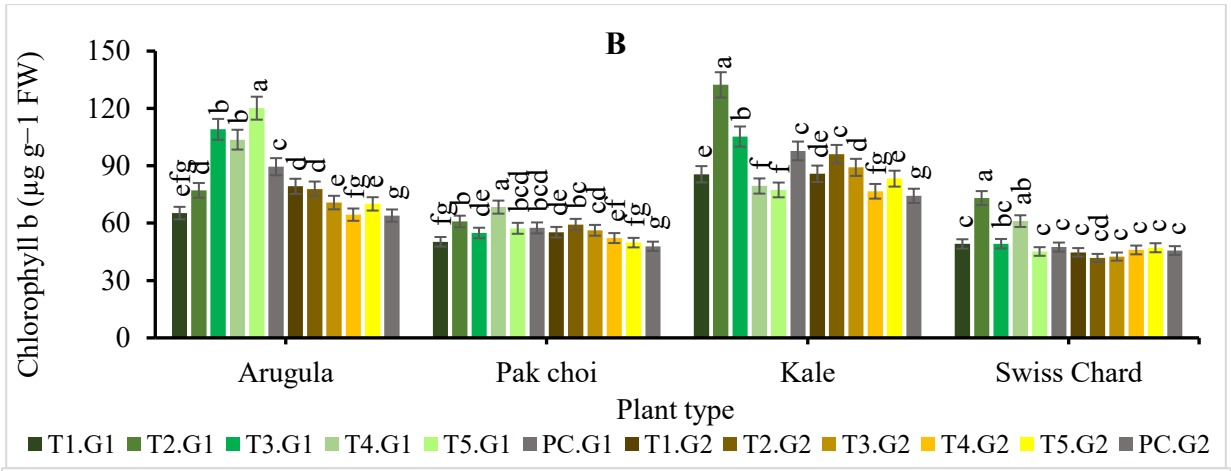
Also, the root volume of arugula was significantly enhanced by ca. 135.7% in T4.G1 followed by T2.G1 and T4.G2 in comparison with their PC counterparts (Fig 5. 2E). Furthermore, the root volume of pak choi, kale, and Swiss chard exposed to B20: R80 LED ratio were significantly enhanced by 106.2%, 85.1%, 121.4% in G1 compared to PC.G1 (Fig 5. 2E). Arugula yield exposed to B20: R80 LED ratio was significantly enhanced by 29.3%% in G2, while that of pak choi, kale, and Swiss chard yield were improved by 7.2%, 17.4%, and 44.5% in G1 respectively, compared to their counterparts cultivated in the PC (Fig 5. 2F). Interestingly, the lowest yield was reported in all the microgreens grown in G2 and exposed to B80: R20 and B60: R40 LED ratios (Fig 5. 2F).

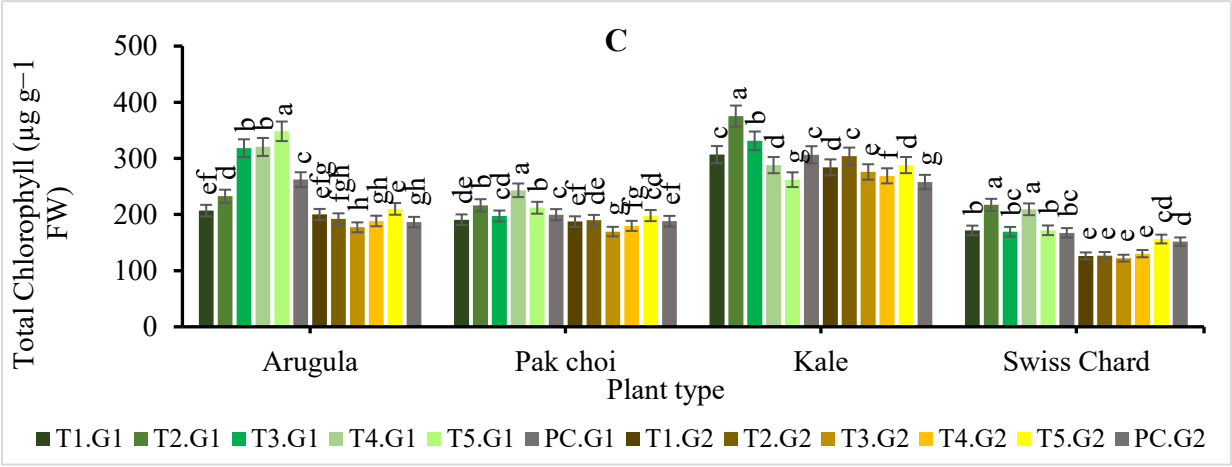
5.4.2 Microgreens biochemical composition

The results of ANOVA indicated that different B: R LED ratios, mixed growing media, and their interactive effects significantly ($P < 0.01$) altered the plant phytochemicals and antioxidant enzymes activities (POD and APEX). Arugula Chl a, Chl b, and Chl t grown in G1 were enhanced by 33% under the B50:R50 LED ratio, but that of pak choi was enhanced by 21.1% under B60: R40 LED ratio in G1 compared to their PC.G1 counterparts (Fig 5. 3A-C). In addition, Chl a, Chl b, and Chl t were considerably increased by 17%, 36%, and 23% in kale and 30%, 54%, and 30% in Swiss chard, respectively under B20: R80 LED ratio growing in G1 compared to

respective PC.G1 (Fig 5. 3A-C). Moreover, among the different plant species, kale (by 22.5%) followed by arugula (by 33%) exhibited the highest Chl t of plants grown in G1 under B20: R80 and B50: R50 LED ratios compared to those in PC.G1 (Fig 5. 3C). Total carotenoids of pak choi, Swiss chard, and arugula were significantly enhanced by average 42%, 41%, and 16% in both B40: R60 and B50: R50 LED ratios in G2, whereas kale total carotenoids grown in G2 was increased by 44% under B60: R40 LED ratio compared to their PC.G2 counterparts (Fig 5. 3D). B20: R80 LED ratio × G1 had a similar effect as B40: R60 LED ratio × G2 and B50: R50 LED ratio × G2 in increasing carotenoids of arugula but the effect varied for other microgreen species (Fig 5. 3D). Consistently, PC.G1 gave the least total carotenoid content of all the microgreens except for kale which was least under B80: R20 LED ratio × G1 (Fig 5. 3D). In general, the trend for the total carotenoids was kale (69.31 µg/g FW) > arugula (67.36 µg/g FW) > Swiss chard (50.20 µg/g FW) > pak choi (49.87 µg/g FW) (Fig 5. 3D).




B
Chlorophyll b (µg g−1 FW)
150
120
90
60
30
0
Arugula
Pak choi
Kale
Swiss Chard
Plant type
T1.G1 T2.G1 T3.G1 T4.G1 T5.G1 PC.G1 T1.G2 T2.G2 T3.G2 T4.G2 T5.G2 PC.G2


C
Total Chlorophyll (µg g−1 FW)
500
400
300
200
100
0
Arugula
Pak choi
Kale
Swiss Chard
Plant type
T1.G1 T2.G1 T3.G1 T4.G1 T5.G1 PC.G1 T1.G2 T2.G2 T3.G2 T4.G2 T5.G2 PC.G2

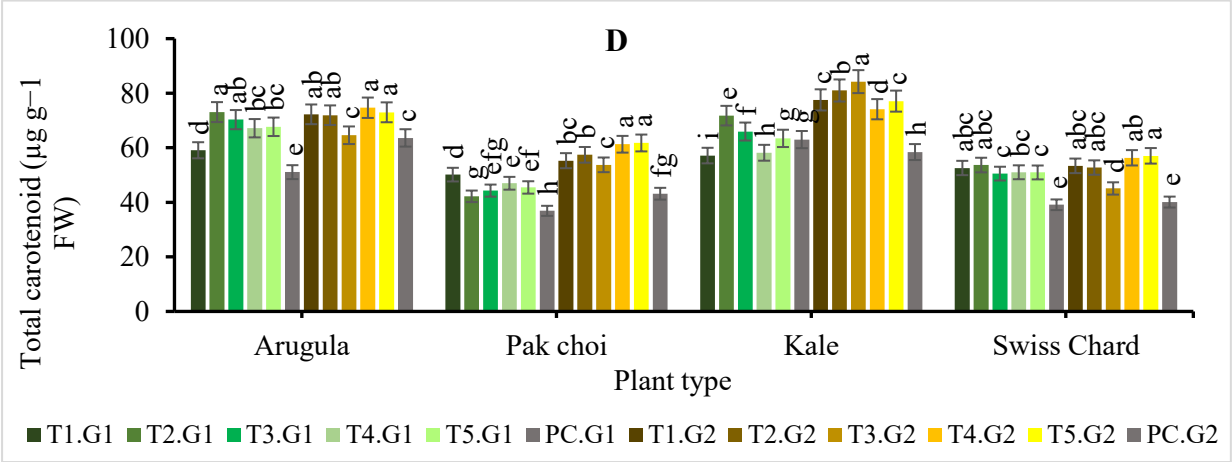
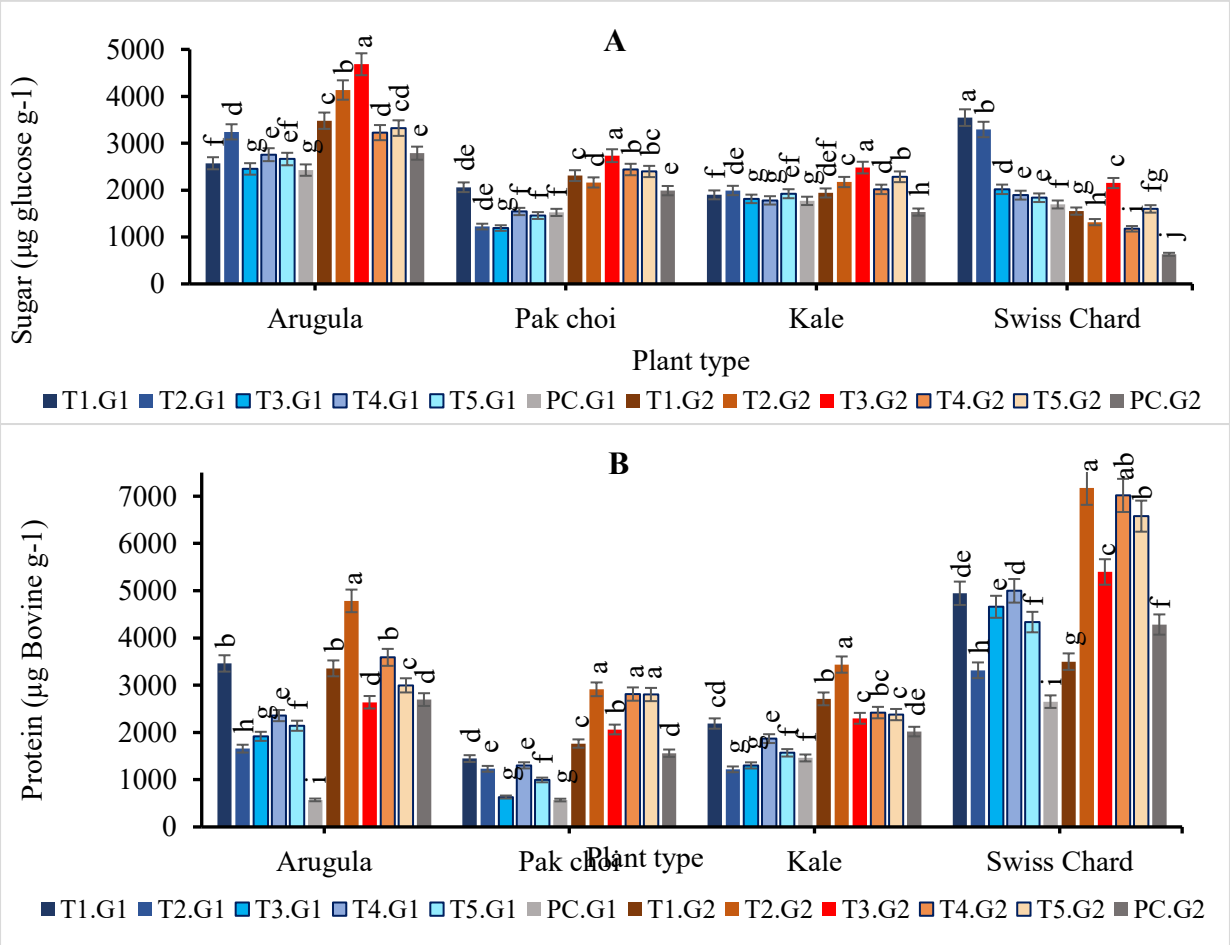

D
Total carotenoid (µg g−1 FW)
100
80
60
40
20
0
Arugula
Pak choi
Kale
Swiss Chard
Plant type
T1.G1 T2.G1 T3.G1 T4.G1 T5.G1 PC.G1 T1.G2 T2.G2 T3.G2 T4.G2 T5.G2 PC.G2

Figure 5. 3. Chl a (A), Chl b (B), Chl t (C), and carotenoid (D) contents of arugula, pak choi, kale, and Swiss chard plant species in response to LED spectrums and mixed growing media variation. Error bars indicate the standard deviations. Values are the means of three replicates and different alphabetical letters demonstrate significant differences based on Fisher's least significant (LSD) post hoc test within each plant species at $p < 0.05$.

The maximum sugar concentration was observed in arugula cultivated in G2 under B60: R40 followed by B20: R80 LED treatments in comparison with other treatments (Fig 5. 4A). Sugar contents of arugula, kale, and pak choi gown in G2 significantly enhanced by 68%, 62%, and 38% under B60: R40 compared to PC.G2, while sugar content of Swiss chard grown in G1 was increased by 109% under B80: R20 LED ratio compared to PC.G1 (Fig 5. 4A). Overall, the trend for sugar concentration expressed as µg glucose/g was arugula (3145.34) > kale (1966.76) > pak choi (1919.34) > Swiss chard (1892.01) (Fig. 4A). Similarly, B20: R80 LED treatment × G2 statistically ($P < 0.01$) enhanced protein level in pak choi, arugula, kale, and Swiss chard by ca. 87%, 78%, 70%, and 67% respectively in comparison with their PC.G2 counterparts (Fig 5. 4B). Also, B20: R80, B40: R60, and B50: R50 LED ratios similarly increased protein contents of pak choi and Swiss chard grown in G2 although the effect varied between microgreen species (Fig 5. 4B). In general, the trend for the protein accumulation expressed as µg Bovine/g was Swiss chard (4904.74) > arugula (2680.67) > kale (2072.58) > pak choi (1674.42) (Fig 5. 4B). The highest total phenolics were reported in all the plants grown in G2 under B80: R20 LED ratio compared to other treatments (Fig 5. 4C). Also, B60: R50 LED ratio increased phenolics of kale but did not significantly ($P > 0.05$) affect phenolics contents of other microgreens (Fig 5. 4C). The highest phenolics content was recorded in arugula followed by kale microgreens grown in G2 under B80: R20 LED ratio compared to the other treatments. Interestingly, total phenolics of pak choi, arugula,

Swiss chard, and kale significantly increased by ca. 101%, 78%, 76%, and 51% in G2 under B80: R20 LED ratio, respectively in comparison with their PC.G2 counterparts. The overall trend for the concentration of the phenolic expressed as mg GAE/g in the microgreens was arugula (339.82) > kale (307.71) > pak choi (184.04) > Swiss chard (163.30) (Fig 5. 4C).



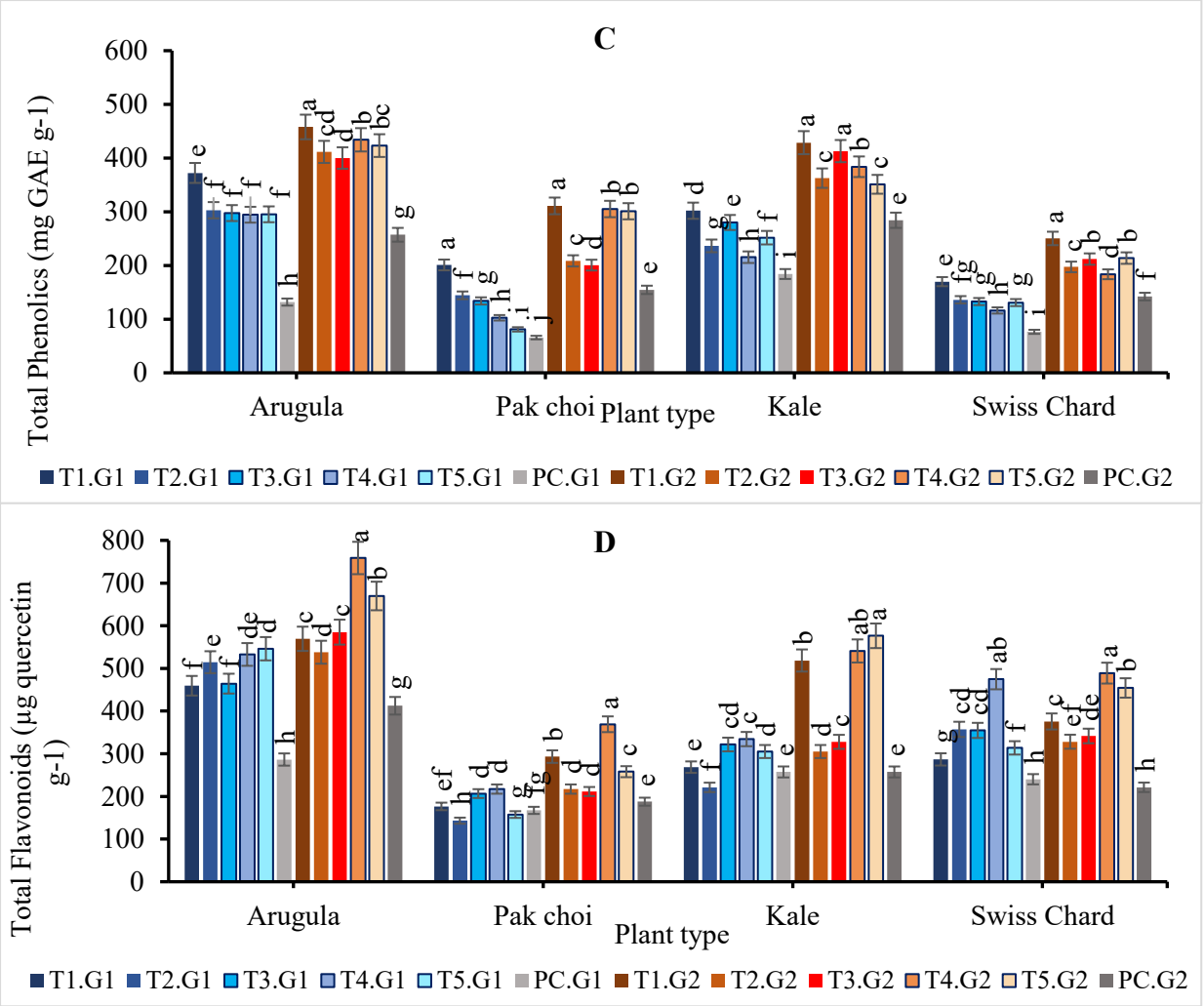


Figure 5. 4 Sugar (A); protein (B); total phenolics (C); total flavonoids (D) of arugula, pak choi, kale, and Swiss chard plant species in response to LED spectrums and mixed growing media variation. Error bars indicate the standard deviations. Values are the means of three replicates and different alphabetical letters demonstrate significant differences based on Fisher's least significant (LSD) post hoc test within each plant species at $p < 0.05$.

Total flavonoids enhanced by an average of 101% in all the microgreens grown in G2 under B40: R60 LED ratio compared to those cultivated in PC.G2, except for kale (Fig 5. 4D). Kale flavonoids was increased by 124% in G2 under B50: R50 LED ratio compared to PC.G2. Total flavonoids of

arugula, pak choi, and Swiss chard were significantly enhanced by 84%, 97%, and 121% respectively in G2 under B40: R60 LED ratio in comparison with their PC.G2 counterparts. Comparatively, the trend for the flavonoid accumulation expressed as μg quercetin/g was arugula (528.03) > Swiss chard (353.07.34) = kale (352.89) > pak choi (217.05) (Fig 5. 4D).

The increase in peroxidase activities in all microgreens by T4.G2 was on average 2.3- and 4.2-folds superior to their microgreens cultivated in the PC.G2. Peroxidase activities in arugula, pak choi, Swiss chard, and kale were statistically ($P< 0.01$) enhanced by ca. 136%, 177%, 320%, and 234%, respectively in G2 under B40: R60 LED ratio compared to PC.G2 (Table 5. 3). Furthermore, kale followed by Swiss chard and pak choi had the highest POD activities whereas arugula had the minimal POD activity (Table 3). The general trend for POD action expressed as Unit/ mg FW was kale (0.81) > Swiss chard (0.75) > pak choi (0.73) > arugula (0.64). Moreover, APEX activities were notably ($P < 0.01$) increased in all tested plants grown in G2 under B80: R20 and B40: R60 LED treatments in comparison with other treatments (Table 5. 3). APEX activities in arugula, pak choi, Swiss chard, and kale were considerably ($P < 0.01$) enhanced by 44%, 103%, 70%, and 94% in G2 under B80: R20 LED ratio as well as 38%, 164%, 69%, and 113% in G2 under B40: R60 LED ratio in comparison with their PC.G2 counterparts (Table 5. 3). Among the microgreens, the general trend for APEX actions expressed as Unit/ mg FW was pak choi (0.117) > arugula (0.101) > kale (0.097) > Swiss chard (0.089) (Table 5. 3).

Table 5. 3. The effects of B: R LED variations × growing media on peroxidase activity and ascorbate peroxidase activity

Treatment	Peroxidase Activity (U/ mg FW)				Ascorbate Peroxidase Activity (U/ mg FW)			
	Arugula	Pak choi	Swiss Chard	Kale	Arugula	Pak choi	Swiss Chard	Kale
T1 × G1	0.57de	0.59fg	0.74cd	0.58ef	0.10ef	0.13bc	0.06fg	0.11bc
T2 × G1	0.39g	0.35h	0.60e	0.53fg	0.09efg	0.09de	0.06fg	0.08ef
T3 × G1	0.32i	0.34hi	0.61e	0.50gh	0.08gh	0.08ef	0.06fg	0.10c
T4 × G1	0.60d	0.59fg	0.80c	0.64de	0.12bc	0.14b	0.11bc	0.09d
T5 × G1	0.54e	0.55g	0.65de	0.54fg	0.10ef	0.11c	0.09de	0.08de
PC × G1	0.35h	0.33i	0.21h	0.47h	0.07h	0.07f	0.05g	0.06g
T1 ×G2	1.07ab	1.17b	1.18b	1.40b	0.14a	0.15b	0.12ab	0.13a
T2 × G2	0.54e	0.79d	0.69de	0.67d	0.06i	0.10cd	0.08ef	0.08ef
T3 × G2	0.82c	0.70e	0.42f	1.14c	0.10de	0.12bc	0.12ab	0.12ab
T4 × G2	1.17a	1.72a	1.41a	1.75a	0.13ab	0.19a	0.13a	0.13a
T5 × G2	0.95b	1.04c	1.37a	1.01c	0.11c	0.13bc	0.10cd	0.11bc
PC × G2	0.49f	0.63f	0.34g	0.52fg	0.08gh	0.07f	0.06fg	0.07f
p value								
LED light	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Growing media	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000
Interaction	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Peroxidase activity and ascorbate peroxidase activity in response to LED spectrums and mixed growing media variation. T: LED light treatments; G: growing media, and T × G: the interaction effects between LED light × growing media (N = 4). Values are the means of three replicates and different alphabetical letters demonstrate significant differences based on Fisher's least significant (LSD) post hoc test within each plant species ($p < 0.05$).

5.4.3 Association between media, LED spectrum, and phytochemicals

A multivariate two-dimensional PCA biplot illustrated the association among plant yield and selected biochemical compounds affected by the interactive effects of varying ratios of red: blue LED light quality and mixed growing media (Fig 5. 5A-D). The PCA results showed that T4.G2 can be positively connected to enhanced yield and phytochemicals of all microgreens due to being closer to the origin of the axes compared to the other treatments are found on peripheral position, except for kale which was T3.G2 (Fig 5. 5C). The interactive effects of LED light and mixed growing media on arugula are associated with total phenolics and flavonoids followed by carotenoids and protein (Fig 5. 5A).

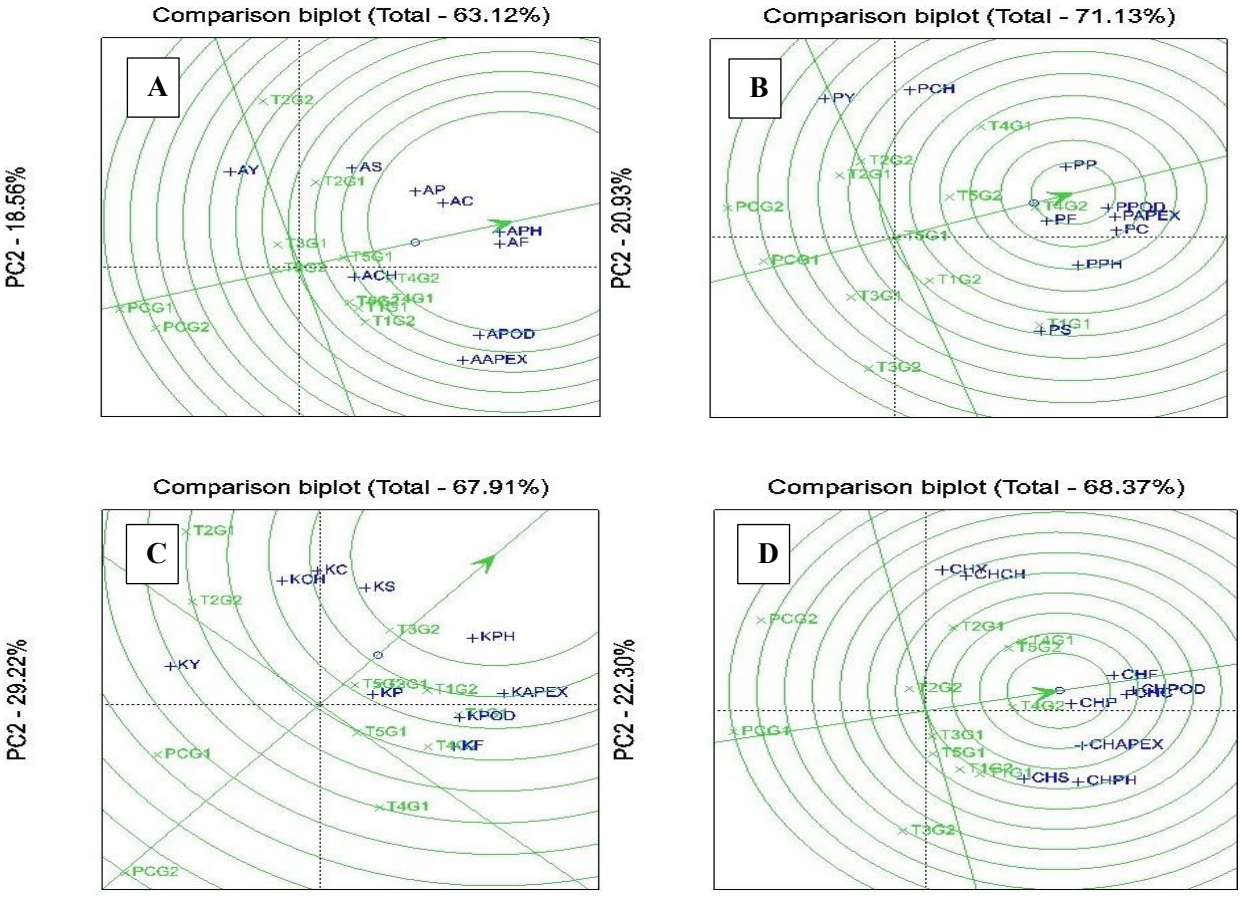


Figure 5. 5. A multivariate two-dimensional PCA biplot for comparison of LED treatment (T) × mixed growing media (G) interaction effects on yield and biochemical variations of arugula (A); pak choi (B); kale (C); Swiss chard (D). The PCA explained 61%, 71%, and 68% of the total variations in the dataset of A, B, C, and D, respectively. AA: arugula ascorbate; ACH: chard ascorbate; AK: kale ascorbate; AP: pak choi ascorbate; APXA: arugula APEX Activity; APXCH: chard APEX Activity; APXK: kale APEX Activity; APXP: pak choi APEX Activity; CA: arugula carotenoids; CCH: chard carotenoids; CK: kale carotenoids; CP: pak choi carotenoids; PA: arugula POD Activity; PCH: chard POD Activity; PK: kale POD Activity; PP: pak choi POD Activity; YA: Arugula yield; YCH: Chard yield; YK: kale yield; YP: pak choi yield. Light treatments were: T1: B80:R20; T2: B20:R80; T3: B60:R40; T4: B40:R60; T5: B50:R50, and white LED light as a positive control (PC) at 100 μmol m−2 s−1 light intensity. Growing media were G1: 20%sawdust: 20%perlite and G2: 30%PittMoss: 10%perlite, all containing 30%vermicast and 30%mushroom compost.

While pak choi flavonoids and protein followed by antioxidant enzymes activities were strongly affected by the interaction between LED light spectrum × mixed growing media (Fig 5. 5B). On the contrary, kale phenolics and sugar followed by APEX activities was strongly influenced by the interaction between LED light spectrum and mixed growing media (Fig 5. 5C). Furthermore, the interaction effects LED light spectrum × mixed growing media on Swiss chard can be attributed to protein content followed by flavonoids, carotenoids, and POD activities (Fig 5. 5D). Moreover, compared to biochemical compounds and antioxidant enzymes activities, yield index was not strongly influenced by the interaction between different LED light quality and growing media in all the microgreens. Overall, the interaction between different red: blue ratios of

LED light quality and growing media can be strongly associated with biochemical parameters and antioxidant enzymes activities compared to yield for all the plants.

5.5 Discussion

5.5.1 Seed germination and growth indices

The effects of different blue: red LED ratios, different growing media and their interactive effects on seed germination and growth traits of several microgreens were studied under controlled environmental conditions. In this study, microgreens grown in G1 exhibited a higher growth rate and yield in compared to that of in G2. It has been shown that the growing medium G1 containing higher percentage of perlite and sawdust can improve plant growth by modulating the physicochemical properties of the growing media (Emami and Astaraei, 2012; Vahid Afagh et al., 2019). Previous studies confirmed that high salinity, total dissolved solids (TDS) and electrical conductivity (EC) levels in growing media significantly can negatively affect the physicochemical characteristic of growing substrates and reduce plant growth parameters and fresh biomass in most plant species (Warrence et al., 2002). However, the negative effects of high salinity, TDS, and EC can be compensated by combining wood-based substrates like sawdust and perlite with other growing substrates as porosity aeration, and water holding capacity enhancers (Zhang et al., 2009; Lee et al., 2011a). Therefore, higher microgreens growth and biomass growing in G1 can be ascribed to the presence of sawdust and higher percentage of perlite compared to G2 with no sawdust. These results corresponded with the work by of Lin et al. (2020) in which the combination of 60% vermicast plus 40% sawdust significantly ($P < 0.05$) increased plant growth parameters and yield of Swiss chard, pak choi, and kale due to improved physicochemical properties and nutrient mineralization in this mixed growing medium. Vahid Afagh et al. (2019) attributed to enhanced morphophysiological characteristics and biomass of German chamomile (*Matricaria*

recutita L.) to ameliorated physicochemical properties, bioavailability of essential elements, and promising growing medium microbes which supported by adding 15% spent mushroom compost in a mixture of sandy loam soil.. Saleh et al. (2022) illustrated that improved structure and functionality of G1 improved plant growth in kale, pak choi, Swiss chard, and arugula microgreens. The author reported the bulk density (0.12 g/cm3) below the root-restriction threshold in G1 which is closely associated with an improved root system, thereby increasing nutrient and water uptake followed by enhanced growth parameters in G1. . Based on the results of this study, root volume was improved in microgreens grown in G1 compared to G2, which may relate to the interactive effects between different red: blue LED light ratios and growing media. Furthermore, it was shown that B80:R20 had the greatest effect on seed germination of all microgreens, except for the kale, which might be related to the higher ratio of blue light. In support of our results, seed germination of lettuce was increased by 56% and 42% by increasing blue fraction and only blue light, respectively compared to lower B: R ratio and only red LED light (Hernández-Adasme et al., 2022). Cocco et al. (2022) attributed reduced germination under a higher R: B LED ratio to increasing seed dormancy through phytochrome activation/inactivation, which may explain higher germination under a higher B: R LED ratio in this study. As confirmed by Gubler et al. (2008), blue light has a stimulatory effect on expression genes involved in the biosynthesis of abscisic acid (ABA) and gibberellin (GA) in breaking seed dormancy. In current study, microgreens grown in growing medium containing sawdust (G1) and treated with higher red: blue LED ratio (R80: B20) showed an improvement in plant growth components including plant height, root and shoot length, root and shoot surface area, and yield compared to other treatments. Moreover, B20:R80 × G1 containing sawdust had a great influence on chlorophyll of kale and Swiss chard, while B40: R60 × G1 and B50: R50 × G1 increased chlorophyll of pak choi and arugula, respectively. These

findings agree with Metallo et al results in which kale seedlings grown under the 20B: 80R LED ratio had the highest root and shoot length, fresh biomass, and chlorophyll compared to 5B: 95R LED and white LED lights. In another study, 9B: 91R LED and 5B: 95R LED treatments significantly enhanced the fresh and dry mass, chlorophyll of lettuce, spinach, basil, peppers, and kale (Naznin et al. 2019).

It was assumed that the presence of sawdust and higher proportion of perlite had a positive role in enhancing the field capacity of G1 followed by increasing nutrient and water intake from G1 by root, which may explain higher plant growth and chlorophyll content in all the microgreens. Also, Jin et al. (2023) found a strong correlation between a higher R: B LED ratio and an enhanced root system causing higher organic matter accumulation in the root area. This might be the reason for the synergetic effect of higher red fraction and ingredients of G1 on enhanced growth factors, yield, and chlorophyll content in microgreens growing in G1 and exposed to 80R: 20 B ratio. However, the crucial role of blue light intercepted by PHOT1 (phototropin-1) and Cryptochrome Circadian Clock 1 (CRY1) photoreceptors on gene expression involved in the biosynthesis of gibberellic acid resulting in cellular division, endoreplication, and cell growth. According to previous studies, morphological features and pigment accumulation were significantly improved in leafy vegetables grown under an appropriate ratio of blue and red in comparison with white LED and other monochromatic spectra. However, the role of red and blue light on plant performance is plant species dependent (Gangadhar et al., 2012; Kopsell and Sams 2013; Bian et al., 2015). Red LED light intercepted via cryptochrome photoreceptors has a stimulatory effect on cellular division, leaf development, and shoot surface area of various plant species which may explain the enhanced growth factors caused by the higher red ratio in this study (Metallo et al., 2018; Wang et al., 2020). Moreover, higher photosynthetic activity led to enhancing plant growth

and chlorophyll in kale, paper, and spinach in response to higher percentage of red LED (Naznin et al., 2019).

5.5.2 Nutritional values and biological properties

The interaction effect between different LED treatments and mixed growing substrates on microgreens biochemical compounds and antioxidant enzymes potentials were significant. There are several scientific studies on the impact of various soilless growing substrates and the variations in LED quality on the biochemical quality of microgreens. Nevertheless, it should be highlighted that the majority of the literature available on the interaction effects of LED spectrum × mixed growing media on bio-functional properties of microgreen species are very scanty. Irrespective of different LED treatment, G2 containing PittMoss was the most effective media in increasing phytochemicals and antioxidant enzymes activities which can be attributed to the nutrient availability and higher C/N ratio in this medium (Ibrahim et al., 2011). In promise with the findings of this study Ghiasy-Oskoee et al. (2018) who found that a strong correlation between enhanced phenolics, flavonoid, and antioxidant potential in Blessed thistle (*Cnicus benedictus* L.) with higher C/N ratio. Contrary to this, G1 containing sawdust was effective at increasing germination, growth indices, chlorophyll content of all microgreens, which might be due to optimal growth condition and higher nitrogen content (N) in this medium as explained by Gonani et al. (2011). Correlation data analysis confirmed a negative relationship between enhanced growth parameters and elevated phytochemical compositions in all microgreens in this study (S. Table 1). According to the results, a higher carotenoid content was reported in almost all plants grown in B40:R60 × G2 (containing PittMoss) and B50:R50 × G2, except for kale carotenoid. However, several studies revealed that there is a positive correlation between increasing blue LED fraction and elevated

carotenoid levels in greens leafy vegetables (Bian et al., 2015; Kopsell and Sams 2013). Similarly, Metallo et al. (2018) revealed that increasing the ratio of blue to 20% significantly stimulated carotenoid accumulation in kale most possibly due to the enhanced cryptochrome photoreceptor activity under increasing blue fraction, as confirmed by Naznin et al (2019). On the contrary, Brazaitytė et al. (2021) reported that the total carotenoid concentration of basil, mustard, kale (*Brassica napus* 'Red Russian'), and parsley (*Petroselinum crispum*) microgreens were unaffected by increased proportion of blue light, and highest total carotenoid content was observed in plant grown under high proportion of red light.

Plant carotenoid accumulation in response to different blue: red LED ratios is mostly likely associated with the gene expression engaged in carotenoids accumulation and also the cultivation condition. This is a possible reason that may explain enhanced carotenoid content in microgreens cultivated in B40: R60 × G2 and B50:R50 × G2 in the current study (Lobiuc et al., 2017; Alrifai et al., 2021; Brazaitytė et al., 2021). In this study, all LED treatments with higher ratio of blue light significantly increased kale soluble sugar compared to other treatments. Among the treatments, B60: R40 × G2 showed the highest sugar content of microgreens, possibly due to the blue light-induced photosynthetic, metabolic activity, detoxification pathways in response to reactive oxygen species (ROS) accumulation induced by higher B: R LED ratio(Eckstein et al., 2012; Metallo et al., 2018). However, protein content of all microgreens enhanced by increasing red fraction that most likely relate to enhance the activity of phytochrome photoreceptor regulating gene expression involved in biosynthesis of chloroplast protein (Cocco et al., 2022). Moreover, higher protein content in pak choi was attributed to the downregulation of the senescence-related genes caused by increasing red LED doses (Song et al., 2020). In addition, the improved protein content under a higher R: B ratio can be associated with differential physiological and biochemical responses to

red light, photosystem II (PSII) efficiency, and photosynthetic performance (Wang et al., 2020). In the present study, irrespective of plant species, B80:R20 × G2 treatment enhanced phenolics content in all the microgreens. Consistency, Vaštakaitė et al. (2015) observed that total phenolics of basil was increased by 33% by increasing blue light doses. This can be ascribed to stimulatory effect of higher proportion of blue light on the photoinduction of phenolics synthesis, their precursors, and phenylalanine ammonia lyase (PAL) enzyme involved in phenolics biosynthetic pathway (Lobiuc et al., 2017; Vaštakaitė et al., 2015). Iwai et al. (2010) attributed the enhanced phenolic compounds in red perilla (*Perilla frutescens* var. *purpurea* cv. Akajiso) grown under blue light intercepted via cytochrome P450 defense mechanisms involved in scavenging ROS induced by blue light. Also, the results of this study demonstrated that higher flavonoid content was obtained from microgreens grown in G2 and exposed to B40: R60 and B50: R50 compared to other treatments. Consistent with the results of this study, higher flavonoid content was observed when basil, kale, and Chinese cabbage plants grown under 1red: 1blue LED ratios (Lobiuc et al., 2017). The higher flavonoid content in these treatment might be attributed to the regulatory systems of polyphenols and flavanols biosynthesis mediated by red photoreceptor and higher expression of genes encoding PAL; 4 coumaroyl CoA-ligase (4C)L; and chalcone synthase (CHS) enzymes in phenylpropanoid pathway (Lobiuc et al. 2017; Lee et al .2016; Liu et al. 2018). The difference in blue: red LED ratio and mixed growing media significantly affected POD and APEX enzymes activities in all tested microgreens. It is widely known that there is a significant correlation between phytochemical compounds and antioxidant functions (Arumugam et al., 2016). In this study, the highest POD and APEX activities were reported in microgreens grown B40: R60 × G2 followed by B80: R20 × G2, which strongly associated with the enhanced carotenoids and flavonoids in B40: R60 × G2 and the elevated phenolics in B80: R20 × G2. The

enhanced activities of POD and APEX antioxidant enzymes in response to the LEDs is ascribed to the enhanced carotenoids in leafy greens (Metallo et al., 2018; Naznin et al., 2019), enhanced phenolics compounds (Son et al., 2013; Lobiuc et al., 2017) as well as the elevated polyphenols and flavonoids (Lee et al., 2016). In support of our results, Son et al. (2013) reported that lettuce exposed to higher ratio of blue light had a higher phenolic content and antioxidant capacity but decreased growth characteristics which might be related to the growth-defense trade-off mechanism. Therefore, the increased antioxidant enzyme activities may be related to higher pigment concentration including phenolic compounds and flavonoids in plants treated with a specific combination of blue and red LED ratio in this study.

5.6 Conclusion

Conventional agriculture used synthetic chemical fertilizers and pesticides to provide the global high-nutrient foods demand for the growing population which has major implications for the environment and human health. Therefore, a research project needs to be conducted to increase food production with enhanced health-promoting compounds by developing innovative and climate-smart agricultural management practices is highly needed. In the current study, the interaction effects of various blue: red LED ratios × mixed growing substrates on the edible quality and bio-functional properties of several microgreen species were evaluated. It was demonstrated that variations in LED spectrum and growing media differentially altered the studied characteristics of microgreens. Growth indicators were significantly improved in microgreens grown in sawdust based growing media (G1) under the application of 20B: 80R LED ratio compared to other treatments. Whereas almost all measured biochemical compounds and antioxidant enzymes capacity were significantly enhanced in microgreens grown in PittMoss-based growing media (G2) with a slightly higher red LED fraction i.e., 40B: 60R LED ratio.

However, the highest phenolics and sugar content were found in microgreens grown in G2 and exposed to 80B:20R and 60B:40B LED ratios, respectively compared to other treatments. . The efficacy of 40B: 60R × G2 on the yield and phytochemical compounds was confirmed by the PCA analysis. Considering the high global appeal and use of microgreens as vegetable crops in different cuisine or diets, the output from this work could improve our understanding of how variation in blue and red LED ratios as well as different mixed growing media can enhance productivity and nutrients and non-nutrients bioactive compounds. Compared to other conventional artificial lighting sources, Light-emitting diodes (LEDs) have substantial advantages like higher efficiency with lower heat emissions and a variety of narrow wavebands. Also, natural mixed growing substrates applied in this study are cost-effective and easily accessible alternatives to current media for growing microgreens to increase plant productivity and quality. Thus, according to the findings of this study, we recommend that the application of natural growing media and the mixture of blue and red LEDs most likely has a vital role in increasing crop phytonutrients and benefiting consumer health.

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CHAPTER 6: CONCLUSIONS

6.1 Thesis findings

Global warming and climate change have had adverse impacts on plant production and food security. During the last decade, synthetic chemical fertilizers and pesticides have been extensively used in conventional agriculture to meet global food and nutrition demands. However, it is well acknowledged that the application of synthetic chemicals in agriculture negatively affect the environment, ecosystem services and all life forms. Therefore, regulating preharvest parameters can be a practical and sustainable strategy to increase food productivity and the bioactive composition of high-quality crops for the growing population without compromising the environment and agroecological systems. The literature showed that plant morphophysiological responses and biosynthesis of phytochemicals are largely influenced by preharvest factors (Chapter 2). The main objective of this study was to develop an innovative and climate-smart approach using an optimized soilless mixed growing medium and a specific of ratio blue: red LED light spectrum to enhance the growth and quality of microgreens grown in an indoor production system.

It was shown that variations in physicochemical parameters and the effectiveness of different proportions of mixed media can impact the growth and yield of microgreens (Chapter 3). The media containing Shiitake mushroom compost (MC2) substantially promoted plant growth and yield in all microgreen plant species due to improved physicochemical parameters of the growing media and possibly superior nutrient status. In contrast, the reduced plant growth in media containing White oyster mushroom compost (MC1) may be attributed to higher salinity, EC, and TDS levels. Overall, T2.2 (30% vermicast + 30% sawdust + 10% perlite + 30% MC2) and T4.2 (30% vermicast + 20% sawdust + 20% perlite + 30% MC2) were the most effective treatments in

improving germination rate, plant height, and yield in all microgreens due to enhanced media physical features and nutrients availability in these mixed growing media.

The next experiment evaluated the effect of optimized mixed growing media obtained from chapter 3 on the chemical composition and antioxidant enzymes activities of microgreens (Chapter 4). Also, substitution of mushroom compost with PittMoss was designed to compare the effects of mushroom compost versus PittMoss (PM) on plant growth and productivity and biochemical compounds. Overall, the growing media containing mushroom compost i.e., T2.2, (30% vermicast + 20% sawdust + 20% perlite + 30% MC2) was found to be the most favorable that was further confirmed through the PCA analysis. These findings provide evidence that T2.2 can be used as an alternative to conventional media such as Pro-mix BX™ potting medium for growing microgreens to improve productivity and phytonutrients composition of microgreens.

The interactive effects of mixed growing media × different red: blue LED ratios on the edible quality and bio-functional properties of different microgreen species was further investigated in Chapter 5. It was found that growth indicators were markedly improved in microgreens grown in growing media containing sawdust with an increasing red LED ratio i.e., T2.G1 (20% blue + 80% red × 30% vermicast + 20% sawdust + 20% perlite + 30%MC2) compared to other treatments. Whereas the quality and antioxidant enzymes capacity were markedly enhanced in microgreens grown in PittMoss based growing media with a slightly higher red LED fraction i.e., T4.G2 (40% blue + 60% red × 30% vermicast + 30% PittMoss + 10% perlite + 30%MC2). The efficacy of T4.G2 on yield and quality traits was further confirmed through the PCA analysis.

Considering the high global appeal and use of microgreens as functional food in different cuisine or diets, the output from this work could improve our understanding of how variation in

blue and red LED ratios as well as different mixed growing media can enhance productivity and nutritional values. Compared to other conventional artificial lighting sources, LEDs have substantial advantages like higher efficiency with lower heat emissions and a variety of narrow wavebands. Also, natural mixed growing media applied in the present study are cost-effective and the additives are locally available. Therefore, it was recommended that the application of natural growing media in combination with blue and red LEDs most likely has a vital role in increasing crop phytonutrients and benefiting consumer health.

6.2 Thesis limitations and recommendations

Although the objective of this thesis was accomplished, there were experimental limitations. For example, the nutrient presented in white oyster, shiitake mushroom compost, and PittMoss were not analyzed due to financial limitations. Therefore, it is unknown what C: N ratio was presented in mixed media containing the two different mushroom compost and PittMoss (Chapter 3 and 4). Previous studies reported a strong correlation between increased chlorophyll content and photosynthetic activity and higher N content (Gonani et al., 2011; Zhang et al., 2017) whereas there was a positive correlation among enhanced phenolics, flavonoid, and antioxidant potential and higher C content (Ghiasy-Oskoee et al., 2018). Hence, enhanced germination rate, plant height, and yield of microgreens grown in mixed media made by Shiitake may attribute to higher N content in this study (Chapter 3). Moreover, due to time constraints microbiome assessment of the optimized growing media was not accomplished which could provide more important details and explained the positive effects of optimized mixed media on growth and certain biochemical accumulation. Similarly, germination, growth indices, chlorophyll content of all the microgreens were likely attributed to more nutrient availability and high-N input from

vermicast and mushroom compost (Chapter 4). In addition, C might have improved the carbon-based phenolic compounds and their precursors involved in plant defense mechanisms and responses to environmental stress (Treutter, 2010; Ibrahim et al., 2011). Also, a negative correlation between reduced phenolic content and N-rich growing media was reported by Singh et al. (2016). Therefore, the elevated phenolic content in the microgreens grown in T1.1 and T2.1 can be ascribed to the high-carbon content of the PittMoss (Chapter 4). However, possible reasons given in discussion sections can explain enhanced yield and growth parameters in mixed growing media containing Shiitake mushroom compost (chapter 4) or enhanced certain biochemicals in mixed media containing PittMoss (chapter 5), but further studies should be conducted on the microbiome and essential nutrients presented in optimized growing media to find precise mechanisms and the relationship between media and enhanced plant performance.

Another experimental limitation was related to regulation of intensity in LED panels which had fixed light intensity in last experiment (Chapter 5). The maximum intensity of LED was 100 µmol m^{-2} s^{-1} which was provided by the combination of red and blue LED wavelength. It was shown that manipulation of intensity has direct effect on seed germination, plant growth, photosynthesis, flowering, and the accumulation of secondary metabolites (Hou et al., 2010; Montgomery, 2016). Previous studies confirmed that light intensity of 250 µmol m^{-2} s^{-1} provided by red and blue LED can significantly increase plant productivity and the quality of a variety of leafy vegetables (Bian et al., 2015; Zhang et al., 2018). Lettuce yield and secondary metabolites accumulation were increased by increasing light intensity from 150 µmol m^{-2} s^{-1} to 300 µmol m^{-2} s^{-1} (Ahmed et al., 2020). Moreover, many studies attributed enhanced yield and growth parameters under optimal light intensity (200-300 µmol m^{-2} s^{-1}) to higher photosynthetic activities, whereas enhanced biochemical composition under high and low light intensity (800 and 100 µmol

m^{-2} s^{-1}) was linked to the environmental stress induced by light intensity. Therefore, applying light intensity of 100 µmol m^{-2} s^{-1} less than the optimal level most likely affected the results presented in this study.

Although this study demonstrated significant effect of the combination of optimal mixed growing media and specific blue: red LED ratio on enhanced yield and creating biochemical compounds, further commercial technologies and techniques are needed to minimize the limitations of this study in order to increase high-quality plant production in commercial scale and support validate the results obtained. For example, the use of LED panels doesn't have any restrictions on setting the light intensity to test the light intensity higher than 100 µmol m^{-2} s^{-1} on microgreen yield and biochemical compositions. Also, further studies should be conducted to test the application of other spectra (i.e., far-red spectrum) along with blue: red LED ratios for optimization of the LED spectrum.

In addition, due to time constraints, the lipidomic experiments on microgreens were not done. Therefore, it will be interesting to test the effects of different blue; red LED ratios on essential lipids which possess health benefits in microgreens. So, further studies should be conducted to assess the effects of different blue: red LED ratios and light intensity on yield, phytochemicals, and lipidomic of other different microgreen species for optimization of the LED spectrum.

6.3 CONTRIBUTIONS TO KNOWLEDGE

My PhD thesis presents the development of an effective and innovative soilless growing medium combined with a specific LED light spectrum to increase microgreens production in an indoor production system with enhanced health-promoting compounds. The suggested cost-effective and easily available mixed growing media and precise blue: red LED ratio can be a

practical alternative to traditional substrates such as peatmoss and Pro-mix BX™ to solve food insecurity and to produce high-quality functional food for the growing global population with minimal environmental risks. Many researchers have tested the effect of various natural amendments on plant productivity (Hernánde et al., 2021; Abbott et al., 2018), but few studies have considered the effects of mixed several natural amendments on physicochemical properties and beneficial microbial activities of growing media as well as microgreen growth indices, biochemical compounds, and biological properties.

The study of different amendments and LED light spectra on morphophysiological responses of various plant species has become more common, as these important preharvest indicators have been used to assess plant productivity and quality of microgreens (Abbey et al., 2017; Ahmed et al., 2020; Brazaitytė et al., 2021). However, there is limited information about the interactions of different LED light spectrum × mixed growing media or the potential synergistic effects of these preharvest factors on microgreen plant growth and biochemical compounds. Therefore, investigating the factorial experiment (i.e., LED x growing media) under controlled conditions has undoubtedly provided insight into the potential impact of LED x growing media on yield and health-promoting compounds of microgreens from a research and development perspective of climate-smart agricultural management practices.

Knowledge of the organic soilless growing media combined with the optimized LED spectrum could be adapted for use and application for other crop cultivation systems with lowering input requirements and expenses i.e., avoidance of using synthetic chemical fertilizers and pest repellent as well as more appropriate water and soil management. The clean technology suggested by this study can be used in places with severe plant production problems like Newfoundland, due to a lack of soil or high saline soil. Furthermore, data collected from this study could be compiled

for other crop species to determine the effectiveness of optimal mixed-growing media and blue: red LED ratio being applied. The data could also be used for a deep understanding of the growth-defense trade-off mechanism and the correlation between yield and bio-functionality of various crops to propose best management practices.

Canadian field vegetables focus on increasing harvestable yields with high quality at lower production costs, which can be achieved by the suggested effective, innovative and climate-smart soilless growing medium combined with a specific LED light spectrum. The ingredients used to make the mixed growing media in this study are reasonably inexpensive and locally available. Therefore, they can be used as an alternative to conventional media such as Pro-mix BX™ potting medium for growing microgreens to improve productivity and nutrient and non-nutrient bioactive compounds. This PhD research project is unique, as it directly addresses one of the main problems in industrial agricultural systems. The results of this study demonstrated a 230% increase in plant yield and over 50% increase in total ascorbate, flavonoids, and antioxidant enzymes activities of the microgreens obtained from optimized mixed-growing media compared to their Pro-mix BX™ counterparts.

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Appendix

Supplementary table I

Table A. 1 Pearson coefficient correlation between physicochemical growing media and growth parameters

	Pak choi height	Pak choi yield	Pak choi Root Length	Pak choi Root Surf Area	Pak choi Root Volume	Pak choi Shoot Length	Pak choi Shoo Surf Area	Pak choi chla	Pak choi chlb	Pak choi chlt	Pak choi car	Pak choi Flavonoid	Pak choi Phenolics	Pak choi Sugar	Pak choi Protein	Pak choi POD	Pak choi APEX
Pak choi GR																	
Pak choi height	1																
Pak choi yield	.465**	1															
Pak choi Root Length	.542**	0.216	1														
Pak choi Root	0.151	0.239	.408*	1													

Surf Area																	
Pak choi Root Volume	0.011	-0.017	.338*	0.031	1												
Pak choi Shoot Length	.549**	.792**	0.234	0.101	0.006	1											
Pak choi Shoo Surf Area	.429**	.422*	0.063	0.037	-0.183	.669**	1										
Pak choi chla	-0.157	.369*	-0.181	-0.135	-0.249	0.002	-0.273	1									
Pak choi chlb	-0.297	0.162	-0.094	-0.124	0.089	-0.190	-0.301	.485**	1								
Pak choi chlt	-0.217	.351*	-0.176	-0.147	-0.179	-0.054	-0.314	.966**	.695**	1							
Pak choi car	-0.215	-.603**	0.000	-0.328	.642**	-.370*	-0.116	-.399*	-0.037	-.340*	1						
Pak choi Flavonoid	-0.210	-.420*	-.342*	-.512**	.506**	-0.197	-0.064	-.331*	-0.139	-0.313	.802**	1					

Pak choi Phenolics	-0.133	-.589**	-0.010	-0.264	.632**	-0.294	-0.140	-.445**	-.354*	-.470**	.889**	.796**	1			
Pak choi Sugar	-0.071	-.621**	-0.014	0.047	.451**	-.360*	-0.107	-.549**	-0.156	-.497**	.773**	.608**	.768**	1		
Pak choi Protein	0.227	-.396*	0.235	-0.242	.500**	-0.081	0.112	-.414*	-0.211	-.403*	.814**	.649**	.790**	.786**	1	
Pak choi POD	-0.085	-.428**	-0.234	-.357*	.526**	-0.227	0.006	-.350*	-0.221	-.353*	.849**	.932**	.841**	.705**	.760**	1
Pak choi APEX	-0.057	-0.119	-0.270	-0.301	.349*	-0.049	0.085	-0.277	-0.169	-0.278	.503**	.779**	.486**	.357*	.468**	.801**

	Kale height	Kale yield	Kale Root Length	Kale Root Surf Area	Kale Root Volume	Kale Shoot Length	Kale Shoo Surf Area	Kale chla	Kale chlb	Kale chlt	Kale car	Kale Flavonoid	Kale Phenolics	Kale Sugar	Kale Protein	Kale POD	Kale APEX
Kale GR																	
Kale height	1																
Kale yield	0.179	1															
Kale Root Length	.489**	.472**	1														
Kale Root Surf Area	0.257	0.186	.818**	1													
Kale Root Volume	.400*	-0.140	0.207	0.180	1												
Kale Shoot Length	.610**	.757**	.711**	.473**	0.053	1											
Kale Shoo Surf Area	.569**	.534**	.572**	.502**	0.134	.828**	1										
Kale chla	0.265	.662**	.615**	.428**	0.045	.622**	.372*	1									

Kale chlb	.362*	.693**	.404*	0.073	0.103	.567**	.358*	.821**	1								
Kale chlt	0.326	.709**	.540**	0.272	0.076	.624**	.383*	.959**	.949**	1							
Kale car	0.166	-.363*	-0.163	-0.303	.409*	-0.190	-0.120	-0.124	0.191	0.026	1						
Kale Flavonoid	-0.278	-.595**	-.348*	-0.118	0.222	-.399*	-0.270	-0.309	-.411*	-.374*	.471**	1					
Kale Phenolics	0.104	-.791**	-0.175	-0.103	.352*	-.496**	-.346*	-.379*	-0.270	-.343*	.753**	.635**	1				
Kale Sugar	-0.065	-.341*	-0.052	-0.073	0.216	-0.208	-0.091	-0.081	0.108	0.009	.829**	.357*	.604**	1			
Kale Protein	0.318	-.663**	0.075	0.148	.516**	-0.203	-0.034	-.360*	-.385*	-.390*	.545**	.446**	.737**	.416*	1		
Kale POD	-0.048	-.595**	-0.261	-0.127	0.187	-.344*	-0.219	-.394*	-.346*	-.389*	.594**	.808**	.781**	.437**	.506**	1	
Kale APEX	-.394*	-.555**	-0.148	0.018	-0.133	-.541**	-.572**	-0.033	-0.162	-0.098	.341*	.561**	.637**	.402*	0.181	.515**	1

	Arugula height	Arugula yield	Arugula root Length	Arugula Root Surf Area	Arugula Root Volume	Arugula Shoot Length	Arugula Shoot Surf Area	Arugula chla	Arugula chlb	Arugula chlt	Arugula car	Arugula Flavonoid	Arugula Phenolics	Arugula Sugar	Arugula Protein	Arugula POD	Arugula APEX
Arugula GR																	
Arugula height	1																
Arugula yield	0.207	1															
Arugula root Length	-0.142	0.308	1														
Arugula Root Surf Area	-0.144	0.088	.821**	1													
Arugula Root	0.260	0.124	.538**	.561**	1												

Volume																	
Arugula Shoot Length	0.303	0.073	0.227	0.300	.712**	1											
Arugula Shoot Surf Area	.657**	0.180	0.023	0.194	.594**	.796**	1										
Arugula chla	-.373*	.608**	0.078	-0.112	-.418*	-0.218	-.362*	1									
Arugula chlb	-0.313	.523**	-0.037	-0.121	-0.282	-0.049	-0.189	.902**	1								
Arugula chlt	-.362*	.594**	0.043	-0.117	-.384*	-0.170	-0.315	.991**	.952**	1							
Arugula car	-.363*	-0.171	.411*	.493**	.440**	.489**	0.139	-0.110	-0.032	-0.088	1						
Arugula Flavonoid	-.519**	-.393*	.476**	.638**	.424*	0.166	-0.170	-0.266	-0.232	-0.261	.746**	1					
Arugula Phenolics	-.407*	-.606**	0.156	.494**	.393*	0.287	0.083	-.540**	-.419*	-.513**	.647**	.819**	1				

Arugula Sugar	0.045	-.427**	0.174	0.286	.656**	.398*	0.315	-.666**	-.413*	-.601**	.353*	.472**	.621**	1			
Arugula Protein	0.029	-.422*	0.077	.482**	.474**	.491**	.440**	-.563**	-.447**	-.539**	.474**	.554**	.807**	.504**	1		
Arugula POD	-.329*	-.680**	0.148	.423*	0.214	-0.059	-0.176	-.486**	-.427**	-.478**	.458**	.796**	.765**	.444**	.509**	1	
Arugula APEX	-.590**	-.446**	0.237	.351*	-0.274	-.389*	-.524**	-0.003	-0.123	-0.041	0.280	.536**	.415*	-0.075	0.048	.709**	1

	Swiss Chard height	Swiss Chard yield	Swiss Chard Root Length	Swiss Chard Root Surf Area	Swiss Chard Root Volume	Swiss Chard Shoot Length	Swiss Chard Shoot Surf Area	Swiss Chard chla	Swiss Chard chlb	Swiss Chard chlt	Swiss Chard car	Swiss Chard Flavonoid	Swiss Chard Phenolics	Swiss Chard Sugar	Swiss Chard Protein	Swiss Chard POD	Swiss Chard APEX
Chard height	1																
Chard yield	.728**	1															
Chard Root Length	-0.201	-0.079	1														
Chard Root Surf Area	-.467**	-0.261	.699**	1													
Chard Root Volume	-0.260	-0.169	.776**	.359*	1												
Chard Shoot Length	.897**	.770**	-0.175	-.611**	-0.052	1											
Chard Shoot Surf Area	.840**	.682**	-0.201	-.550**	-0.081	.864**	1										
Chard chla	0.208	.659**	-.414*	-.335*	-0.271	.379*	.367*	1									

Chard chlb	-0.078	0.191	-0.213	-0.131	0.008	0.075	0.053	.581**	1								
Chard chlt	0.162	.608**	-.402*	-0.316	-0.232	.342*	.327*	.985**	.713**	1							
Chard car	-0.319	-0.004	.717**	.780**	.555**	-.347*	-0.226	-0.119	0.120	-0.077	1						
Chard Flavonoid	-.658**	-0.289	.685**	.698**	.714**	-.523**	-.531**	-0.215	0.125	-0.158	.814**	1					
Chard Phenolics	-.503**	-.524**	.366*	.634**	0.162	-.703**	-.495**	-.551**	-0.191	-.515**	.624**	.482**	1				
Chard Sugar	-.682**	-.624**	.398*	.640**	0.119	-.786**	-.697**	-.370*	-0.194	-.360*	0.241	0.317	.549**	1			
Chard Protein	-0.120	0.043	.704**	0.298	.811**	0.062	0.215	-0.098	-0.064	-0.098	.533**	.573**	0.042	0.030	1		
Chard POD	-.448**	-0.051	.500**	.604**	.509**	-.387*	-.376*	-0.029	0.295	0.038	.863**	.886**	.486**	0.059	.376*	1	
Chard APEX	-0.317	-0.145	.386*	.549**	0.306	-.370*	-.423**	-0.276	0.183	-0.198	.729**	.713**	.597**	0.029	0.073	.879**	1