

Effects of Fertigation Practices on Postharvest Quality and Microbial Safety of Microgreens

by

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Abstract

Microgreens are young edible plants harvested at the cotyledon or first true leaf stage. They are valued for their high nutritional content, vibrant color, unique texture, and rapid growth cycle. As demand increases, optimizing fertilization practices to enhance crop quality while maintaining food safety is critical, particularly given that organic and synthetic fertilizers differ in nutrient release, microbial inputs, and potential impacts on microbial persistence. This thesis evaluated the effects of fertilizer type (organic vs. synthetic) on microgreen growth, postharvest quality, and nutritional attributes. Extended storage (up to 21 days) was included to better capture changes in quality and nutritional composition over time. Emphasis was also placed on *Salmonella* survival as a key food safety concern, assessing pathogen persistence under conditions relevant to commercial handling.

In the first experiment, four microgreen species (amaranth, beet, cilantro, and kale) were grown under controlled greenhouse conditions. Two commercially-available liquid fertilization treatments were used: a synthetic fertilizer (Miracle-Gro) and an organic liquid fish emulsion. Growth parameters including fresh yield and hypocotyl length were evaluated at harvest. Postharvest storage was evaluated for up to 21 days at 4 °C, with data collected at 0, 7, 14, and 21 days. Quality and nutritional attributes were assessed by measuring weight loss, color, anthocyanins, phenolics, antioxidant activity, and chlorophyll and carotenoid content. Both microgreen species and fertilizer treatment significantly influenced microgreen growth, yield and quality. Synthetic fertilization generally increased fresh yield and hypocotyl length, although responses differed among species. Kale had the highest fresh yield (1658.92 g·m⁻²), while cilantro

had the lowest ($322.5 \text{ g}\cdot\text{m}^{-2}$). Weight loss increased throughout storage, with kale exhibiting the lowest loss (0.18%). Organic fertilization increased anthocyanin, phenolic, and antioxidant activity levels by 26%, 45%, and 20%, respectively, compared to synthetic fertilization.

In beet microgreens, anthocyanins, phenolics, and antioxidant activity increased by 8%, 14%, and 2%, respectively, during the first 14 days of storage before declining by day 21. Chlorophyll content generally decreased during storage, while carotenoid content remained stable or increased depending on the species.

In the second experiment, the role of organic Earth Juice (plant-based), organic Fish Emulsion (animal-based) and synthetic fertilizer (Miracle-Gro) as potential sources of contamination were evaluated. *Salmonella* (*S.*) *enterica* survival was assessed in kale microgreens grown in controlled conditions under full spectrum light emitting diodes (LED). Fertilizer solutions were inoculated with a two-strain *Salmonella enterica* cocktail and were applied through bottom irrigation after seed germination. The *S. enterica* populations were monitored in grow mats and edible kale microgreen tissues during production. Similarly, populations in kale microgreen tissues were evaluated during postharvest storage at 4 °C on days 0 and 7 (d). *Salmonella* populations were significantly influenced by fertilizer type during production, with higher populations observed in organic fertilizers ($7.2 \text{ log CFU}\cdot\text{g}^{-1}$) compared to synthetic fertilizers ($6.17 \text{ log CFU}\cdot\text{g}^{-1}$). *Salmonella* decreased over time by $0.85 \text{ log CFU}\cdot\text{g}^{-1}$ in grow mats and $1.89 \text{ log CFU}\cdot\text{g}^{-1}$. However, the pathogen persisted throughout production and postharvest storage regardless of fertilizer treatment, indicating that contaminated fertilizer solutions can serve as a potential source of pathogen contamination in

microgreen production systems. Overall, these results demonstrate fertilizer type plays an important role in influencing microgreen quality, growth, nutritional value and food safety.

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In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Microsoft 365 Copilot and Grammarly. These tools were used primarily for grammar checking and language refinement. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

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Table of Contents

Abstract	2
Artificial Intelligence (AI) Use Disclosure Statement	5
Digital Accessibility Disclosure Statement	6
Acknowledgements	7
Table of Contents	9
List of Tables	13
List of Figures	14
List of Abbreviations	17
Chapter I. Literature Review	19
1.1 Introduction	19
1.2 Types of Microgreens	22
1.3 Microgreens: A Nutritional Powerhouse and Superfood	22
1.3.1 Minerals	23
1.3.2 Vitamins	24
1.3.3 Carotenoids	25
1.3.4 Anthocyanins	27
1.4 Production of Microgreens	28
1.5 Growing Media, Sowing Practices and Harvesting	30
1.6 Fertilization in Microgreens	31
1.7 Shelf-life and Quality Changes of Microgreens	34

1.8. Storage Temperature and Microgreen Quality.....	34
1.9. Postharvest Storage and Nutritional Quality	36
1.10 Food Safety Issues in Microgreens.....	37
1.11 Inoculation Methods in Microgreens Food Safety Research.....	38
2. Gaps in Postharvest Research	40
3. Conclusion	41
4. Research Objective(s) and Hypothesis.....	42
References	43
Tables.....	52
Chapter II. Postharvest Shelf-Life and Phytonutritional Quality of Four Microgreen Species Grown Under Different Fertigation Systems	53
Abstract.....	53
1. Introduction	54
2. Materials and Methods	58
2.1 Plant Materials and Growing Conditions.....	58
2.2 Fertilizer Treatments and Application	59
2.3 Mineral Analysis of Liquid Fertilizer and Growing Media	60
2.4 Microgreen Harvest	61
2.5 Postharvest Experimental Design.....	61
2.6 Weight Loss and Colorimeter Assessments	62

2.7 Sample Preparation and Extraction of Total Anthocyanins, Phenolics and Antioxidant Activity.....	62
2.8 Microgreen Preparation for Total Chlorophyll and Total Carotenoid Content	65
2.9 Data Analysis.....	65
3. Results	66
3.1 Fresh yield and Hypocotyl length.....	66
3.2 Weight loss (%) and Colorimeter Assessments	67
3.3 Phytonutrient Content and Total Antioxidant Activity	68
3.4 Chlorophyll and Carotenoid Content Among the Microgreens and Storage Timepoints	70
4. Discussion.....	70
4.1 Fresh Yield and Hypocotyl Length	70
4.2 Weight Loss and Colorimetric Changes.....	72
4.3 Phytonutrient Content and Antioxidant Activity	75
4.4 Chlorophyll and Carotenoid Content.....	78
5. Conclusion	79
References	81
Tables	88
Figures	95
Chapter III. Effect of Fertilization Type on <i>Salmonella enterica</i> Survival in Microgreens During Production and Postharvest.....	102

Abstract.....	102
1. Introduction	103
2. Materials and Methods	106
2.1 Microgreen Production.....	106
2.2 Fertilizer Treatments.....	107
2.3 Preparation of Bacterial Cocktail.....	107
2.4 Fertilizer Inoculum Preparation	109
2.5 Experimental Design.....	109
2.6 Sample Collection and Microbial Analysis	110
2.7 Statistical Analysis	111
3. Results	112
3.1 Survival of <i>S. enterica</i> in Grow Mats During Production	112
3.2 Survival of <i>S. enterica</i> in Microgreens During Production.....	112
3.3 Survival of <i>S. enterica</i> in Microgreens During Postharvest.....	113
4. Discussion.....	113
5. Conclusion	115
References.....	117
Tables	121
Figures	123

List of Tables

Table 1.1. Overview of microgreen plant families, harvest pigmentation and representative species.	52
Table 2.1. Average day and night temperatures and relative humidity (RH) recorded under greenhouse conditions during microgreen production.....	88
Table 2.2. The pH and electrical conductivity (EC) of organic and non-organic growing media used for microgreen production.	89
Table 2.3. Germination and cultivation duration of four microgreen species.....	90
Table 2.4. The pH, electrical conductivity (EC), and macronutrient and micronutrient composition of organic and synthetic liquid fertilizers used during production of four microgreen species.	91
Table 2.5. Soil pH, electrical conductivity (EC) and mineral analysis at the time of microgreen harvest among the four species and two fertilization treatments.	92
Table 2.6. Effect of fertilizer treatments on the color characteristics (L^* , a^* , b^* , C^* , hue) of four microgreen species.	93
Table 2.7. Effect of species x storage timepoint on the color characteristics (L^* , a^* , b^* , Chroma (c^*), hue angle) of four microgreen species.	94
Table 3.1. The pH, electrical conductivity (EC), and macronutrient and micronutrient composition of fertilizer solutions used during kale microgreen.	121
Table 3.2. P-values for the effects of fertilizer type, sampling time, and their interaction on <i>Salmonella enterica</i> populations in grow mats and microgreens during production and in microgreens during postharvest storage.	122

List of Figures

- Figure 2.1** Interaction effects of species and fertilizer treatment on fresh yield ($\text{g}\cdot\text{m}^{-2}$) of four microgreen species. Bars represent means $\pm$ standard error (SE). Different letters indicate significant differences among means according to Tukey's HSD test ($p < 0.05$). 95
- Figure 2.2** Hypocotyl length of microgreens as affected by fertilizer treatment (a), species (b), and the species $\times$ fertilizer type interaction (c) across two production experiments. Bars represent mean hypocotyl length (cm), and error bars indicate standard error of the mean. Different letters denote significant differences among treatments according to Tukey's HSD test ($p < 0.05$). 96
- Figure 2.3** Percentage of weight loss (%) differed in the microgreens across storage days (a) and among the four microgreen species(b). Different letters show significant differences among means according to Tukey's (HSD) test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest. 97
- Figure 2.4** Total anthocyanin content ($\text{g}\cdot 100\text{g}^{-1}$) of amaranth, beet, cilantro, and kale microgreens (a) and the interaction effect of species and storage time (b). Bars represent means $\pm$ standard error (SE). Amaranth microgreens were evaluated only through day 14; therefore, anthocyanin data for amaranth at day 21 are not reported. Different letters indicate significant differences according to Tukey's honest significant difference (HSD) test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest..... 98
- Figure 2.5** Total phenolic content ($\text{g}\cdot\text{kg}^{-1}$) differed among the microgreen storage timepoints (a) and interaction of species $\times$ fertilizer treatment (b). Bars represent means

± standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest..... 99

Figure 2.6 Total Antioxidant Activity ($\text{nM}\cdot\text{g}^{-1}$) differed in the interactions of microgreen species x storage timepoint (a) and species x fertilization treatment (b). Bars represent means ± standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest..... 100

Figure 2.7 Total chlorophyll ($\mu\text{g}\cdot\text{g}^{-1}$) (a) and total carotenoids ($\mu\text{g}\cdot\text{g}^{-1}$) (b) differed in the species x storage timepoint interaction. Bars represent means ± standard error (SE). Different letters indicate significant differences among means according to Tukey's HSD test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest. 101

Fig. 3.1 *Salmonella enterica* populations on grow mats during production, affected by fertilizer treatment (a) and over the production period across sampling days (b), as affected by fertilizer treatment. Bars represent mean *Salmonella* populations ($\log \text{CFU}\cdot\text{g}^{-1}$) ± standard error (SE). Different letters indicate significant differences among means according to Tukey's HSD test ($p < 0.05$). The dashed horizontal line represents the initial inoculum level ($7 \log \text{CFU}\cdot\text{mL}^{-1}$). 123

Fig. 3.2 *Salmonella enterica* populations on kale microgreens during production, shown by fertilizer treatment (a) and over the production period across sampling days (b). Bars represent mean populations ($\log \text{CFU}\cdot\text{g}^{-1}$) ± standard error (SE). Different letters

indicate significant differences among means according to Tukey's HSD test ($p < 0.05$).

The dashed horizontal line represents the initial inoculum level ($7 \log \text{CFU} \cdot \text{mL}^{-1}$). 124

Fig. 3.3 Mean *Salmonella enterica* populations in kale microgreens during postharvest storage. Bars represent mean populations ($\log \text{CFU} \cdot \text{g}^{-1}$) $\pm$ standard error (SE). Means were separated using Student's *t*-test ($p < 0.05$). The dashed horizontal line represents the initial inoculum level ($7 \log \text{CFU} \cdot \text{mL}^{-1}$). Days (d) in cold storage are expressed as 1d and 7d, respectively. 125

List of Abbreviations

a*	Redness/Greenness coordinate (CIELAB color space)
ATCC	American Type Culture Collection
b*	Yellowness/Blueness coordinate (CIELAB color space)
BPW	Buffered Peptone Water
c*	Chroma
Ca	Calcium
CAGR	Compound Annual Growth Rate
CEA	Controlled Environment Agriculture
CDC	Centers for Disease Control and Prevention
CFIA	Canadian Food Inspection Agency
CFU	Colony Forming Units
cm	Centimeter
Cu	Copper
d	Days
°C	Degree Celsius
DPPH	2,2 Diphenyl-1-picrylhydrazyl
EC	Electrical Conductivity
FDA	U.S. Food and Drug Administration
Fe	Iron
FW	Fresh weight
g	Gram
GAE	Gallic Acid Equivalents

h	Hours
ICP-OES	Inductively Coupled Plasma–Optical Emission Spectroscopy
K	Potassium
kg	Kilogram
L*	Lightness (CIELAB color space)
LED	Light Emitting Diode
mL	Milliliter
mg	Milligram
Mn	Manganese
µg	microgram
µmol	Micromole
OMRI	Organic Materials Review Institute
%	Percent
PAL	Phenyl Ammonia Lyase
PBS	Phosphate-Buffered Saline
RCBD	Randomized Complete Block Design
s	Seconds
STEC	Shiga toxin-producing <i>Escherichia coli</i>
TPC	Total Phenolic Content
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
TSBNR	Tryptic Soy Broth supplemented with Nalidixic acid and Rifampicin
UV-B	Ultraviolet-B radiation

Chapter I. Literature Review

1.1 Introduction

Over the last decade, microgreens have emerged as a popular culinary ingredient due to their vibrant colors, distinctive textures, and unique flavors (Pinto et al., 2015). They are classified as young, edible vegetables harvested 7 to 21 days after germination when the cotyledonary leaves are fully developed (Choe et al., 2018; Di Gioia et al., 2017; Trandel-Hayse et al., 2025; Zhang et al., 2021). Several different edible species can be used for microgreen production. This includes vegetable crops belonging to the Brassicaceae (arugula, broccoli, radish, cabbage, kale, mustard), oleaginous (sunflower), leguminous (peas, alfalfa) and herbs such as basil and cilantro (Xiao et al., 2012). Among these, broccoli, lettuce, cabbage, spinach, cilantro, beetroot, mustard, and radish are the most cultivated microgreens (Bhatt and Sharma, 2018).

Microgreens are primarily marketed to high-end grocery stores, restaurants and urban farmers markets (Treadwell et al., 2020). The global microgreen market has grown significantly in recent years. Currently, the Compound Annual Growth Rate (CAGR) is 8.23%. The market size is expected to increase from \$2.86 billion in 2024 to \$5.83 billion by 2033 (Global Growth Insights, 2025). North America leads the microgreen industry, holding approximately 40% of the global market share ($\approx$ \$1.14 US billion), with Canada, Mexico, and the United States contributing significantly due to the expansion of indoor vertical farming systems and rising health consciousness. Europe, the second-largest market, accounts for 30% of the global share ($\approx$ \$0.86 US billion), with France and Germany responsible for over 50% of the region's Brassicaceae microgreen production. Other European countries such as Italy, Spain, the United Kingdom, and the Netherlands are key producers of microgreens like kale, radish, and

arugula. The Asia-Pacific region holds 20% of the market share (\$0.57 US billion), with Japan emerging as a key player due to the integration of microgreens into urban agriculture, health foods, and convenience retail sectors. Lastly, the Middle East and Africa contribute 10% of the global microgreens market ($\approx$ \$0.29 US billion). Countries such as South Africa, Kenya, and the United Arab Emirates have increased in hydroponic and greenhouse-based microgreen production (Microgreen Market, 2024; Global Growth Insights, 2025).

Microgreens are commonly consumed raw, often used as garnishes for salads, sandwiches, and soups, or incorporated as key ingredients in both sweet and savory dishes (Choe et al., 2018; Renna et al., 2017). Consuming them fresh helps preserve their chemical composition, ensuring that consumers receive the maximum nutritional and health benefits (Sharma et al., 2022). These young greens are recognized for their superior nutritional profiles, particularly in vitamins, minerals, anthocyanins, phenolics, and carotenoids, when compared to their mature plant counterparts (Xiao et al., 2012; Yadav et al., 2019). The intake of these bioactive phytonutrients has been associated with a range of health benefits, including improved cardiovascular and eye health, better blood sugar regulation, enhanced cognitive function, and a reduced risk of certain cancers (Zhang et al., 2021).

Many microgreen growers have shifted to indoor production since it offers the ability to grow crops year-round with fewer pathogen issues and better control over irrigation, temperature and radiation levels (Avercheva et al., 2009; Kyriacou et al., 2016). These young crops can be cultivated using traditional soil substrates or alternative inert media, such as peat moss, coconut coir, perlite, vermiculite, rockwool,

and synthetic fibrous materials like Sure-to-Grow (Di Gioia et al., 2017; Kyriacou et al., 2016; Muchjajib et al., 2015). They are commonly fertilized using synthetic fertilizers such as urea, ammonium nitrate, and calcium nitrate (Murthy and Pill, 2010), while organic fertilizers are rarely used. Microbial processes must convert organic nitrogen into forms that plants can absorb, but this conversion is often too slow for the microgreens short production cycle (Gaskell et al., 2007). Moreover, organic fertilizers might increase the risk of pathogen contamination such as *Salmonella* and *E. coli* (Mukherjee et al., 2004).

Numerous studies have demonstrated the influence of controlled environment agriculture and light quality on the growth and nutritional composition of microgreens at harvest (Meas et al., 2020; Ying et al., 2021; Kamal et al., 2020; Ying et al., 2020). However, relatively few investigations have explored the quality and nutritional degradation of microgreens during cold storage periods (e.g., 21 to 28 days). Due to the delicate and immature tissue structure of microgreens, they are considered to have a very short shelf-life (e.g., 10 days) (Mir et al., 2017). Additionally, there is a lack of comparative data on how different production systems and fertilization methods affect the postharvest shelf life of microgreens and the risk of food-borne pathogen contamination. This literature review seeks to address the impact of various cultivation techniques and fertilization strategies on the long-term nutritional quality, shelf life, and sustainability of microgreens. This review will also address how common food borne pathogens enter and persistent in microgreens from production through postharvest handling and storage.

1.2 Types of Microgreens

Microgreens can be cultivated from a diverse range of plant families, each offering unique flavors, colors, growth characteristics, and nutritional profiles. Common botanical families used in microgreen production include Brassicaceae (e.g., broccoli), Asteraceae (e.g., lettuce), Apiaceae (e.g., carrot), Amaryllidaceae (e.g., onion), Amaranthaceae (e.g., amaranth), and Cucurbitaceae (e.g., melon). In addition to these, non-vegetable species such as cereals (e.g., wheat), legumes (e.g., chickpeas), oilseed crops (e.g., sunflower), and aromatic herbs (e.g., basil, cilantro, fennel) are also widely cultivated for microgreen production (Renna et al., 2017). Commonly cultivated microgreens along with their botanical classification and pigmentation traits are summarized in **Table 1.1** (Adapted from Xiao et al., 2012).

1.3 Microgreens: A Nutritional Powerhouse and Superfood

Microgreens are recognized as a “next-generation superfood” due to their abundant content of health-promoting phytonutrients, including minerals, vitamins, antioxidants, and other bioactive compounds (Bhaswant et al., 2023). Anthocyanins and carotenoids are natural pigments found in microgreens and are related to antioxidant activity and free radical scavenging (Qian et al., 2016). Microgreens exhibit several advantages over mature plants as they contain 4 to 40 times more vitamins and antioxidants (Xiao et al., 2012). Research on the nutritional content of microgreens at harvest has expanded over recent years and other secondary metabolites including glucosinolates, flavonoids and volatile compounds have been quantified (Kyriacou et al., 2016; Xiao et al., 2012; Di Gioia et al., 2023; Teng et al., 2023; Trandel-Hayse et al.,

2025). These bioactive compounds vary significantly by species and growing conditions and contribute to the health-promoting potential of microgreens.

1.3.1 Minerals

Several studies have examined the macro and micro-mineral composition across different microgreen species (Di Gioia et al., 2023; Singh et al., 2023; Xiao et al., 2016). A recent study by Di Gioia et al. (2023) analyzed 17 microgreen species grown in a soilless system under controlled greenhouse conditions, using a nutrient solution without added micronutrients. While Singh et al. (2023) analyzed minerals across 20 celery microgreens and mature plant genotypes. Both studies indicated that nitrogen and potassium were the most abundant macronutrients. These were followed by calcium, phosphorus, sulfur, magnesium, and sodium (Di Gioia et al., 2023; Singh et al., 2023). Di Gioia specifically identified that scallion, red cabbage, amaranth, and Genovese basil had the highest content of calcium, while amaranth was the only species particularly rich in magnesium. Regarding micronutrient composition, iron (Fe) was the most abundant element among all species followed by zinc (Zn), manganese (Mn), boron (B), and copper (Cu). Similarly, Xiao et al. (2016) evaluated the mineral content of 30 microgreen species within the Brassicaceae family. Potassium was the most abundant macronutrient, followed by phosphorus, Ca, Mg, and sodium (Na). Among micronutrients, Fe was the highest, with notable levels of Zn, Mn, and Cu. These findings highlight substantial genotypic variation in both macro- and micronutrient concentrations among microgreen species. This suggests that specific microgreens can be selected or bred for their richness in health-promoting minerals.

1.3.2 Vitamins

Microgreens are also highly abundant in several vitamins including C, E and K (Alloggia et al., 2025; Ghoola et al., 2020; Xiao et al., 2015; Yadav et al., 2019). Vitamin C is the most abundant in microgreens and plays a crucial role in strengthening the immune system, acting as a powerful antioxidant, and is essential for bone formation and wound healing (Chambial et al. 2013). Previous research on nine microgreen species indicated radish and poi had the highest content at $52.31 \text{ mg} \cdot 100\text{g}^{-1}$ fresh weight (FW) and $46.50 \text{ mg} \cdot 100\text{g}^{-1}$ FW, respectively (Yadav et al., 2019). While palak and bottle gourd had the lowest at $6 \text{ mg} \cdot 100\text{g}^{-1}$ FW. This study also indicated microgreens had 10 to $17.45 \text{ mg} \cdot 100\text{g}^{-1}$ FW more vitamin C compared to mature plants. Alloggia et al. (2025) investigated the effects of two substrates (perlite and a commercial mixture of coconut fiber, perlite, and peat moss) and four fertigation treatments (distilled water, tap water, a half-strength Hoagland nutrient solution, and nutrient solution enriched with sulfur) on vitamin C accumulation in four *Brassica* microgreens. The study found red cabbage ($54.86 \text{ mg} \cdot 100\text{g}^{-1}$ FW) and radish ($49.01 \text{ mg} \cdot 100\text{g}^{-1}$ FW) had the highest vitamin C content, while mustard had the lowest ($30.78 \text{ mg} \cdot 100\text{g}^{-1}$ FW). Microgreens grown on perlite showed slightly higher vitamin C levels ($45.33 \text{ mg} \cdot 100\text{g}^{-1}$ FW) than those grown on a coconut fiber-based commercial mix ($41.36 \text{ mg} \cdot 100\text{g}^{-1}$ FW). Among fertigation treatments, distilled water led to the greatest vitamin C concentration ($49.77 \text{ mg} \cdot 100\text{g}^{-1}$ FW), whereas nutrient-enriched solutions yielded lower levels (approximately $39.7\text{--}39.9 \text{ mg} \cdot 100\text{g}^{-1}$ FW). These findings highlight not only the species-dependent variability in vitamin C content but also the influence of growing conditions, such as substrate and nutrient availability, on the nutritional quality of microgreens.

Vitamin E (α -tocopherol) plays an important role in the prevention and treatment of heart disease, cancer, and Alzheimer's disease due to its antioxidant properties (Tucker and Townsend, 2005). In a study by Ghoola et al. (2020), ten microgreen species were examined, including vegetable (spinach, radish, roselle, and onion), leguminous (fenugreek), oleaginous (sunflower), and aromatic species (French basil and fennel). Among them, radish microgreens contained the highest level of α -tocopherol, with $58.6 \text{ mg} \cdot 100\text{g}^{-1}$. Other microgreens like sunflower, mustard, and fennel also showed significant amounts of α -tocopherol, while fenugreek microgreens had the lowest at $5.0 \text{ mg} \cdot 100\text{g}^{-1}$.

Vitamin K (phylloquinone) is essential for blood clotting and has been linked to bone health, reduced vascular calcification, cancer prevention, improved wound healing, immune regulation, and hormone production (Fakhree et al., 2021). Xiao et al. (2015) evaluated the phylloquinone content in six microgreen species and reported values ranging from 2.1 to $4.0 \mu\text{g} \cdot \text{g}^{-1} \text{ FW}$. Among the tested species, opal basil exhibited the highest concentration, while bull's blood beet had the lowest.

1.3.3 Carotenoids

Carotenoids are the naturally occurring lipophilic pigments produced by plants and microorganisms, including fungi, bacteria, and algae (El-Agamey et al., 2004; Milani et al., 2017). Total carotenoids play a key role in strengthening the immune system, reducing the risk of cancer, and neutralizing harmful free radicals (Brazaitytė et al., 2015). The carotenoids most abundantly found in microgreens include β -carotene, lutein, violaxanthin, and neoxanthin (Perera and Yen, 2007; Trandel-Hayse et al., 2025).

β -Carotene is a red-orange pigment found in plants and fruits that serves as a source of provitamin A (Gul et al., 2015). It is a strong antioxidant that helps to improve the immune system and lowers the risk of oxidative stress, cancer, metabolic disorders, cardiovascular diseases, and eye conditions (Tufail et al., 2024). Lutein and zeaxanthin are often green to orange in color and are the important components of the macular pigment of the eye and delay the development of macular degeneration and cataracts (Eggersdorfer and Wyss, 2018).

In a study conducted by Xiao et al. (2012), 25 commercially available microgreens were analyzed for β -carotene, lutein and zeaxanthin content with significant variation found. Concentrations of β -carotene ranged from 0.6 to 12.1 mg·100 g⁻¹ FW, with red sorrel exhibiting the highest concentration (12.1 mg·100 g⁻¹ FW), followed by cilantro (11.7 mg·100 g⁻¹ FW), red cabbage (11.5 mg·100 g⁻¹ FW), and pepper cress (11.1 mg·100 g⁻¹ FW). In contrast, golden pea tendrils and popcorn shoots recorded the lowest levels, averaging around 0.6 mg·100 g⁻¹ FW. Lutein and zeaxanthin concentrations varied between 1.3 and 10.1 mg·100 g⁻¹ FW, with cilantro showing the highest levels of lutein/zeaxanthin (10.1 mg·100 g⁻¹ FW), followed by red sorrel (8.8 mg·100 g⁻¹ FW), red cabbage (8.6 mg·100 g⁻¹ FW), and garnet amaranth (8.4 mg·100 g⁻¹ FW).

In a recent study, Trandel-Hayse et al. (2025) evaluated the effects of various red, green and blue LED light ratios on the phytochemical composition of *Amaranthus cruentus* microgreens. The results showed that 70red:10green:20blue (70R:10G:20B; 70:10:20) LED lighting significantly enhanced carotenoid accumulation compared to ambient light and white LED light. Total carotenoid content increased from 0.33 mg·g⁻¹

under ambient light to $0.52 \text{ mg} \cdot \text{g}^{-1}$ under 70R:10G:20B lighting. Lutein concentration rose from $117.28 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ to $137.36 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ (a 17.1% increase), while β -carotene increased from $118.93 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ to $120.72 \text{ } \mu\text{g} \cdot \text{g}^{-1}$. Canthaxanthin levels more than doubled, rising from $0.53 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ under white LED to $1.09 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ under 70R:10G:20B.

1.3.4 Anthocyanins

Anthocyanins are naturally occurring flavonoid pigments that impart blue, purple, red, and orange coloration to a variety of fruits and vegetables (de Pascual-Teresa and Sanchez-Ballesta, 2008). Anthocyanins are recognized for their significant health benefits, primarily attributed to their potent antioxidant activity. These compounds have been shown to provide protective effects against a range of chronic conditions, including cardiovascular diseases, certain types of cancer, diabetes, as well as visual impairments, liver dysfunction, and damage induced by UV-B radiation (Oancea and Oprean, 2011).

Total anthocyanin content in microgreens has been widely studied at harvest using the DPPH free radical scavenging assay across various plant families, including Amaranthaceae, Brassicaceae, and Chenopodiaceae (Trandel-Hayse et al., 2025; Gunjal et al., 2024; Quin et al., 2021). Gunjal et al. (2024) evaluated seven microgreen species grown in either sandy loam soil and cocopeat media and found significant variation in total anthocyanin levels. Concentrations ranged from 2.04 to $45.87 \text{ } \mu\text{mol} \cdot 100 \text{ g}^{-1}$ fresh weight, with radish microgreens grown in cocopeat showing the highest levels and flax microgreens the lowest. These findings underscore the influence of growing media on phytonutrient accumulation. Similarly, Trandel-Hayse et al.

(2025) reported that amaranth microgreens grown under specific LED treatments (70R:10G:20B and 80R:20B) had the highest anthocyanin content (13.42 and 12.88 mg·L⁻¹, respectively), compared to those grown under white LED or ambient light. Despite these insights, limited information is available on the specific anthocyanin compounds present and how they vary across microgreen species at harvest and through postharvest storage.

1.4 Production of Microgreens

Microgreens can be cultivated in both outdoor fields and controlled environments, such as container production and large warehouses via vertical farms, greenhouses and high tunnels (Ying et al., 2021; Zhang et al., 2021). Microgreens thrive in cooler production temperatures, with optimal yields and quality observed when grown in environments ranging from 20–22 °C (Murphy et al., 2010). Due to the extreme and often unpredictable conditions of outdoor field production, indoor and controlled environment agriculture (CEA) has become the preferred method for microgreen cultivation. These systems enable continuous, year-round production by precisely regulating key environmental factors such as temperature, humidity, lighting, and irrigation. (Ebert, 2022; Zhang et al., 2021; Riggio et al., 2019; Santosh and Shukla 2024).

Vertical farming is the most used CEA system for commercial-scale leafy green and microgreen production. It involves growing plants in vertically stacked layers, allowing for efficient use of space and resources (Budavári et al., 2024). It offers better control of light, temperature, and humidity, leading to higher-quality crops with reduced use of water and fertilizers (Silva et al., 2024). In vertical farming, LED lighting is mostly preferred.

Greenhouses are another common method and are typically suitable for medium to large-scale producers (Brazaitytė et al., 2017). They utilize either natural or supplemental artificial lighting to maintain optimal environmental conditions for horticultural crop production (Shamshiri et al., 2018). Common types of artificial lighting used in greenhouses include high-pressure sodium (HPS) lamps and LED lights (van Iersel and Gianino, 2017). Greenhouses enhance predictability by maintaining a controlled environment and minimizing the effects of external weather conditions, such as temperature and rainfall, while also reducing production costs and improving overall crop yields (Shamshiri et al., 2018). In greenhouse systems, microgreens are commonly cultivated in sterile, loose, and soilless growing media to minimize contamination and promote effective seedling development. In this method, trays are partially filled with the growing medium to a depth of ½ inch to 1 or 2 inches, depending on the specific irrigation regime (Treadwell et al., 2020). Common growing media used in greenhouse microgreen production include recycled fibrous materials, coconut coir, vermicompost (Muchjajib et al., 2015; Di Gioia et al., 2017) and sterilized soil (Xiao et al., 2012).

High tunnels, which are hoop house structures covered with polyethylene film, are increasingly used for seasonal microgreen production. These passive systems operate without the need for electricity-based heating or cooling, offering a low-cost alternative for small to medium-scale growers. Common growing media in high tunnel vegetable systems include peat-based mixes, coir, pine bark, and sand due to their good drainage and aeration (Agehara et al., 2023). Irrigation in these structures is commonly delivered through low-pressure systems such as drip tape, emitters, or

soaker hoses, which efficiently provide water directly to the root zone while minimizing water loss and runoff (Rader and Karlsson, 2006).

1.5 Growing Media, Sowing Practices and Harvesting

The selection of appropriate growing media is essential for microgreens cultivation, as it significantly influences their growth, yield, and nutritional composition (Sukewijaya et al., 2025). Traditionally, peat and peat-based media have been the most widely used substrates for microgreen production (Kyriacou et al., 2016). However, peat can be expensive, especially in countries lacking peat moss resources (Abad et al., 2001). As a result, coconut coir dust, sugarcane filter cake, and vermicompost have been identified as alternatives to peat for growing microgreens (Muchjajib et al., 2015). Gunjal et al. (2024) reported that cocopeat is a highly suitable medium for microgreens, offering improved productivity and better nutritional and bioactive properties compared to synthetic substrates.

A study by Di Gioia et al. (2017) on Rapini microgreens involved cultivation using cost-effective and renewable substrates such as recycled textile fiber and jute kenaf fiber. These were compared with non-renewable, expensive substrates like peat and synthetic mats (Sure to Grow, Beachwood, OH, USA). The results showed that microgreens grown on textile and jute fibers had higher yields and lower nitrate content, indicating their potential as suitable alternatives to peat and synthetic mats.

Microgreens are sown either in rows or using the broadcast method. Dense seeding is often used to increase yield; but excessive crowding can result in elongated stems and a greater risk of disease (Mir et al., 2017). To help growers achieve uniform and efficient microgreens production, Penn State Extension developed a microgreens

seed density calculator, which considers factors such as tray dimensions, seed size, and germination percentage to promote efficient, uniform planting (Penn State Extension, 2020). Pre-treating seeds with hydrogen chloride, sodium hypochlorite, or hydrogen peroxide can improve microgreen yields (Lee et al., 2004). Murphy and Pill (2010) demonstrated that arugula seeds pregerminated for 24 hours at 20 °C in fine-grade exfoliated vermiculite moistened to 200% of its dry weight, and then sown as a vermiculite–seed mixture, produced a 21% increase in shoot fresh weight at 14 days after planting compared to non-pregerminated seeds.

Microgreens are typically harvested at an early growth stage, once the cotyledons have fully emerged and one or two true leaves have developed on the stem before the plants reach full maturity (Turner et al., 2020). Harvesting is commonly preferred in the morning, as seedlings exhibit lower respiration rates and retain higher moisture content during this period (Saini et al., 2017). The harvest time may vary depending on the species. For example, Swiss chard and rocket are usually ready for harvest around 17 days after sowing, whereas basil requires a longer crop cycle of approximately 27 days (Bulgari et al., 2017). Despite general recommendations, there is currently no published data that specifically investigates the importance of harvest timing on microgreen quality or shelf-life, indicating a key gap in the existing literature.

1.6 Fertilization in Microgreens

Fertilizers, organic or synthetic, serve as a primary source of nutrients for plants, supporting their growth and physiological development (Choe et al., 2018). Synthetic fertilizers are composed of synthetic chemical compounds such as urea, anhydrous ammonia, potassium chloride, and triple superphosphate (Hazra, 2016). Organic

fertilizers are a type of fertilizer that typically comes from animal waste, human excreta, or plant-based materials such as compost and manure. One key advantage of organic fertilizers is their slow nutrient release compared to chemical fertilizers, which can benefit long-term soil health and plant development (Assefa & Tadesse, 2019). Another important benefit is the growing global demand for organic-certified products, driven by increasing concerns over the potential impacts of synthetic agricultural practices on human health, food safety, and environmental sustainability (Gregory, 2000; Schifferstein & Oude Ophuis, 1998). Additionally, many consumers believe that organic foods offer superior taste, improved nutritional quality, and greater freshness which strongly influences consumer purchasing decisions (Yiridoe et al., 2005).

Synthetic fertilizers are widely used in the microgreen industry. They are commonly applied either as a media pre-fertilization or a post-emergence solution fertilization. Murphy and Pill (2010) studied the effects of various nitrogen fertilizers on arugula (*Eruca vesicaria* subsp *sativa*) microgreens. They evaluated calcium nitrate, ammonium nitrate, and urea in both solid and liquid forms, applied to the growing medium at concentrations of 500, 1,000, 2,000, or 4,000 mg N·L⁻¹ before sowing. Liquid fertilizers produced higher fresh weight per plant compared to solid forms, likely due to more uniform distribution and faster plant uptake. Among the treatments, calcium nitrate led to the highest fresh weight, followed by ammonium nitrate and urea.

They also evaluated the effects of combining pre-germinated and non-germinated seeds with either pre-plant incorporation of solid calcium nitrate at 2,000 mg N·L⁻¹ or daily application of a 21-2.2-16.6 (N-P-K) fertilizer solution at 150 mg N·L⁻¹. They recommended that daily fertigation with 150 mg N·L⁻¹, or daily fertigation with 75

mg N·L⁻¹ combined with pre-plant incorporation of 1,000 mg N·L⁻¹ from calcium nitrate (Ca (NO₃)₂), can increase the shoot fresh weight of arugula microgreens (Murphy and Pill, 2010).

Organic fertilization can be applied in two primary forms: solid and liquid. Common commercial solid organic fertilizers include Chilean nitrate, blood meal, fish meal, seabird guano, bone meal, soybean meal, alfalfa meal, pelleted chicken manure, soft rock phosphate, and potassium magnesium sulfate (Gaskell et al. 2007). Liquid organic fertilizers are typically derived from plant and animal residues, such as fish and seaweed emulsions, hydrolyzed fish products, and extracts from oilseeds (Burnett et al., 2016). Compared to solid fertilizers, liquid organic fertilizers release nutrients more quickly, making them more suitable for crops with shorter growth cycles such as microgreens (Gunawan et al., 2021).

To date, no studies have specifically evaluated the use of organic-certified fertilizers in microgreen production. Though, related research has been conducted on other leafy greens. For example, Shaik et al. (2022) compared six OMRI-approved organic fertilizers with a synthetic inorganic fertilizer and a no-fertilizer control in lettuce (*Lactuca sativa* cv. Rex). Their results showed the inorganic fertilizer produced the highest growth, fresh and dry biomass, and leaf area. Among the organic treatments, the fish-based fertilizer (OF1, Alaska Fish Fertilizer) and the hybrid fish- and plant-based fertilizers (OF2 and OF6) had plant yields most comparable to the synthetic fertilizer. These findings suggest certain organic formulations may offer viable alternatives to synthetic inputs, depending on crop type and production goals, and may be suitable for microgreen production.

1.7 Shelf-life and Quality Changes of Microgreens

The shelf-life of fruits and vegetables is typically assessed by their visual quality, such as freshness, color, and lack of decay or disorders, as well as by textural characteristics like firmness, juiciness, and crispness (Zavala et al., 2004). In the case of microgreens, harvesting at the cotyledon stage interrupts their growth prematurely, resulting in limited shelf-life (Kou et al., 2014). Under ambient storage conditions, the typical shelf-life of microgreens ranges from 1 to 3 days (Mir et al., 2017). Shelf-life of freshly harvested produce such as microgreens, is influenced by various parameters, including storage temperature, relative humidity, type of packaging material, initial microbial load, filling weight in the container, and volume-to-surface ratio (Chandra et al., 2012). Among these, storage temperature and atmospheric composition are the most critical factors in determining postharvest shelf-life (Hodges and Toivonen, 2008).

1.8. Storage Temperature and Microgreen Quality

Storage temperature is a key factor in preserving the postharvest quality and shelflife of microgreens. Temperatures between 1 and 5 °C are generally effective in slowing respiration, delaying tissue aging, and reducing microbial activity that leads to spoilage (Dayarathna et al., 2023). However, the optimal temperature can vary depending on the species. For example, Kou et al. (2013) found that 5 °C was the most suitable temperature for maintaining quality in buckwheat microgreens over a 21- day storage period. Similarly, Patil et al. (2024) reported broccoli microgreens stored at 5 °C retained better nutritional and physiological quality than those stored at 1 °C. Broccoli stored at 5 °C exhibited lower weight loss (12.84%) and better color retention, while samples stored at 1 °C experienced greater weight loss (20.17%) and increased

yellowing. In contrast, Xiao et al. (2014) demonstrated that 1 °C was most effective for daikon radish microgreens, helping preserve chlorophyll content, minimize off-odor development, and suppress microbial growth without causing chilling injury. These findings suggest most microgreens benefit from cooler storage temperatures around 4 to 5 °C, some species-specific sensitivity to chilling injury must be considered when determining optimal postharvest conditions.

Warm storage temperatures also influence the physiological and visual quality of microgreens. Dayarathna et al. (2023) evaluated mustard microgreens stored at 5, 10, 15, 20, and 25 °C for up to 14 days and found chlorophyll degradation occurred across all treatments, with the slowest decline at 5 °C. This indicates cooler temperatures effectively delay pigment loss and senescence. Weight loss also increased with both temperature and storage duration, with microgreens stored at 5 °C and 10 °C showing relatively low moisture loss (4.65% and 5.65%, respectively), while those stored at higher temperatures exhibited significantly greater moisture loss.

Visual quality, particularly color, is a key indicator of freshness and consumer appeal. Komerowski et al. (2024) examined the color stability of arugula microgreens over a 10-day storage period under different packaging systems. Declines in lightness (L^*), greenness (a^*), and yellowness (b^*) were observed across treatments, reflecting chlorophyll degradation and senescence. Modified atmosphere packaging (MAP) coupled with cold storage was most effective in preserving visual quality, maintaining green pigmentation and overall appearance for up to 7 days. Despite its benefits for color retention, MAP-treated microgreens experienced the highest cumulative weight loss - approximately 24% by day 10 - compared to other packaging methods. This

highlights the trade-off between maintaining visual appeal and minimizing moisture loss during storage.

1.9. Postharvest Storage and Nutritional Quality

Postharvest storage conditions can significantly impact the levels of anthocyanins, phenolic compounds, and the antioxidant capacity in fruits and vegetables. Mudau et al. (2015) highlighted the significant influence of both storage temperature and duration on the nutritional stability of baby spinach. At 4 °C, carotenoid levels remained high for up to six days, and phenolic content declined gradually. In contrast, samples stored at 22 °C showed a sharp decline in carotenoids after just two days and in phenolics after four days, dropping from 3.8 to 0.45 mg·g⁻¹ FW. Similar patterns were observed for antioxidant activity and vitamin C: both remained relatively stable under cold storage but declined rapidly at ambient temperature after two days. The study confirms that lower temperatures help preserve the nutritional quality of leafy vegetables during early postharvest storage, with 4 °C storage maintaining higher levels of bioactive compounds compared to 22 °C.

Chlorophyll and carotenoid dynamics during storage have also been studied in microgreens. Niroula et al. (2019) found that chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in wheat and barley microgreens increased until day 16. Peak levels were recorded between days 7 to 10 in wheat and between days 10 to 13 in barley, suggesting species-specific variations in postharvest pigment development.

In addition to storage effects, production methods can also influence nutritional quality. Weber (2016), for example, compared cabbage and lettuce microgreens grown

on hydroponic pads versus vermicompost and found that those cultivated in vermicompost had higher nutrient content. Similarly, Tan et al. (2020) investigated the antioxidant properties and sensory attributes of microgreens produced in hydroponic (commercial) and soil-based (local farm) systems. Their results indicated that soil-grown microgreens contained significantly higher levels of vitamin C compared to those produced hydroponically.

1.10 Food Safety Issues in Microgreens

Food safety plays a critical role in protecting public health and sustaining life, as safe and nutritious food helps prevent illnesses caused by harmful bacteria, viruses, parasites, or chemical substances (WHO, 2024). With the growing interest in fresh, nutrient-rich produce, microgreens have become increasingly popular both in restaurants and among health-conscious consumers. Since they are typically consumed raw and undergo minimal postharvest processing, they are considered a high-risk category for foodborne illnesses like sprouts and leafy greens (Chen et al., 2023; Riggio et al., 2019).

The most common pathogens linked to fresh produce include *Salmonella enterica*, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* (Bennett et al., 2018). Of these, *Salmonella* is the most common, accounting for 53% of outbreaks in the United States and 50% in the European Union (Sivapalasingam et al., 2004). Common produce items linked to *Salmonella* infections include salad greens, sprouts, and melons, while *E. coli* O157:H7 outbreaks have frequently been traced back to lettuce and unpasteurized juices (Sivapalasingam et al., 2004).

A recent outbreak highlights the continued risk of *Salmonella* in sprouted and minimally processed foods. In July 2025, the U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) reported a *Salmonella Anatum* outbreak linked to Deep-brand frozen sprouted mat (moth) and moong (mung) beans. Routine testing in May 2025 confirmed contamination, and whole genome sequencing matched the strain to that found in infected individuals. Eleven cases were reported across ten U.S. states, with four hospitalizations and no deaths. This incident reinforces the need for strict safety controls in similar ready-to-eat products, including microgreens (FDA, 2025)

Although no major outbreaks have been directly linked to microgreens, several product recalls have been reported by regulatory agencies such as the Canadian Food Inspection Agency (CFIA) and the U.S. Food and Drug Administration (FDA) (CFIA, 2018; FDA, 2017). These recalls have primarily involved contamination with the pathogens *Listeria monocytogenes* and *Salmonella* (Xavier et al., 2025). The microgreens most frequently associated with recalls include broccoli, arugula, radish (including daikon), pea shoots, sunflower, spicy microgreen mixes, coriander, alfalfa, onion, cilantro, and various mixed microgreen products (Xavier et al., 2025). For example, in December 2022, the U.S. Food and Drug Administration (FDA) announced a voluntary recall by Wegmans Food Markets due to the potential contamination of their organic microgreens, sweet pea leaves, and cat grass products with *Salmonella* (FDA, 2022).

1.11 Inoculation Methods in Microgreens Food Safety Research

Seed inoculation is a common method used to study microbial contamination in microgreens. Typically, seeds are inoculated with the pathogen such as *E. coli* O157:H7

or *E. coli* O104:H4 strains by soaking them in bacterial suspensions at low and high concentrations (Xiao et al., 2014). The inoculated seeds are then air-dried and stored before being used for both sprouting and microgreen production. Microgreens can be grown on peat-based or soil-based media under controlled conditions including hydroponic systems. Pathogen sampling occurs on day 0 and after 7 days of production (Xiao et al., 2014; Xiao et al., 2015). Pathogen levels can be assessed using selective media and confirmed by Green Fluorescent Protein markers.

Although irrigation water is a recognized route of contamination in commercial production, it has been less frequently researched. In a more recent study, five rifampicin-resistant *Salmonella enterica* serotypes and five nalidixic acid-resistant EHEC strains were used to contaminate Daikon radish microgreens. These bacterial strains were originally isolated from leafy green outbreak cases (Chen et al., 2023). In this setup, non-inoculated seeds were cultivated on three substrates (soil-compost mix, vermiculite-perlite, and Biostrate pads) and irrigated with water spiked with either a high (5 log CFU·ml⁻¹) or low (3 log CFU·ml⁻¹) pathogen cocktail. Irrigation water was applied initially and flood-irrigated every three days thereafter under controlled conditions (Chen et al., 2023).

Soil media inoculation is another method used to evaluate pathogen survival and translocation in microgreens. A study conducted by Misra (2020) applied *Salmonella enterica* and *Listeria monocytogenes* directly to peat/vermiculite and Biostrate substrates using a high inoculum (10⁶ CFU·mL⁻¹) before sowing seeds. The study showed that pathogen persistence varied by substrate type, with organic media

supporting higher survival rates than inert materials, highlighting the importance of growing media in food safety research.

2. Gaps in Postharvest Research

Most postharvest studies on microgreens have primarily focused on extending shelf-life through packaging and handling techniques such as modified atmosphere packaging (MAP), washing, and sanitizing (Kim et al., 2004; Xiao et al., 2014; Kou et al., 2013; Dayarathna et al., 2023). Significant knowledge gaps on species specific storage guidelines persist on microgreens. Cooler storage temperatures were noted to preserve microgreen quality (Dayarathna et al., 2023; Kou et al., 2013; Patil et al., 2024). Thus, identifying optimal temperature conditions and shelf-life variability (e.g., 7, 14, or 21 days in storage) among species is critical to decrease loss and increase consumer acceptance. Many studies have assessed the nutritional quality of microgreens at harvest (Di Gioia et al., 2023; Trandel-Hayse et al., 2025; Xiao et al., 2014). Yet, there is limited data on how nutritional compounds such as antioxidants, vitamins and other phenolic compounds degrade during storage and across different microgreen species. Previous research also indicated growing media and the use of organic or synthetic fertilization does affect nutrient content, but how these influence postharvest stability is underexplored.

The aforementioned food safety findings indicated that multiple contamination routes are relevant in microgreen production, each with distinct implications for pathogen survival and transfer (Chen et al., 2023; Xiao et al., 2014; Xiao et al., 2015). Substrate type also plays a critical role in pathogen persistence, with the organic materials posing a higher risk for contamination (Misra, 2020). Still, few studies have

systematically examined the survival, internalization, and translocation of key foodborne pathogens such as *Salmonella enterica*, *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* within different microgreen species and production systems (Xiao et al., 2015; Chen et al., 2023). These findings underscore the need for comprehensive risk assessments in microgreen production systems to enhance our understanding of food safety.

3. Conclusion

Microgreens are gaining popularity for their nutritional benefits and culinary uses. This review focused on key aspects of microgreen production, including growing methods in greenhouse and hydroponic systems, as well as fertilization techniques. There are significant gaps in the current research, particularly concerning postharvest quality and shelf-life. While most of the research has concentrated on optimizing growth and yield, there is a lack of detailed studies on how production practices, fertilization methods, and harvest timing affect the long-term nutritional quality and shelf-life of microgreens. Addressing these gaps through further research will provide valuable insights into how to enhance the overall sustainability and nutritional value of microgreens. Additionally, food safety concerns, including microbial contamination, must be addressed to ensure the safe consumption of microgreens. As microgreens are often eaten raw, it is crucial to understand the potential risks from pathogens like *E. coli*, *Salmonella*, and *Listeria*. Further research is needed to develop standardized protocols for safe production and handling, ensuring that microgreens are both nutritious and safe for consumers.

4. Research Objective(s) and Hypothesis

The main goal of this research is to compare how different fertilization regimes affect the shelf-life, quality and food safety risks of microgreen. The specific objectives are listed below:

The first objective of this study was to evaluate the effects of fertigation type (organic and synthetic) on the postharvest shelf-life of four commercially grown microgreen species (amaranth, beet, cilantro and kale). Additionally, this study also aimed to determine how the fertigation type influences phytochemical composition during refrigerated storage at 4 °C and evaluated at harvest and after 7, 14, and 21 days of storage. It was hypothesized that microgreens grown with organic fertilizers will have a short shelf-life but higher nutritional quality due to nutrient stress/slower nutrient availability, which activates defense mechanisms, leading to increased production of antioxidants, vitamins and secondary metabolites.

The second objective of this study was to evaluate the effects of synthetic and organic based fertigation on *Salmonella enterica* survival in substrate mats and during production of kale microgreens. The study further evaluated the persistence of *Salmonella enterica* on harvested kale during 7 days of postharvest storage when produced using different fertigation treatments. It was hypothesized that animal-based and plant-based organic fertilizers would support greater survival of *Salmonella enterica* than synthetic fertilizer due to the increased availability of organic matter and enhanced microbial activity associated with organic fertilization.

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Tables

Table 1.1. Overview of microgreen plant families, harvest pigmentation and representative species.

Plant family	Common pigmentation	Scientific name	Example microgreens
Chenopodiaceae	Reddish	<i>Spinacia oleracea</i>	Magenta spinach
	Reddish green	<i>Atriplex hortensis</i> <i>Beta vulgaris</i>	Red orach Red beet, Bull's blood beet
Apiaceae	Green	<i>Coriandrum sativum</i> <i>Apium graveolens</i>	Cilantro Celery
Brassicaceae	Green	<i>Brassica oleracea</i> var. <i>italica</i>	Broccoli
		<i>Eruca sativa</i>	Arugula
		<i>Lepidium bonariense</i>	Peppercress
		<i>Raphanus sativus</i> var. <i>longipinnatus</i>	Green daikon radish
		<i>Brassica rapa</i> ssp. <i>Nipposinica</i>	Mizuna
	Purple red	<i>Wasabia japonica</i>	Wasabi
		<i>Raphanus sativus</i>	China rose radish
	Greenish purple	<i>Raphanus sativus</i>	Opal radish
		<i>Brassica oleracea</i> var. <i>gongylodes</i>	Kohlrabi
	Purplish green	<i>Brassica juncea</i>	Red mustard, purple mustard
		<i>Brassica oleracea</i> var. <i>capitata</i>	Red cabbage
Amaranthaceae	Red	<i>Amaranthus hypochondriacus</i>	Garnet amaranth
Lamiaceae	Green	<i>Ocimum basilicum</i>	Green basil
	Greenish purple	<i>Ocimum basilicum</i>	Opal basil
Fabaceae	Yellow	<i>Pisum sativum</i>	Golden pea tendrils
Polygonaceae	Green	<i>Pisum sativum</i>	Pea tendrils
	Reddish green	<i>Rumex acetosa</i>	Red sorrel
	Green	<i>Rumex acetosa</i>	Sorrel
Poaceae	Yellow	<i>Zea mays</i>	Popcorn shoots

Chapter II. Postharvest Shelf-Life and Phytonutritional Quality of Four Microgreen Species Grown Under Different Fertigation Systems

Abstract

Microgreens contain high levels of vitamins, minerals, and phytonutrients, yet little is known about how fertigation practices influence their shelf-life and nutritional quality. This study compared effects of synthetic and organic fertilizers on the quality and nutrient composition of four microgreens. After harvest, microgreens were placed in destructive and non-destructive clamshells and stored at 4 °C for 21 days. Hypocotyl length (cm) and yield ($\text{g}\cdot\text{m}^{-2}$) were measured at harvest and postharvest assessments included weight loss, color (L, a, b, c, hue), and phytonutrient assays of anthocyanins ($\text{mg}\cdot\text{L}^{-1}$), phenolics ($\text{g}\cdot\text{kg}^{-1}$), and antioxidant activity ($\text{nM}\cdot\text{g}^{-1}$) at 0, 7, 14, and 21 days. Synthetic fertilizer increased fresh weight in amaranth, beet, and cilantro by 63%, 103%, and 187%, respectively, compared to organic fertilization. Kale had the highest yield under both organic ($1658.92\text{ g}\cdot\text{m}^{-2}$) and synthetic fertilization ($1377.64\text{ g}\cdot\text{m}^{-2}$). Hypocotyl length varied by species, with the longest in kale and amaranth (5.39 and 6.01 cm). Weight loss increased during storage, with kale showing the lowest loss (0.18%). Organic fertilization increased color intensity (a and c) in amaranth, whereas synthetic fertilization increased lightness (L) in kale and cilantro. Cilantro and kale maintained stable color during storage, while beets and amaranth showed greater changes. Organic fertilization increased anthocyanins (26% in beet, 18% in amaranth), antioxidant activity (20% in beet), and phenolics (45% in beet). Beets had the highest anthocyanins ($41.82\text{ mg}\cdot\text{L}^{-1}$), phenolics ($27.43\text{ g}\cdot\text{kg}^{-1}$), and antioxidants ($39.65\text{ nM}\cdot\text{g}^{-1}$). Anthocyanin and antioxidant activity increased at Day 7 (16% and 5%) of storage before decreasing, mainly in the beet. Cilantro had the highest chlorophyll content (742.34

mg·g⁻¹), whereas kale had the highest carotenoid content (891.55 mg·g⁻¹). Chlorophyll content generally decreased during storage, while carotenoid content remained stable or increased depending on species. These findings show that fertilizer-species combinations can enhance yield, shelf-life, and phytonutrient content in microgreens.

1. Introduction

In recent years, interest in functional foods has grown as consumers seek health benefits beyond basic nutrition and reduced disease risk (Ponte et al., 2025; Sun-Waterhouse, 2011). Fresh vegetables provide essential nutrients, including vitamins, minerals, and dietary fiber, along with bioactive compounds such as antioxidants and phytochemicals that support health (Ramya and Patel, 2019). Among these, microgreens have emerged as a specialty crop recognized for their high nutritional value and suitability for fresh consumption (Sharma et al., 2022; Weber, 2017).

Microgreens are immature plants harvested shortly after germination, typically within 7 to 21 days, once the cotyledons are fully developed (Sun et al., 2013; Xiao et al., 2012; Treadwell et al., 2020). They are characterized by intense flavor, vibrant color, and high concentrations of phytonutrients, including vitamins, minerals, antioxidants, and phenolic compounds (Choe et al., 2018; Janovská et al., 2010; Xiao et al., 2012).

As demand for nutrient-dense produce increases, production and fertigation strategies for microgreens are receiving greater research attention (Murthy and Pill, 2010). Among these approaches, organic systems have gained prominence due to perceived health benefits and reduced reliance on synthetic agrochemicals (Yiridoe et al., 2005). Within these systems, organic fertilizers rely on plant and animal-derived

nutrient sources that release nutrients more gradually and can enhance substrate biological activity. Whereas synthetic fertilizers supply readily available nutrients for rapid uptake (Bulgari et al., 2017; Di Gioia et al., 2017). These differences in nutrient availability and uptake dynamics can influence plant growth, morphology, and the accumulation of secondary metabolites (Wang et al., 2008). In some cases, organically fertilized crops exhibit elevated concentrations of phytochemicals. For example, organically fertilized celery had higher vitamin C by 23%, total phenolic content by 26% and antioxidant activity by 24% than synthetically fertilized celery (Babalar et al., 2023). Although, responses remain highly dependent on species and production conditions (Lima et al., 2018; Zhao et al., 2006).

Microgreens are well-suited to these comparative nutrient management approaches. They are primarily produced in controlled-environment agriculture systems, including greenhouses and indoor production systems enabling consistent, year-round cultivation (Trandel-Hayse et al., 2025; Zhang et al., 2021). Within these systems, microgreens can be produced using soil-based substrates or soilless media such as peat, perlite, and vermiculite, as well as hydroponic systems where nutrients are supplied through solution (Xiao et al., 2012; Treadwell et al., 2020; Ebert, 2022; Riggio et al., 2019; Santosh and Shukla, 2024). This range of production systems enables precise control over environmental conditions and inputs, making fertilization strategy a key determinant of growth, yield, visual quality, and nutritional composition (Di Gioia et al., 2017; Meas et al., 2020).

As a result, nutrient management remains a central factor in optimizing microgreen production. The supply of essential macronutrients, including nitrogen (N),

phosphorus (P), potassium (K), and sulfur (S), directly influences both biomass accumulation and nutritional quality (Wang et al., 2008). Despite the rapid expansion of microgreens, fertilization practices are not well standardized and crop-specific nutrient requirements remain poorly defined (Bulgari et al., 2017). Both preplant media fertilization and postemergence nutrient application have been shown to enhance growth and yield (Murphy and Pill, 2010), indicating the need to refine nutrient management strategies, particularly when comparing organic and synthetic fertilization approaches.

Microgreens are highly perishable due to their early harvest stage, delicate tissues, and high respiration rates, typically remaining marketable for only 1 to 3 days under ambient conditions (Chandra et al., 2012; Kou et al., 2014; Mir et al., 2017). Consequently, postharvest research has focused on storage temperature, packaging, and washing treatments, with low temperature storage playing a central role in slowing quality loss and maintaining visual and nutritional quality. For example, buckwheat microgreens stored at 5 °C under modified atmospheric conditions exhibited extended shelf-life up to three weeks (Kou et al., 2013), while radish microgreens stored at 1 °C maintained tissue integrity for up to 28 days (Xiao et al., 2014). Low temperature storage also supports nutritional retention; radish sango microgreens stored at 5 °C retained 168% higher total phenolic content, 105% higher anthocyanin content and 39% higher flavonoid content and antioxidant activity by 100% compared to those stored at 25 °C (Gunjal et al., 2025). Additionally, combining organic acid treatments with modified atmosphere storage at 5 °C has been shown to enhance bioactive compound retention by 59-78% and extend shelf-life up to 12 days (Patil et al., 2024).

Most studies have focused on extending microgreen shelf-life through postharvest treatments such as organic acids, modified atmospheric packaging, and calcium applications (Azarpazhooh et al., 2026; Chandra et al., 2012; Kou et al., 2014; Kou et al., 2015). While relatively few studies have evaluated both physiological and nutritional quality over extended postharvest storage (14 to 21 days) (Patil et al., 2024). Moreover, production-related factors, including lighting systems, cultivation methods (hydroponic vs. soil-based), and growing substrates, have been more extensively studied, with emphasis on nutritional quality at harvest (Bulgari et al., 2021; Samuolienė et al., 2013; Tan et al., 2020; Trandel-Hayse et al., 2025). In contrast, direct comparisons between organic and synthetic fertigation systems and their combined effects on both nutritional quality and shelf-life remain limited. Although extended storage of microgreens (up to 21–28 days) has been investigated (Xiao et al., 2014), integrated assessments of weight loss, visual quality, and nutritional changes over time are still lacking.

Based on these gaps, we hypothesized microgreens grown with organic fertilization would exhibit shorter shelf-life but enhanced nutritional quality due to slower nutrient availability, which may induce mild nutrient stress and activate plant defense mechanisms, resulting in increased concentrations of total antioxidants, phenolics, and anthocyanins. To address this, the objective of this study was to evaluate the effects of organic (fish-based) and synthetic (water-soluble) fertigation systems on yield, postharvest shelf-life, and phytonutrient composition of four commercially relevant microgreen species during cold storage at 0, 7, 14, and 21 days.

2. Materials and Methods

2.1 Plant Materials and Growing Conditions

The experiment was conducted in Greenhouse No. 5 at the Paterson Greenhouse Complex, Auburn University, Auburn, Alabama (32° 36' 35.4816" N, 85° 28' 50.8152" W; 450 Duncan Dr, Auburn, AL 36849). The greenhouse was covered with polycarbonate panels and equipped with an evaporative cooling system to maintain controlled environmental conditions. Four commercially grown microgreen species were selected for the experiment: cilantro (*Coriandrum sativum*), bulls blood beet (*Beta vulgaris*), garnet red amaranth (*Amaranthus cruentus*), and kale (*Brassica oleracea* L. var. *acephala*). The experiment was conducted in two production experiments using a randomized complete block design (RCBD) with four blocks and four replications per treatment. The first experiment occurred from 21 Feb. to 14 Mar. 2025, and the second from 28 Mar to 18 Apr 2025. Seeds were purchased from Johnny's Selected Seeds (Winslow, Maine, USA) and sown into standard plastic trays (50.8 × 25.4 cm) with small drainage holes at the bottom to allow for irrigation.

Seeding rates were determined using the microgreen seed rate calculator developed by Penn State Extension (<https://extension.psu.edu/microgreens-seed-density-calculator>). Based on these calculations, cilantro was seeded at 10.23 g per tray, beets at 20.50 g per tray, amaranth at 7.72 g per tray, and kale at 15.14 g per tray. During both production replications, temperature and relative humidity were continuously monitored using a HOBO data logger (model no. UX100-003, Onset Computer Corporation, Bourne, MA) (**Table 2.1**). Light intensity was monitored throughout the production period using a HOBO data logger (model no. H21-USB) equipped with two photosynthetically active radiation sensors (model no. S-LIA-M0003).

Two types of growing media were used in the experiment to compare the effects of organic and synthetic treatments on microgreen yield and nutritional quality. The organic media was Pro-Mix MP Mycorrhizae Organix, consisting of sphagnum peat moss (75-80% by volume), perlite, dolomitic and ground limestone (for pH adjustment), a wetting agent, and beneficial microorganisms. The synthetic media was Jolly Gardener Pro-Line Custom Growing Mix, a non-organic soilless substrate formulated with Canadian sphagnum peat moss, processed pine bark, and horticultural perlite. The pH and electrical conductivity (EC) of the growing media were tested by mixing roughly 50 mL of the media with 100 mL of tap water. The mixture was thoroughly stirred with a spatula, and then the pH and EC were measured using a Portable pH/EC/TDS/Temperature Meter (model no: HI9813-6, Hanna Instruments). These values are reported in **Table 2.2**. The sowing schedule was adjusted based on species-specific germination and growth durations, as determined from preliminary trials. To ensure a uniform harvest date, microgreens were sown in a staggered sequence as reported in **Table 2.3**.

2.2 Fertilizer Treatments and Application

To evaluate the effects of nutrient sources on microgreen yield and postharvest quality, two fertilizer treatments were applied. The synthetic treatment consisted of Miracle-Gro All Purpose Water-Soluble Plant Food, diluted in 3.78 L (1 gallon) of deionized (DI) water at a concentration of $0.312 \text{ g}\cdot\text{L}^{-1}$. This fertilizer contains 24% total nitrogen (3.5% ammoniacal nitrogen and 20.5% urea nitrogen), 8% available phosphate (P_2O_5), and 16% soluble potash (K_2O). The organic treatment utilized was liquid fish

fertilizer (Indian River Organics), diluted at $7.8 \text{ mL} \cdot \text{L}^{-1}$ in 3.78 L of DI water, providing 2% total nitrogen (1.7% water-soluble and 0.3% water-insoluble), 3% available phosphate (P_2O_5), and 1% soluble potash (K_2O).

Before application, both fertilizers were tested at various concentrations and analyzed for mineral composition. Final concentrations were selected based on these preliminary analyses to ensure optimal nutrient availability. Fertilizer solutions were applied via subirrigation by adding them to the bottom of the holding trays twice a day, in the morning and evening. No overhead fertigation was applied.

2.3 Mineral Analysis of Liquid Fertilizer and Growing Media

Mineral analysis of the fertilizer solutions and growing media was conducted at the Soil, Forage, and Water Testing Laboratory at Auburn University (Auburn, AL). The fertigation samples were analyzed using the EPA 200.7 method by inductively coupled plasma-optical emission spectrometry (ICP-OES) (iCAP 6500, Thermo Scientific, Waltham, MA, USA) after microwave-assisted digestion (MARS Express, CEM Corp., Matthews, NC, USA) to determine the concentrations of P, K, Ca, Mg, Fe, B, Cu, Zn, Mn, Na, and Ni. The nitrate content was determined using EPA Method 353.2 by automated colorimetry (U.S. EPA, 1993). Mineral analysis of liquid fertigation is reported as an average for each experiment in **Table 2.4**.

For growing media, roughly 60 g of the samples were collected from the bottom of the flats, stored in pre-labelled bags, and analyzed using the Saturated Media Extract (SME) method following Warncke (1986) and SERA standardized procedures. Moist samples were saturated with deionized water and allowed to equilibrate, after which the pH of the saturated media was measured. The samples were vacuum filtered to obtain

the extract, and the filtrate was measured for electrical conductivity (EC). The extracts were later analyzed for macro- and micronutrients using ICP-OES following the same methods as liquid fertilizer samples. The resulting pH, EC, and mineral values are summarized in **Table 2.5**.

2.4 Microgreen Harvest

All four microgreen species were harvested at the commercial harvesting stage (fully expanded, colored, and turgid cotyledons and the initial growth of true leaves), following the methods of Trandel-Hayse et al. (2025). Before harvest, trays were transported to the Postharvest Laboratory, located in Funchess Hall at Auburn University (Auburn, AL). Hypocotyl length (cm) was measured by randomly selecting 10 plants from each tray and averaging their lengths. Harvesting was performed using sterilized scissors by cutting shoots 1 cm above the soil surface. Total fresh yield ($\text{g} \cdot \text{m}^{-2}$) was recorded immediately after harvest using a digital scale.

2.5 Postharvest Experimental Design

Postharvest shelf-life evaluation was conducted using an RCBD, with the four production blocks treated as postharvest replications. A total of 64 plastic clamshells (4 species $\times$ 2 fertilizer treatments $\times$ 4 blocks $\times$ 2 clamshell types) were used for the postharvest study ($n = 64$). The microgreens were placed in plastic clamshells and stored at 4 °C and 75% relative humidity in cold storage for postharvest evaluation at Days 0, 7, 14, and 21. For each treatment and replication, samples were divided into two clamshells: one for destructive sampling and the other for non-destructive quality measurements. At each time point, 5 g of microgreens were collected from the

destructive clamshells, placed in pre-labeled sample bags, and immediately stored at –80 °C until further analysis. Non-destructive clamshells were used to evaluate weight loss (%), colorimeter parameters (L^* , a^* , b^* , c^* and hue angle), and visual quality over time.

2.6 Weight Loss and Colorimeter Assessments

Weight loss was determined by weighing the non-destructive clamshell (with microgreens) on a digital scale (Fristaden Lab, model no. HC2000X) at days 0, 7, 14 and 21 in storage. The percentage of weight loss was calculated using the formula:

$\frac{D0-DX}{D0} \times 100$, where $D0$ was the clamshell's initial weight at harvest (day 0, $d0$), and the DX was the weight at a specific storage timepoint of day 7, 14 and 21 ($d7$, $d14$, and $d21$).

Color assessments during storage were conducted using a Konica Minolta CM-600 colorimeter (Konica Minolta Business Solutions U.S.A. Inc., Ramsey, NJ). At each storage timepoint, five random points were measured per non-destructive clamshell to record L^* , a^* , b^* , chroma (c^*), and hue angle values in CIELAB Units.

2.7 Sample Preparation and Extraction of Total Anthocyanins, Phenolics and Antioxidant Activity

Before phytochemical assays, samples were removed from a –80 °C freezer and freeze-dried using a Harvest Right commercial freeze dryer (model no. HRFDXL; Harvest Right LLC., Salt Lake City, UT, USA). The freeze dryer was pre-cooled to –20 °F and samples were freeze-dried under a vacuum pressure of 500 mTorr for 72 h with a final drying temperature of 50 °F.

Freeze-dried samples were ground into a fine powder using a Bead Bug Microtube Homogenizer (model no. D1030; Benchmark Scientific, Sayreville, NJ, USA). Briefly, 50–75 mg of freeze-dried tissue was placed into 2 mL centrifuge tubes containing 6 x 2mm stainless steel beads. Samples were homogenized at 60 strokes min⁻¹ for 120 s until a fine powder was obtained. Ground samples were stored at –80 °C until analysis. All subsequent assays followed the methods described by Trandel-Hayse et al. (2025).

Approximately 20 mg of the powdered sample was weighed into a 2 mL black microcentrifuge tube (Sure Seal™ Microtubes, Ward's Science, Rochester, NY, USA), and 1.5 mL of methanol-formic acid-water solvent was added for extraction. Samples were vortexed for 5 min using a Vortex Genie 2 mixer (Scientific Industries, Inc., Bohemia, NY, USA), followed by sonication for 20 min using an ultrasonic bath (Model M3800, Branson 3800, Emerson, Danbury, CT, USA). The extracts were centrifuged at 10,000 g for 5 min at 4 °C using a Mikro 200R centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The collected extracts were filtered through 0.45 µm nylon syringe filters (25 mm; VMR International, Radnor, PA, USA) and were stored at –80 °C until further analysis.

Total anthocyanin content was determined using the pH-differential method. Extracted samples were mixed with potassium chloride buffer (pH 4.5) and sodium acetate buffer (pH 1.0) in separate tubes, vortexed for 20 s and allowed to stand in the dark at 4 °C for 2 h. Absorbance was measured at 510 nm and 700 nm using a UV-vis spectrophotometer (Shimadzu UV-2600i, Shimadzu Corporation, Kyoto, Japan). Total

anthocyanin content was calculated using the formula: $[(\text{Absorbance} \times \text{Molecular Weight} \times \text{Dilution Factor} \times 1000)/26,900]$ and are reported as $\text{g} \cdot 100\text{g}^{-1}$.

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method and expressed as gallic acid equivalents (GAE). In this method, the extracted samples were diluted with deionized water and vortexed for 30s. The Folin–Ciocalteu reagent was then added. After 3 min, 1N sodium carbonate was added and the samples were vortexed again for 30s. The mixtures were incubated at room temperature for 2h. Absorbance was measured at 726, 700 and 640 nm and phenolic concentration was calculated using a gallic acid standard curve ($y = 0.0061x + 0.0396$, $R^2 = 0.9991$). Total phenolic content was calculated as $\text{TPC} = (C \times V) / M$ and expressed as $\text{g GAE} \cdot \text{kg}^{-1}$, where C is the gallic acid concentration, V is the extract volume, and M is the sample mass.

Antioxidant activity was measured using the DPPH assay. A Trolox calibration curve (100–800 nM) was prepared prior to sample analysis. Sample extracts were combined with DPPH solution, vortexed, and incubated for 15 min at room temperature before measuring absorbance at 515 nm. Absorbance values were corrected against the DPPH blank with universal solvent added and was measured at the beginning and end of each replication to account for variation in reagent stability. The corrected absorbance (blank – sample) was used to calculate antioxidant concentration based on the Trolox standard curve ($y = 0.0008x + 0.787$, $R^2 = 0.972$), incorporating sample mass, extraction volume, and dilution factor. Results were expressed as $\text{nM} \cdot \text{g}^{-1}$.

2.8 Microgreen Preparation for Total Chlorophyll and Total Carotenoid Content

Total chlorophyll and total carotenoids were quantified using the methods described by Trandel-Hayse et al. (2025) and Lichtenthaler (1987). Approximately 5 mg of ground microgreen tissue was weighed into black microcentrifuge tubes, and 80% (v/v) acetone was added to each sample. The samples were vortexed for 5 min, sonicated for 10 min, and then centrifuged at 10,500 rpm for 7 min at 4 °C. After centrifugation, the samples were filtered through 0.45 µm nylon membrane filters into a new black microcentrifuge tube. Absorbance of the supernatant was measured at 750, 665, 649, 520, and 470 nm using a spectrophotometer. Chlorophyll and carotenoid concentrations were calculated using the following equations: Chlorophyll A (CA) = $(13.95 \times (\text{Absorbance } 664 - \text{Absorbance } 750)) - (6.88 \times (\text{Absorbance } 647 - \text{Absorbance } 750))$ = CA1 mg·mL⁻¹; Chlorophyll B (CB) = $(24.96 \times (\text{Absorbance } 647 - \text{Absorbance } 750)) - ((7.32 \times (\text{Absorbance } 664 - \text{Absorbance } 750)))$ = CB1 mg·mL⁻¹; Total Chlorophyll = CA1 + CB1; Total Carotenoids (TC) = $(1000 \times \text{Absorbance } 470) - (2.05 \times \text{CA1}) (114.8 \times \text{CB1}) / 209$ = TC1 mg·mL⁻¹ and are reported in mg·g⁻¹.

2.9 Data Analysis

Statistical analyses were performed using JMP version 18.1 (SAS Institute, Cary, NC, USA). Harvest variables (hypocotyl length and fresh yield) were analyzed using a two-way analysis of variance (ANOVA), with species, fertilizer treatment, and their interaction specified as fixed effects. Experiment and replication were initially included as random effects; however, replication was not significant for any harvest variable and was therefore removed from the final model. Hypocotyl length and fresh yield were treated as dependent variables.

Postharvest variables (weight loss and colorimeter measurements) and phytonutrient concentrations were analyzed using a three-way ANOVA, with fertilizer treatment, species, storage timepoint, and their interactions specified as fixed effects. Experiment and replication were included as random effects but were not significant and were subsequently excluded from the final analysis. Weight loss, colorimetric parameters, and phytonutrient measurements were considered dependent variables. All statistical tests were evaluated at a significance level of $\alpha = 0.05$. When significant main effects or interactions were detected ($p < 0.05$), mean separations were performed using Tukey's honest significant difference (HSD) test.

3. Results

3.1 Fresh yield and Hypocotyl length

Microgreen fresh yield was affected by the interaction of species x fertilizer type (**Fig. 2.1**). Organically and synthetically produced kale had the highest total fresh yields (1658.92 and $1377.64 \text{ g}\cdot\text{m}^{-2}$), respectively. While organically produced beet ($385.87 \text{ g}\cdot\text{m}^{-2}$) and cilantro ($322.5 \text{ g}\cdot\text{m}^{-2}$) were the lowest. Overall, the synthetically fertilized microgreens had a higher total fresh yield ($1061.66 \text{ g}\cdot\text{m}^{-2}$) compared to those grown with organic fertigation ($769.70 \text{ g}\cdot\text{m}^{-2}$) with an increase of $291.96 \text{ g}\cdot\text{m}^{-2}$.

Hypocotyl length differed significantly between production experiments, in production experiment 1 species and fertilizer treatment significantly differed with no significant interaction effect. While in production experiment 2 the interaction of species x fertilizer type was significantly different. In experiment 1, synthetic fertilizer produced longer hypocotyls (5.1 cm) compared with organic fertilizer (3.5 cm) (**Fig. 2.2a**). Among

species, kale had the longest hypocotyls (5.3 cm), followed by amaranth (4.5 cm), while the lowest values were reported in beet (3.0 cm) and cilantro (3.4 cm) (**Fig. 2.2b**). In Experiment 2, significant differences among species x fertilizer type combinations were observed, with organic kale and organic amaranth producing the longest hypocotyls (4.84 cm) and synthetic beet producing the shortest hypocotyls (**Fig. 2.2c**).

3.2 Weight loss (%) and Colorimeter Assessments

Percent (%) weight loss differed by storage timepoint and species, but no differences were seen in fertilizer treatment or the interactions. Weight loss increased throughout storage among all species and was highest on Day 21 (0.68%) (**Fig. 2.3a**). Among the cultivars, beets had the highest %weight loss (0.28%), followed by cilantro (0.26%) and amaranth (0.25%), while kale was lowest (0.18%) (**Fig. 2.3b**).

Color characteristics (L^* , a^* , b^* , c^* and hue angle) differed among the interactions for species x fertilizer type (**Table 2.6**) and species x storage timepoint (**Table 2.7**). Beet (35.14 vs 31.39) and cilantro (42.42 vs 40.73) grown using synthetic fertilizer had highest L^* values relative to organic fertilizer, whereas kale had higher L^* under organic fertilization compared to synthetic (41.42 vs 39.45). While no differences in L^* were seen in amaranth grown under synthetic or organic fertilization. Redness (a^*) and chroma (c^*) were lowest in amaranth grown using synthetic fertilizer at 8.72 and 9.13 compared to organically fertilized amaranth at 11.20 (a^*) and 11.37 (c^*), respectively. Beet grown using synthetic fertilizer had higher b^* compared to organic (3.68 vs 0.89), with no other differences in b^* for amaranth, cilantro, or kale. Hue angle was higher in organic beet (126.48) than in synthetic beet (65.95) (**Table 2.6**).

The interaction of species x storage timepoint also differed for the color characteristics (**Table 2.7**). Across all species, L^* increased from d0 to d7, then declined through d21, while cilantro had the highest L^* with a peak on d7 at 43.75. Kale had a similar pattern, with d7 L^* (42.15) did not differ from d14 kale (42.12). Amaranth and beet had consistently lower L^* , with beet on d21 (29.29) being the lowest. Redness (a^*) was highest in amaranth on d14 (10.95), whereas beet indicated relatively stable redness patterns across all storage timepoint (6.25). Conversely, Kale (-2.53) and cilantro (-5.3) had the lowest a^* across all storage timepoints. Blueness (b^*) did not significantly differ in amaranth or beet throughout storage. However, both kale and cilantro indicated increased b^* from d0 to d21, with cilantro increasing from 11.81 to 15.16 and kale from 8.27 to 11.75. Chroma (c^*) increased from d0 to d7 in amaranth (8.45 to 11.05) and cilantro (12.71 to 16.37) and increased from d7 to d21 in kale (8.74 to 12.07) but remained low/stable in beet (**Table 2.7**).

3.3 Phytonutrient Content and Total Antioxidant Activity

Anthocyanin content varied in the interaction of species x storage timepoint (**Fig. 2.4a**) and species x fertilizer type (**Fig 2.4b**). Across all storage timepoints beet microgreens recorded the highest anthocyanin concentrations, ranging from 34.7 to 40.2 mg·L⁻¹, with amaranth showing slightly lower values (27.61 to 29.88 mg·L⁻¹). Conversely, kale and cilantro showed the lowest anthocyanin concentrations at 6.17 mg·L⁻¹ and 2.08 mg·L⁻¹, respectively. In beet, anthocyanin content peaked at d7 (40.21 mg·L⁻¹) with intermediate values at d14 (37.54 mg·L⁻¹) and d21 (34.76 mg·L⁻¹). In contrast, anthocyanin content in amaranth remained relatively stable during all storage timepoint (0-14d) at 27.61 mg·L⁻¹. Organically fertilized beet and amaranth had higher

total anthocyanin content by 26% and 18%, respectively, compared to all other species and fertilization treatments.

Total phenolic content differed across storage timepoint (**Fig. 2.5a**) and in the interaction of species x fertilizer type (**Fig. 2.5b**). Total phenolics were highest on d7 of storage timepoint at $21.46 \text{ g} \cdot \text{kg}^{-1}$ compared to d0 ($18.33 \text{ g} \cdot \text{kg}^{-1}$) and d21 ($20.76 \text{ g} \cdot \text{kg}^{-1}$) (**Fig. 2.5b**). Total phenolic content ranged from 12.90 to $27.43 \text{ g} \cdot \text{kg}^{-1}$ across species and fertilizer treatments. Among all treatments, beet microgreens grown with organic fertilizer had the highest phenolic content ($27.43 \text{ g} \cdot \text{kg}^{-1}$), whereas cilantro grown with organic fertilizer had the lowest ($12.90 \text{ g} \cdot \text{kg}^{-1}$) (**Fig. 2.5b**).

Total Antioxidant activity was significantly influenced by species x storage timepoint (**Fig. 2.6a**) and species x fertilizer type (**Fig 2.6b**). Beet microgreens had the highest antioxidant activity throughout all storage timepoints, with values increasing from harvest ($34.25 \text{ nm} \cdot \text{g}^{-1}$) to d7 ($36.10 \text{ nm} \cdot \text{g}^{-1}$) and remaining relatively high at d14 ($34.93 \text{ nm} \cdot \text{g}^{-1}$), followed by a decline at d21 ($29.13 \text{ nm} \cdot \text{g}^{-1}$). In amaranth, antioxidant activity decreased from $31.28 \text{ nm} \cdot \text{g}^{-1}$ to $25.15 \text{ nm} \cdot \text{g}^{-1}$ between d0 and d14 with a reduction of $6.13 \text{ nm} \cdot \text{g}^{-1}$. Kale maintained moderate antioxidant activity, with values ranging from 28.14 to $29.53 \text{ nm} \cdot \text{g}^{-1}$ throughout all storage timepoints. In contrast, cilantro had the antioxidant activity among the species, with values decreasing from $20.84 \text{ nm} \cdot \text{g}^{-1}$ on d0 to $16.64 \text{ nm} \cdot \text{g}^{-1}$ on d21. Antioxidant activity was higher in organic beet ($39.65 \text{ nm} \cdot \text{g}^{-1}$) in comparison with $30.53 \text{ nm} \cdot \text{g}^{-1}$ under synthetic fertilization (**Fig. 2.6b**).

3.4 Chlorophyll and Carotenoid Content Among the Microgreens and Storage Timepoints

Total chlorophyll significantly differed in the species x storage timepoint interaction (**Fig. 2.7a**). Total chlorophyll was highest in cilantro across all storage timepoints, ranging from 732.47 mg·g⁻¹ to 713.15 mg·g⁻¹. In beet, total chlorophyll content was highest at d7 (707.02 mg·g⁻¹) compared with d0 (675.38 mg·g⁻¹), d14 (691.86 mg·g⁻¹) and d21(681.14 mg·g⁻¹). In amaranth, total chlorophyll was highest at d0 (662.41 mg·g⁻¹), decreased at d7 (656.4 mg·g⁻¹) and remained stable at d14 (648.39 mg·g⁻¹). Similarly, total chlorophyll content in kale was highest at d0 (678.26 mg·g⁻¹) and declined throughout storage timepoint, reaching the lowest at d21 (427.79 mg·g⁻¹).

Total carotenoid content was significantly affected by species x storage timepoint interactions (**Fig. 2.7b**). Among all the species, kale had the highest carotenoid concentrations, with values increasing from 552.73 mg·g⁻¹ at d0 to 891.55 mg·g⁻¹ at d21. In beet, carotenoid levels remained stable from d0-d21 (597.34-693.84 mg·g⁻¹). In cilantro and amaranth, carotenoid levels did not differ significantly across storage timepoints but tended to increase as storage progressed by 3% in cilantro and 17% in amaranth.

4. Discussion

4.1 Fresh Yield and Hypocotyl Length

The fresh yields of amaranth, kale, beet, and cilantro microgreens observed in this study were generally within the range reported in previous research (Di Gioia et al., 2023; Xiao et al., 2012; Treadwell et al., 2020). For example, Di Gioia et al. (2023) reported fresh yields of approximately 1444.1, 897.2 and 698.3 g·m⁻² for kale, amaranth, and beet grown under synthetic fertilization, which are comparable to the

yields observed in the present study (1377.64, 1159.37, 782.9 g·m⁻²) who observed similar yield patterns.

Among the species evaluated, kale produced the highest fresh yield, whereas beet and cilantro exhibited the lowest yields. According to previous literature, brassica microgreens often demonstrate greater biomass accumulation relative to other species due to differences in growth rate and seed reserves (Di Gioia et al., 2023; Xiao et al., 2012). These differences in fresh yield among species in this research are likely driven by variation in genotype, seed size, and seedling morphology, which influence early growth dynamics and biomass accumulation in microgreens.

Fresh yield differed significantly among fertilizer treatments. Synthetic fertilizer increased fresh yield in amaranth, beet, and cilantro by 63%, 103%, and 107%, respectively, compared to organic fertilization, due to greater immediate availability of nutrients for plant uptake (Sharma and Chetani, 2017). In contrast, nutrients in organic fertilizers are not readily available for plant uptake as they must undergo microbial mineralization (Treadwell et al., 2007). This process delays the nutrient availability in short-duration crops such as microgreens, which results in lower fresh yield under organic fertilization. Similar findings were reported by Shaik et al. (2022) in lettuce and by Daneshvar et al. (2023) in celery, where synthetic fertilizers resulted in greater fresh biomass by 12-57% in lettuce and yield by 50-52% in celery compared to organic fertilizers.

Hypocotyl length was significantly affected by both species and fertilizer treatments, with responses differing between production experiments. In Experiment 1, microgreens grown using synthetic fertilizer resulted in greater hypocotyl length across

the species. This response can be linked to the rapid availability of nitrate in synthetic fertilizer. Mineral analysis of fertilizer samples collected during production showed higher nitrate concentrations in the synthetic fertilizer (2.05 ppm) compared to organic fertilizer (0.55 ppm) (**Table 2.4**). Nitrate availability enhances cytokinin biosynthesis, and cytokinins play a key role in regulating plant growth processes such as cell division and elongation which contribute to increased hypocotyl extension (Guan et al., 2025; Jia et al., 2022).

In Experiment 2, differences among species x fertilizer combinations further suggest species-specific responses to fertilization strategy. Variability among species can be associated with inherent genetic differences that influence growth characteristics and nutrient utilization under different production conditions (Kyriacou et al., 2019). Hypocotyl length is an important characteristic of microgreens as it represents the edible portion and contributes to the overall appearance of the crops (Bantis, 2021). Microgreens with shorter hypocotyl length are associated with improved visual quality and greater consumer preference (Trandel-Hayse et al., 2025). Therefore, variation in hypocotyl length, including the shorter hypocotyls observed in organically fertilized species, directly affects microgreen marketability.

4.2 Weight Loss and Colorimetric Changes

In this study, weight loss increased with storage time across all species, irrespective of fertilizer type. The lack of fertilizer effect suggests that postharvest water loss is driven primarily by physiological and structural traits rather than preharvest nutrient management (Kissinger et al., 2005). This increase in postharvest weight loss was likely due to the moisture loss through transpiration and continued respiration after

harvest (Nunes and Emond, 2007; Agüero et al., 2011; Shezi et al., 2024). The delicate tissues and a high surface area to volume ratio of microgreens lead to greater moisture loss during storage (Turner et al., 2020). Consistent with our findings, Ghoola and Srividya (2020) reported increases in weight loss stored at 5 °C for 8 days in roselle and radish microgreens packaged in low-density polyethylene (LDPE) and polyethylene terephthalate (PET) clamshell containers packaging. Similarly, Azarpazhooh et al. (2026) also observed increased weight loss in garden cress microgreens stored at both 4 and 12 °C, with greater losses occurring at higher temperatures.

Among the species, kale showed the lowest %weight loss compared to amaranth, beet and cilantro, suggesting differences in species. These differences are due to the structural characteristics of kale leaves, such as thicker tissues and a more developed cuticle layer, which can reduce transpiration and water loss. Nunes and Emond (2007) suggested thicker cuticular layers help to reduce moisture loss. Similarly, studies on Chinese kale have linked thicker leaves and increased cuticular wax deposition to reduced water loss under stress conditions (Issarakraisila et al., 2007). These findings indicated that species-specific structural traits strongly influence postharvest water loss, with kale exhibiting the lowest water loss and greatest shelf-life. This further emphasizes that microgreen species should be carefully selected based on distribution requirements and market objectives.

Color parameters were affected by species, fertilizer type and storage timepoint. Organic fertilization enhanced color intensity, particularly a^* and chroma values in amaranth microgreens as reflected by higher values (**Table 2.6**). The greater color intensity observed under organic fertilization can be linked with enhanced pigment

accumulation under lower nutrient availability (Ibrahim et al., 2013). Reduced nitrogen conditions have been reported to promote anthocyanin synthesis, which can contribute to improved color (Jezek et al., 2018).

In contrast, color responses in beet differed from amaranth, with synthetic fertilization resulting in higher L^* and b^* values and a substantial reduction in hue angle. These changes suggested a shift in pigment composition, potentially reflecting alterations in betalain pigments which are responsible for the characteristic red-violet and yellow-orange coloration of beet tissues (Acharya et al., 2021). This highlights that pigment responses to fertilization are highly species-dependent and influenced by inherent differences in pigment composition and metabolism among microgreen species.

The L^* increased during early storage and declined during later storage indicating progressive pigment changes. The initial increase in L^* values indicated reduced greenness and the development of yellowing during storage. Likewise, higher b^* values observed in cilantro and kale microgreens are related to chlorophyll breakdown and the increased appearance of yellow carotenoid pigments during senescence (Ghoora and Srividya, 2020). These results indicated color characteristics in microgreens are governed by both preharvest nutrient management and postharvest physiological processes (Daneshvar et al., 2023). Fertilization strategy modulated initial pigment accumulation, whereas storage-related processes, including chlorophyll degradation and pigment transformation, largely determine changes in visual quality over time.

4.3 Phytonutrient Content and Antioxidant Activity

Anthocyanins are water-soluble pigments responsible for red, purple, and blue coloration in many fruits and vegetables. In addition to their role in pigmentation, they are synthesized in response to abiotic stress, where they function as protective compounds (Enaru et al., 2021). As a subset of phenolic compounds, anthocyanins are secondary metabolites synthesized through the phenylpropanoid pathway, with phenylalanine ammonia lyase (PAL) playing a key role in their production (Sharma et al., 2019). This biosynthetic pathway is highly responsive to environmental conditions, as factors such as light, nutrient availability, and temperature strongly influence phenolic compound accumulation (Ahmed et al., 2014).

In this study, phytonutrient content and antioxidant activity were influenced by species, storage timepoint, and fertilizer treatment, indicating postharvest responses varied depending on both the compound evaluated and the microgreen species. At harvest, anthocyanin and phenolic concentrations fell within ranges reported for pigmented microgreens in previous studies, supporting the consistency of these results with established literature (Rocchetti et al., 2020; Gunjal et al., 2024).

Total anthocyanin content was significantly affected by the species x storage timepoint interaction. This is consistent with their inherently pigmented tissues, including red hypocotyls and cotyledons. The increase in anthocyanin content during early storage, particularly in beet (peaking at d7), may be attributed to low temperature-induced activation of flavonoid biosynthesis under refrigerated conditions (Xie et al., 2012). Similar responses have been reported in Tartary buckwheat sprouts, where cold stress enhanced anthocyanin accumulation as part of a defense response to mitigate reactive oxygen species (Li et al., 2015). These findings are further supported by Gunjal

et al. (2024), who observed higher anthocyanins in pigmented microgreen species such as beetroot, red amaranth and radish sango.

Organic fertilization further enhanced anthocyanin concentrations in both beet and amaranth, with increases of 26% and 18%, respectively. This response may be linked to reduced nitrogen availability under organic systems, which can stimulate the accumulation of carbon-based secondary metabolites such as anthocyanins (Sarwar et al., 2017). These findings are also consistent with previous reports showing increased anthocyanin content in organically fertilized or nutrient-limited systems (Libutti et al., 2023). Total phenolic content followed a similar pattern, increasing during the first 7 days of storage before stabilizing or slightly declining by d21. This increase is likely associated with the postharvest stresses experienced by microgreens such as low temperature, moisture loss and mechanical injury during storage. Such abiotic stresses are known to increase PAL activity and stimulate phenylpropanoid pathway, resulting in greater accumulation of phenolic compounds (Amodio et al., 2014). After 7 days of storage, a slight reduction (1%) in phenolic content was observed throughout 21 days of storage, although the decrease was not significant. This decline could be due to oxidative degradation of phenolic compounds regulated by polyphenol oxidase (PPO) during prolonged storage (Amodio et al., 2014). Similar trends have been reported in other studies. For example, Tartary buckwheat microgreens stored at 5 °C showed an initial accumulation of phenolic compounds followed by a decline during extended storage (Yan et al., 2022). Similarly, Rocchetti et al. (2020) observed an increase in phenolic content at 7 days, followed by a decrease at later days of storage in amaranth microgreens stored at 4 °C.

Total antioxidant activity closely paralleled changes in phenolic and anthocyanin content throughout storage. Higher antioxidant activity observed during the early stages of storage coincided with increased phenolic content, while the decline during later storage stages may be associated with reductions in total phenolic compounds (Gunjal et al., 2024). Similar trends have been reported in celery, where antioxidant activity initially increased during storage and then declined during extended storage (Daneshvar et al., 2023). The relationship between antioxidant activity and phenolic content observed in this study is consistent with previous findings. For example, antioxidant capacity in minimally processed garden cress increased during early storage along with total phenolic content, reflecting stress-related metabolic responses (Zhan et al., 2009). Similarly, Rocchetti et al. (2020) reported increased phenolic content and antioxidant activity in red beet and amaranth microgreens stored at 4 °C, suggesting that postharvest biochemical activity continues even under refrigerated conditions.

Among the species evaluated beet and amaranth consistently showed higher amounts of anthocyanins, phenolic content and antioxidant activity, likely due to genotype-dependent differences in secondary metabolite accumulation. These species are particularly rich in betalains, including betacyanins and betaxanthins, which contribute to their characteristic pigmentation and antioxidant potential (Rocchetti et al., 2020). Organic fertilization enhanced phenolic compound concentrations, anthocyanin levels, and antioxidant activity compared with synthetic fertigation. Increased accumulation of these compounds may be associated with mild nutrient stress and lower nitrogen availability which can promote synthesis of carbon-based secondary metabolites (Sarwar et al., 2017). Similar responses have been reported in organically

fertilized plants, where reduce nitrogen availability promoted accumulation of bioactive and antioxidant compounds (Sarwar et al., 2017; Libutti et al., 2023).

4.4 Chlorophyll and Carotenoid Content

Chlorophylls and carotenoids are pigments that are important for photosynthesis and plant growth (Simkin et al., 2022; Sun et al., 2022). In addition to this, chlorophyll plays a key role in imparting green color, while carotenoids impart yellow and orange colors. Carotenoids are also important for nutritional value because they possess antioxidant properties (Burns et al., 2003; Sun et al., 2022). At harvest, chlorophyll content ranged from 662 to 732 mg·g⁻¹. These results are comparable to those reported by Paradiso et al. (2018), who observed chlorophyll concentrations ranging from less than 400 to 703 µg·g⁻¹ FW among several microgreen species. In contrast, carotenoid concentrations in the present study ranged from 507 to 597 mg·g⁻¹, which were comparable those reported by Trandel-Hayse et al. (2025) where total carotenoids ranged from 300 to 520 µg·g⁻¹ FW in amaranth microgreens grown under different LED light treatments.

During storage, chlorophyll generally decreased, while carotenoid content increased or remained stable depending on the species (**Fig. 2.7**). The decrease in chlorophyll is likely due to senescence and enzymatic degradation (Heaton and Marangoni, 1996). The slight increase in carotenoid content in beet and cilantro during storage may not necessarily indicate carotenoid synthesis. Rather, the degradation of chlorophyll during storage likely increased the relative visibility of carotenoids (Charoenchongsuk et al., 2015; Sousa, 2022). Consistent with the findings of the present study, increases in carotenoid content during storage have also been reported

in green romaine lettuce stored at 4 °C (Saini et al., 2016). Similar decreases in chlorophyll content during storage have been observed in radish sango and radish microgreens (Gunjal et al., 2025; Xiao et al., 2014).

Species-specific differences in pigment composition and color development were observed during storage. Kale showed greater yellowing, as reflected by the increase in b^* values and the visible loss of green color. This response corresponded with a greater decline in chlorophyll and relatively higher carotenoid levels, suggesting as chlorophyll degraded, carotenoids became more visible and contributed to the observed yellowing. Cilantro retained highest chlorophyll content during storage, suggesting greater chlorophyll stability and slower senescence compared to the other species.

5. Conclusion

Species and fertilizer type significantly shaped microgreen growth, postharvest performance, and phytonutrient composition, with clear tradeoffs between yield and nutritional quality. Synthetic fertigation enhanced fresh yield and hypocotyl elongation, indicated greater nutrient availability and rapid early growth. In contrast, organic fertigation promoted higher accumulation of anthocyanins, phenolic compounds, and antioxidant activity, likely due to reduced nitrogen availability and associated metabolic responses.

Postharvest behavior was primarily driven by species-specific traits rather than fertilization. Weight loss increased during storage across all species; however, kale consistently exhibited the lowest water loss and greatest shelf-life, indicating superior storage potential. In contrast, beet and amaranth maintained higher phytonutrient

concentrations, highlighting their value for nutritional quality. Changes in color and pigment composition further reflected progressive senescence and biochemical activity during refrigerated storage. Overall, these findings demonstrate that optimizing microgreen production requires balancing productivity, nutritional quality, and postharvest performance. Species selection is particularly critical for determining shelf-life and distribution potential, while fertilization strategy can be used to target either biomass production or enhanced phytonutrient content. These results provide a framework for growers and supply chains to align production practices with specific market goals, whether prioritizing yield, nutritional value, or extended storage and transport.

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Tables

Table 2.1. Average day and night temperatures and relative humidity (RH) recorded under greenhouse conditions during microgreen production.

Growing Condition	Growing Condition
Production Experiment 1	
Day Temperature (°C)	23.5
Night Temperature (°C)	18.2
Day RH (%)	46.3
Night RH (%)	59.9
Production Experiment 2	
Day Temperature (°C)	24.6
Night Temperature (°C)	23.7
Day RH (%)	52.9
Night RH (%)	64.4

Daytime values were calculated as the means of measurements recorded from 7:00 am to 7:00 pm; night time values represent the means of measurements recorded outside this interval.

Table 2.2. The pH and electrical conductivity (EC) of organic and non-organic growing media used for microgreen production.

Growing media	Type	pH	EC (dS·m ⁻¹)
Pro-Mix MP	Organic	5.8	0.50
Mycorrhizae Organix	Non-organic	6.1	0.55
Jolly Gardener Pro-			
Line Custom Growing Mix			

Electrical conductivity (EC) expressed as (dS·m⁻¹). Measurements were obtained using a portable pH/EC meter.

Table 2.3. Germination and cultivation duration of four microgreen species.

Microgreen Species	Germination Period (Days)	Growth Period (Days)	Total Cultivation Period (Days)
Cilantro	6	15	21
Beets	3.5	10.5	14
Amaranth	2	12	14
Kale	3	7-8	11

Values are based on two greenhouse production periods conducted from February to April 2025. Germination period = sowing to emergence; growth period = emergence to harvest; total cultivation period = sowing to harvest. Kale growth period is reported as a range (7–8 days) due to variation in harvest readiness.

Table 2.4. The pH, electrical conductivity (EC), and macronutrient and micronutrient composition of organic and synthetic liquid fertilizers used during production of four microgreen species.

Experiment	Treatment	pH	EC	Total N	Nitrate	P	K	Mg	Ca	Na	Fe	Zn	B	Mn	Cu	Ni
Production Experiment 1	Organic Fish Emulsion	4.0	0.96	36.55	0.55	92.5	14.7	2.7	72.4	8.31	0.27	0.21	0.05	0.05	0.13	0.05
Production Experiment 1	Synthetic Miracle Grow	6.7	0.52	34.92	2.05	81.3	89.8	1.1	0.1	1.89	0.28	0.17	0.05	0.09	0.25	0.05
Production Experiment 2	Organic Fish Emulsion	4.1	1.10	33.45	0.57	90.6	20.3	3.0	47.6	8.12	0.34	0.21	0.07	0.04	0.10	0.05
Production Experiment 2	Organic Fish Emulsion	6.7	0.58	38.53	1.16	85.3	90.8	1.0	0.1	1.78	0.35	0.20	0.09	0.14	0.14	0.05

Values are from fertilizer solutions prepared and applied during each of two production replications. Macronutrients (Total N, Nitrate, P, K, Mg, and Ca) and micronutrients (Na, Fe, Zn, B, Mn, Cu, and Ni) are reported as ppm ($\text{mg}\cdot\text{L}^{-1}$) and electrical conductivity (EC) as $\text{mS}\cdot\text{cm}^{-1}$. Production Experiment 1 and Production Experiment 2 correspond to two independent production periods occurring from February to April 2025.

Table 2.5. Soil pH, electrical conductivity (EC) and mineral analysis at the time of microgreen harvest among the four species and two fertilization treatments.

Treatment & Species	pH	EC	Total N	Nitrate	P	K	Mg	Ca	Na	Fe	Zn	B	Mn	Cu
Production Experiment 1														
Organic Amaranth	5.0	0.56	70.1	10.4	37.3	30.1	10.6	48.6	27.5	0.08	0.39	0.15	0.18	0.05
Organic Beet	5.1	0.77	75.3	17.7	31.9	32.9	15.1	70.5	23.2	0.05	0.10	0.10	0.25	0.05
Organic Cilantro	5.3	0.50	79.5	18.4	39.7	20.0	6.1	29.3	21.2	0.08	0.08	0.09	0.09	0.05
Organic Kale	4.9	0.74	82.3	18.5	58.2	33.1	18.5	79.0	50.6	0.08	0.30	0.22	0.29	0.06
Synthetic Amaranth	6.9	0.58	88.5	26.1	6.4	28.3	30.1	54.6	19.5	0.06	0.05	0.11	0.05	0.05
Synthetic Beet	6.7	0.81	91.2	27.1	6.4	32.1	54.9	58.7	20.3	0.05	0.07	0.12	0.05	0.05
Synthetic Cilantro	6.8	0.50	89.6	29.7	5.4	22.3	17.5	43.7	16.5	0.05	0.05	0.10	0.05	0.05
Synthetic Kale	7.0	0.49	88.3	28.0	2.9	30.7	10.6	45.5	10.3	0.05	0.05	0.10	0.05	0.05
Production Experiment 2														
Organic Amaranth	5.2	0.58	79.3	15.6	49.7	33.8	6.1	29.3	24.6	0.11	0.11	0.12	0.11	0.05
Organic Beet	5.5	0.53	77.2	18.9	31.3	32.2	7.7	41.3	21.1	0.05	0.05	0.10	0.07	0.05
Organic Cilantro	5.6	0.74	78.5	12.1	51.3	34.9	7.6	34.1	27.6	0.05	0.09	0.10	0.14	0.05
Organic Kale	5.3	0.50	75.6	16.4	37.7	27.7	3.8	30.6	15.7	0.07	0.05	0.09	0.10	0.05
Synthetic Amaranth	6.2	0.58	78.3	26.2	2.6	20.2	31.1	60.9	24.5	0.05	0.11	0.10	0.11	0.05
Synthetic Beet	6.7	0.74	88.2	26.5	7.7	22.3	29.1	49.2	11.8	0.05	0.05	0.10	0.05	0.05
Synthetic Cilantro	7.0	0.79	89.5	21.8	4.9	26.2	37.3	56.8	18.9	0.05	0.05	0.10	0.05	0.05
Synthetic Kale	6.7	0.33	82.1	25.3	9.2	29.5	4.01	41.4	12.8	0.08	0.20	0.10	0.05	0.05

Production Experiment 1 and 2 represent two independent production runs occurring from February to April 2025.

Electrical conductivity (EC) is reported as $\text{mS}\cdot\text{cm}^{-1}$; macronutrients (Total N, Nitrate, P, K, Mg, and Ca) and micronutrients (Na, Fe, Zn, B, Mn, Cu, and Ni) concentrations are reported as ppm ($\text{mg}\cdot\text{L}^{-1}$ in substrate extract).

Table 2.6. Effect of fertilizer treatments on the color characteristics (L*, a*, b*, C*, hue) of four microgreen species.

Treatment & Species	L*	a*	b*	Chroma	Hue (°)
Organic Amaranth	32.17 ± 0.45d	11.20 ± 0.49a	-0.54 ± 0.17d	11.37 ± 0.49b	247.55 ± 15.83a
Organic Beet	31.39 ± 0.38d	7.21 ± 0.35c	0.89 ± 0.15d	7.67 ± 0.34d	126.48 ± 14.44b
Organic Cilantro	40.73 ± 0.82ab	-4.99 ± 0.23e	12.98 ± 0.59a	13.94 ± 0.63a	110.58 ± 0.21bc
Organic Kale	41.42 ± 0.63ab	-2.58 ± 0.26d	10.15 ± 0.53b	10.66 ± 0.56bc	98.91 ± 1.43bc
Synthetic Amaranth	32.26 ± 0.38d	8.72 ± 0.40b	0.28 ± 0.25d	9.13 ± 0.38cd	212.29 ± 17.92a
Synthetic Beet	35.14 ± 0.54c	6.25 ± 0.41c	3.68 ± 0.37c	8.45 ± 0.27d	65.95 ± 8.90c
Synthetic Cilantro	42.42 ± 0.66a	-5.70 ± 0.18e	14.51 ± 0.45a	15.61 ± 0.48a	111.34 ± 0.24bc
Synthetic Kale	39.45 ± 0.75b	-2.89 ± 0.27d	9.82 ± 0.51b	10.44 ± 0.55bc	101.32 ± 1.41bc

Values are averaged across experimental replications and postharvest time points. All values are reported as the colorimeter measurement ± standard errors. The significant interaction of species x fertilizer was determined using Tukey Honestly Significant Difference; different letters indicate significance ($p \leq 0.05$).

Table 2.7. Effect of species x storage timepoint on the color characteristics (L*, a*, b*, Chroma (c*), hue angle) of four microgreen species.

Species	Storage Timepoint	L*	a*	b*	c*	Hue (°)
Amaranth	Day 0	30.73 ± 0.49e	8.22 ± 0.37b	-0.26 ± 0.23f	8.45 ± 0.36d	229.55 ± 23.75a
	Day 7	33.44 ± 0.38de	10.70 ± 0.63a	-0.17 ± 0.32f	11.05 ± 0.59bc	250.21 ± 17.94a
	Day 14	32.48 ± 0.40de	10.95 ± 0.62a	0.06 ± 0.29ef	11.24 ± 0.58b	209.99 ± 20.91a
	Day 21	—	—	—	—	—
Beet	Day 0	31.91 ± 0.83e	5.91 ± 0.40c	2.22 ± 0.52d	7.26 ± 0.38d	93.09 ± 19.15b
	Day 7	34.91 ± 0.60d	6.95 ± 0.47bc	2.64 ± 0.55d	8.47 ± 0.31d	96.31 ± 15.20b
	Day 14	32.96 ± 0.58de	7.33 ± 0.49bc	2.01 ± 0.42de	8.46 ± 0.42d	99.24 ± 15.67b
	Day 21	29.49 ± 0.81e	6.23 ± 0.76a	2.42 ± 0.71e	8.15 ± 0.56e	139.84 ± 19.07a
Cilantro	Day 0	38.85 ± 0.97c	-4.67 ± 0.32ef	11.81 ± 0.76b	12.71 ± 0.82b	111.36 ± 0.28b
	Day 7	43.75 ± 0.49a	-5.91 ± 0.15f	15.24 ± 0.32a	16.37 ± 0.34a	110.99 ± 0.28b
	Day 14	42.12 ± 0.83ab	-5.46 ± 0.23f	14.19 ± 0.53a	15.25 ± 0.57a	110.54 ± 0.30b
	Day 21	40.01 ± 0.77bc	-5.44 ± 0.23d	15.16 ± 0.60a	16.13 ± 0.63a	109.71 ± 0.47ab
Kale	Day 0	38.43 ± 0.79c	-2.06 ± 0.23d	8.27 ± 0.46c	8.74 ± 0.50cd	98.39 ± 1.19b
	Day 7	42.15 ± 0.60ab	-3.09 ± 0.28de	10.54 ± 0.45b	11.18 ± 0.50b	101.94 ± 1.89b
	Day 14	40.72 ± 0.98bc	-3.05 ± 0.39de	11.15 ± 0.73b	11.73 ± 0.78b	100.00 ± 2.04b
	Day 21	39.51 ± 0.93bc	-1.93 ± 0.36bc	11.75 ± 0.82bc	12.07 ± 0.84c	95.20 ± 1.83b

Values are averaged across experimental replications and are reported as the colorimeter measurement ± standard errors. The significance species x storage timepoint was determined using Tukey Honestly Significant Difference; different letters indicate significance ($p \leq 0.05$). Amaranth was not evaluated at Day 21 due to shelf-life limitations.

Figures

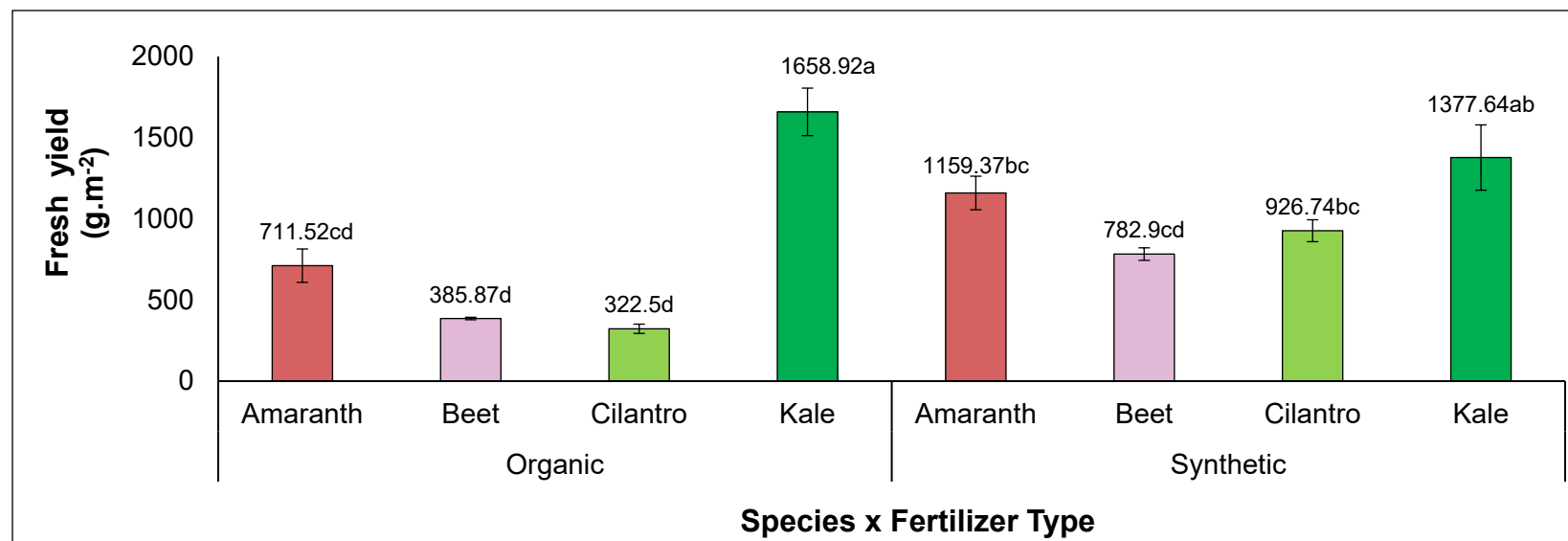


Figure 2.1 Interaction effects of species and fertilizer treatment on fresh yield (g·m⁻²) of four microgreen species. Bars represent means $\pm$ standard error (SE). Different letters indicate significant differences among means according to Tukey's HSD test ($p < 0.05$).

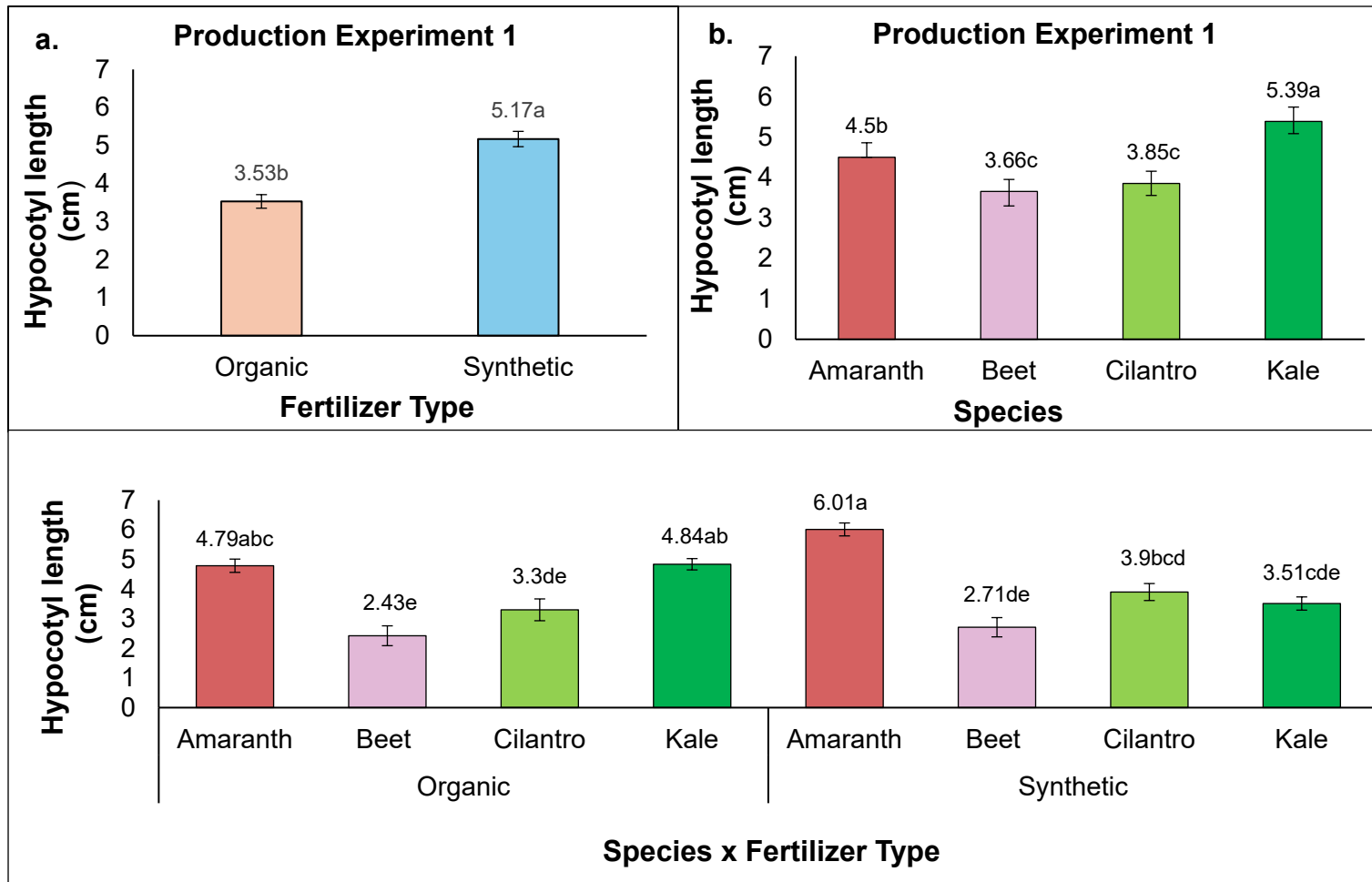


Figure 2.2 Hypocotyl length of microgreens as affected by fertilizer treatment (a), species (b), and the species x fertilizer type interaction (c) across two production experiments. Bars represent mean hypocotyl length (cm), and error bars indicate standard error of the mean. Different letters denote significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

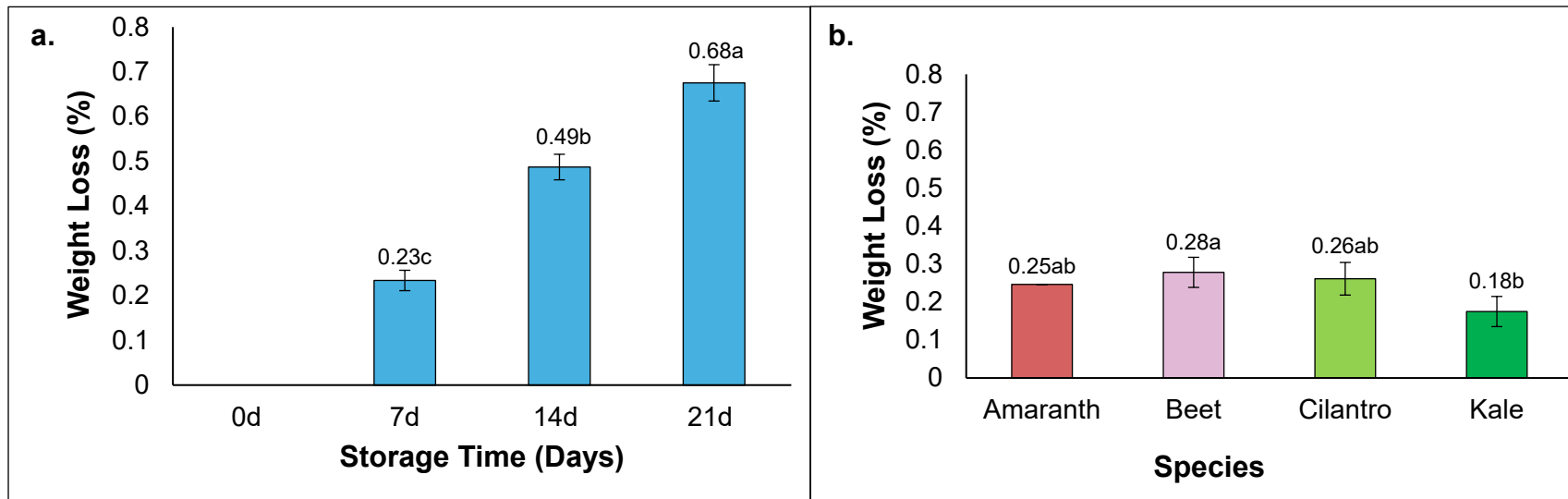


Figure 2.3 Percentage of weight loss (%) differed in the microgreens across storage days (a) and among the four microgreen species (b). Different letters show significant differences among means according to Tukey's (HSD) test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest.

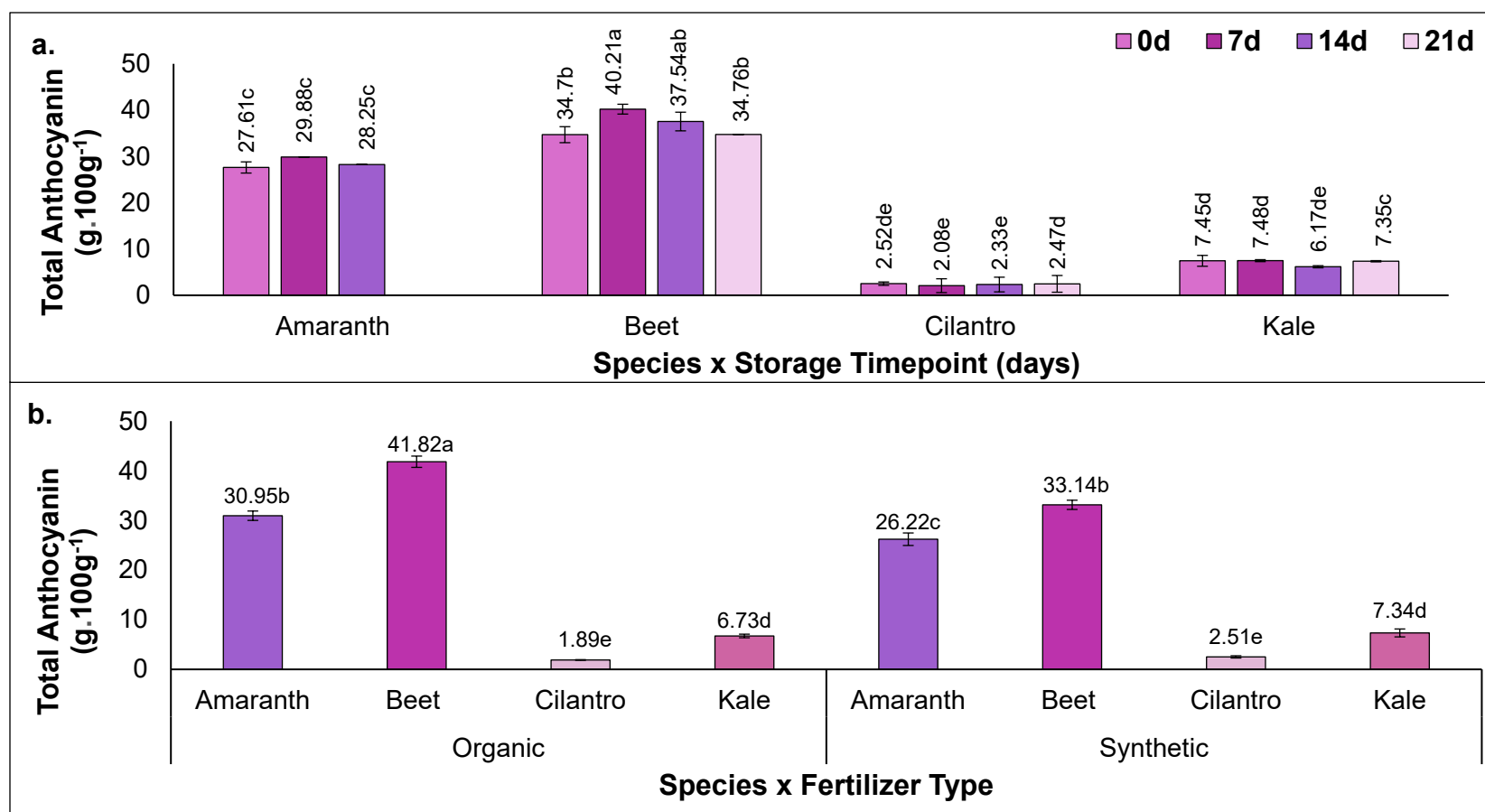


Figure 2.4 Total anthocyanin content ($\text{g} \cdot 100\text{g}^{-1}$) of amaranth, beet, cilantro, and kale microgreens (a) and the interaction effect of species and storage time (b). Bars represent means $\pm$ standard error (SE). Amaranth microgreens were evaluated only through day 14; therefore, anthocyanin data for amaranth at day 21 are not reported. Different letters indicate significant differences according to Tukey's honest significant difference (HSD) test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest.

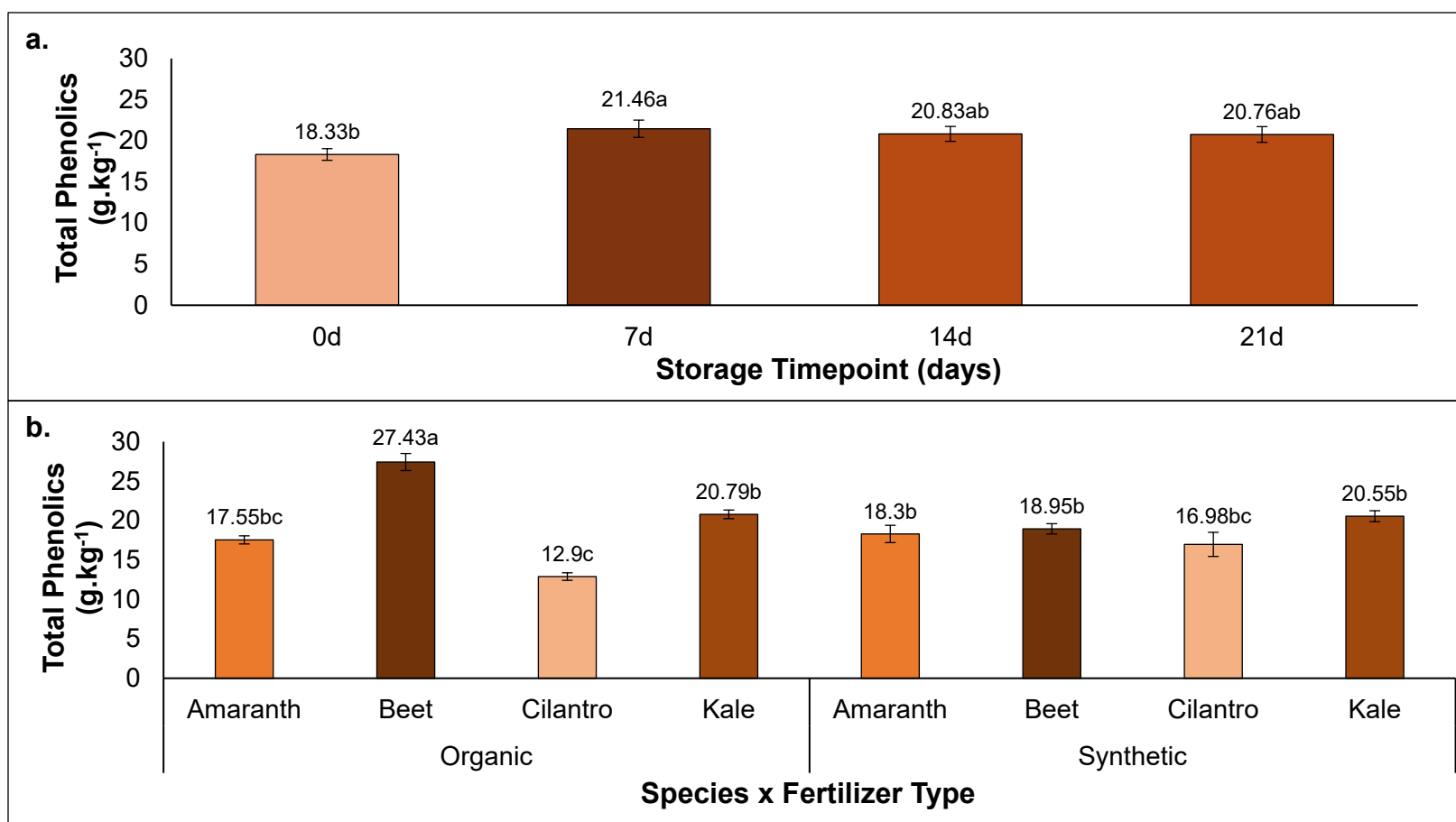


Figure 2.5 Total phenolic content (g.kg⁻¹) differed among the microgreen storage timepoints (a) and interaction of species x fertilizer treatment (b). Bars represent means $\pm$ standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest.

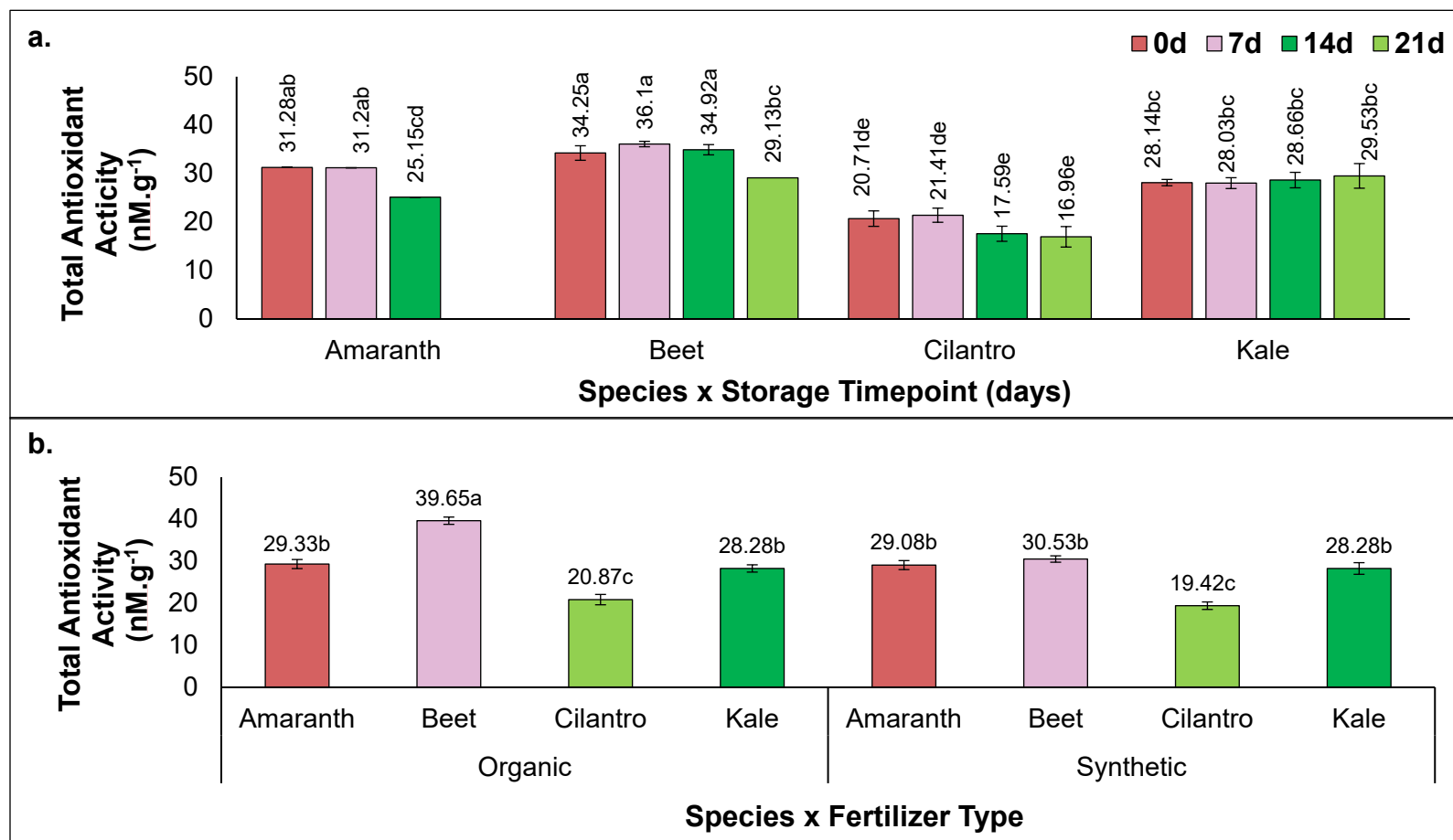


Figure 2.6 Total Antioxidant Activity ($\text{nM}\cdot\text{g}^{-1}$) differed in the interactions of microgreen species x storage timepoint (a) and species x fertilization treatment (b). Bars represent means $\pm$ standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest.

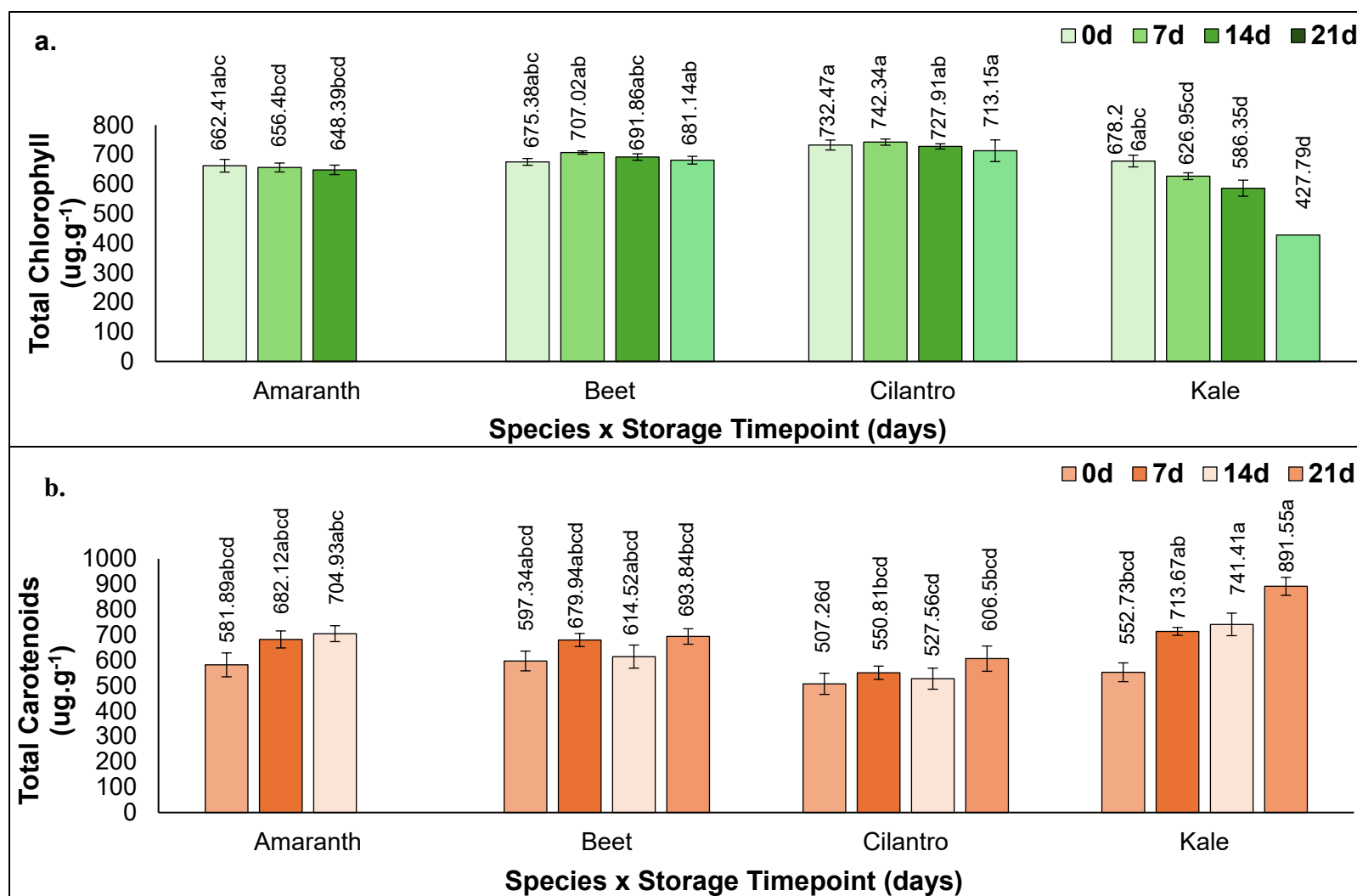


Figure 2.7 Total chlorophyll ($\mu\text{g}\cdot\text{g}^{-1}$) (a) and total carotenoids ($\mu\text{g}\cdot\text{g}^{-1}$) (b) differed in the species x storage timepoint interaction. Bars represent means $\pm$ standard error (SE). Different letters indicate significant differences among means according to Tukey's HSD test ($p < 0.05$). Storage duration is represented by 0d, 7d, 14d, and 21d (days) following harvest.

Chapter III. Effect of Fertilization Type on *Salmonella enterica* Survival in Microgreens During Production and Postharvest

Abstract

Kale microgreens are typically consumed raw, making them particularly vulnerable to food safety risks associated with human pathogens such as *Salmonella*. Recent research has emphasized contamination via seeds, irrigation water, and growing substrates, while little attention has been given to how fertigation practices influence pathogen survival. Thus, the objective of this study was to evaluate the effects of fertilizer type on *Salmonella* survival during microgreen production and postharvest storage. Fertilizer treatments included Earth Juice Grow (organic, plant-based), Fish Emulsion (organic, animal-based), and Miracle-Gro (synthetic). Fertilizer solutions were inoculated with *Salmonella enterica* serotypes Enteritidis and Typhimurium ($7 \log \text{CFU} \cdot \text{mL}^{-1}$) and applied through bottom irrigation after kale seed germination. Grow mat and kale microgreen samples were collected on days 1, 3, 5, and 8 following inoculation throughout the production period. After harvest, microgreens were placed in plastic clamshells and stored at 4°C and sampled on Days 1 and 7 to assess *Salmonella* survival. *Salmonella* was enumerated using selective media and expressed as $\log \text{CFU} \cdot \text{g}^{-1}$. *Salmonella* populations in grow mats were significantly affected by fertilizer treatment, with the highest populations observed in Liquid Earth Juice ($7.2 \log \text{CFU} \cdot \text{g}^{-1}$) and the lowest in synthetic Miracle-Gro ($6.17 \log \text{CFU} \cdot \text{g}^{-1}$). Populations in both grow mats and microgreens remained stable until Day 5 followed by a decline on Day 8, decreasing by $0.85 \log \text{CFU} \cdot \text{g}^{-1}$ in grow mats and $1.89 \log \text{CFU} \cdot \text{g}^{-1}$ in microgreens. During kale postharvest storage, no differences were seen in *Salmonella* populations between the fertilizer treatments or postharvest storage timepoints. Higher *Salmonella*

populations observed in organic fertilizer treatments on grow mats and kale microgreens during production suggest nutrient-rich organic substrates support pathogen persistence. These findings further demonstrated *Salmonella* can be transferred via fertigation solutions to edible microgreen tissues. The persistence of *Salmonella* throughout production and cold storage highlights fertigation as a critical pathway of contamination and elucidates the need to integrate fertilizer management into microgreen food safety strategies.

1. Introduction

Microgreens have gained popularity as young edible seedlings harvested at the cotyledon or first true leaf stage (Xiao et al., 2012). Due to their short production cycle, typically 10 to 21 days, and high commercial value, microgreens have become increasingly important crops in controlled-environment agriculture (CEA) systems (Turner et al., 2020). Despite these advantages, microgreens present food safety concerns, particularly related to *Salmonella enterica*, a leading cause of foodborne illness responsible for approximately 1.35 million infections annually in the United States (CDC, 2024). Microgreens are typically consumed with little to no processing, which increases the likelihood that contamination will persist until consumption (Choe et al., 2018; Renna et al., 2017; Yeni et al., 2016). Moreover, they are commonly produced in CEA systems where warm temperatures, high humidity, and nutrient-rich conditions support plant growth but also favor the survival of foodborne pathogens (Di Gioia et al., 2017; Xavier et al., 2025).

The increasing demand for raw, minimally processed produce has been linked to foodborne outbreaks (Fatica & Schneider, 2011; Reddy et al., 2016). Several *Salmonella* outbreaks have been associated with bean sprouts and leafy greens. While no outbreaks have been affiliated with microgreens, several recalls related to *Salmonella* and *Listeria* contamination have been recently reported in North America (Chen et al., 2023; Topalcengiz et al., 2024). These risks are further compounded by the absence of effective kill steps, allowing contamination introduced during production, harvest, or postharvest handling to persist through consumption (Yeni et al., 2016). *Salmonella* can also internalize into plant tissues and persist within apoplastic spaces, reducing the effectiveness of surface sanitation strategies (Grivokostopoulos et al., 2022). Although microgreens are stored under refrigeration to maintain quality, information on pathogen persistence during postharvest storage remains limited (Mir et al., 2017).

Fresh produce can become contaminated at multiple stages throughout production and postharvest handling (Beuchat & Ryu, 1997; Uyttendaele et al., 2015). In microgreen production systems, *Salmonella* and other foodborne pathogens may be introduced through contaminated seeds, irrigation water, substrates, nutrient solutions, equipment, workers, or inadequate sanitation practices (Riggio et al., 2019; Topalcengiz et al., 2024; Isik et al., 2026). Among these, contaminated seeds and irrigation water have been identified as the primary preharvest sources of foodborne pathogens in sprout and microgreen production (Reed et al., 2018; Chen et al., 2023).

Fertilizers, which are routinely applied to enhance microgreen growth and quality (Kyriacou et al., 2016), represent a less studied but potentially important contamination

pathway. As organic fertilizers become more widely used, concerns have grown about their potential to introduce and sustain foodborne pathogens. Manure-based amendments can harbor *Salmonella* and facilitate its persistence in substrates and soils (Islam et al., 2004; Franz & van Bruggen, 2008; Jacobsen & Bech, 2012), and recent evidence suggested organic fertilizers promote *Salmonella* survival in irrigation systems through enhanced nutrient availability and biofilm formation (Raad et al., 2025). However, limited information is available on how fertilizer type, particularly organic versus synthetic sources, influences *Salmonella* survival in microgreen production systems.

Although numerous studies have evaluated food safety risks in leafy greens and sprouts, research on microgreens remains comparatively limited despite similar production practices and raw consumption (Riggio et al., 2019). Existing studies have largely focused on contamination sources such as seeds, irrigation water, and substrates (Riggio et al., 2019; Reed et al., 2018; Isik et al., 2024; Xavier et al., 2025; Rao et al., 2025), with less emphasis on how production inputs influence pathogen persistence or survival during postharvest storage (Xavier et al., 2026). Therefore, the objective of this study was to evaluate the effects of organic plant-based (Earth Juice Grow), organic animal-based (Fish Emulsion), and synthetic (Miracle-Gro) fertilizers on *Salmonella enterica* survival and transfer during microgreen production and postharvest storage. It was hypothesized that organic-based fertilizers would support greater *Salmonella enterica* survival than synthetic fertilizers due to the increased availability of organic matter and nutrients favoring pathogen persistence.

2. Materials and Methods

2.1 Microgreen Production

The study was conducted in a Biosafety Level 2 (BSL-2) laboratory located at Auburn University, Auburn, Alabama, USA. Kale (*Brassica oleracea* L. var. *acephala*) was used as a model crop in this study. Seeds were purchased from Johnny's Selected Seeds (Winslow, Maine, USA) and stored at 4 °C until use. Coconut coir fiber hydroponic grow mats (10 x 20 inch; True Leaf Market, Salt Lake City, UT, USA) were used as the growing substrate for microgreen production. The grow mats were cut to fit the dimensions of the trays.

Before sowing, standard plastic trays (25 x 13.5 cm; length x width) were disinfected with 70% ethanol. The grow mats were then placed into the trays and pre-moistened with sterile deionized water, and the seeds were sown at a rate of 3.95 g seeds per tray. The seeding rate was calculated using microgreens seed density calculator developed by Penn State Extension (<https://extension.psu.edu/microgreens-seed-density-calculator>). The trays were covered and allowed to germinate for 3 days in the dark at room temperature. Following germination, microgreens were transferred to a controlled growth chamber and grown under full spectrum light-emitting diode (LED) grow lights (Feit Electric, Pico Rivera, CA, USA). Microgreens were grown at a light intensity of 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a 16 h light and 8 h dark photoperiod, as commonly used for microgreen production under controlled-environment systems (Ying et al., 2020; Toscano et al., 2021). Light intensity was monitored using PAR meter and a HOBO light meter (model no. H21-USB) during experimental setup to ensure uniform light distribution across the growing area.

2.2 Fertilizer Treatments

To evaluate the effects of fertigation on *Salmonella enterica* survival in microgreens, three fertilizer treatments were used: one synthetic and two organic fertilizers (animal-based and plant-based). The synthetic fertilizer used was Miracle-Gro All Purpose Water-Soluble Plant Food comprised of 24% total nitrogen, 8% available phosphorus (P_2O_5), and 16% soluble potash (K_2O). Miracle-Gro was diluted in deionized water at a concentration of $0.312\text{ g}\cdot\text{L}^{-1}$. The animal-based fertilizer was a liquid Fish Emulsion (Indian River Organics), containing 2.0% total nitrogen, 3.0% available phosphorus and 1.0% soluble potash (K_2O). This fertilizer was dissolved in deionized water at a concentration of $7.0\text{ mL}\cdot\text{L}^{-1}$. Finally, the plant-based fertilizer was a natural-based liquid plant food (Earth Juice Grow), containing 2% total nitrogen and 1% available phosphate and diluted in deionized water at a concentration of $8\text{ mL}\cdot\text{L}^{-1}$.

Prior to inoculation, fertilizer solution samples were collected and stored at $4\text{ }^{\circ}\text{C}$ until analysis. Concentrations of P, K, Ca, Mg, Fe, B, Cu, Zn, Mn, Na, and Ni were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) following EPA Method 200.7, and nitrate concentrations were measured using EPA Method 353.2 at the Soil, Forage, and Water Testing Laboratory at Auburn University (Auburn, AL). The results are presented in **Table 3.1**.

2.3 Preparation of Bacterial Cocktail

A *Salmonella enterica* cocktail was prepared using two serovars obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA): *S. enterica* serovar Typhimurium (ATCC 14028) and *S. enterica* serovar Enteritidis (ATCC 13076). Each strain was gradually adapted to 50 ppm rifampicin (Sigma-Aldrich, Saint Louis, MO,

USA) and 50 ppm nalidixic acid (Sigma-Aldrich, Saint Louis, MO, USA), following the method described by Pizzo et al. (2023). Briefly, each *Salmonella* strain was cultured in Tryptic Soy Broth (TSB) supplemented with nalidixic acid, with the antibiotic concentration gradually increased from 10 to 50 ppm through successive 24 h transfers. The same procedure was then repeated with rifampicin until the strains were resistant to 50 ppm of both antibiotics. A 500- μ L aliquot of each adapted culture was separately combined with 500 μ L of a sterile glycerol-deionized water solution (1:1, v/v) in a 2-mL screw-cap tube and stored at -80 °C until further use.

For bacterial recovery, a 10 μ L loopful of each pre-adapted strain was streaked separately onto Tryptic Soy Agar (TSA; BD Difco Sparks, MD, USA) supplemented with 50 ppm nalidixic acid and 50 ppm of rifampicin (TSANR). The plates were incubated at 37 °C for 24 h. A single well-isolated colony from each strain was transferred into 10 mL Tryptic Soy Broth (TSB; BD Difco, Sparks, MD, USA) supplemented with 50 ppm of nalidixic acid and 50 ppm of rifampicin (TSBNR). The cultures were incubated at 37 °C for 24 h at 120 rpm in a shaking incubator (Solaris 4000, Thermo Fisher Scientific, Waltham, MA, USA).

Following incubation, 10 μ L of each culture was transferred into fresh TSBNR and incubated under the same conditions. This transfer procedure was performed twice. Then, 10 μ L of each *S. Typhimurium* and *S. Enteritidis* culture was inoculated into the same tube containing 10 mL of fresh TSBNR. The resulting mixed culture was incubated at 37 °C for 24 h at 120 rpm. Next, 10 μ L of the mixed culture was transferred into fresh TSBNR and incubated under the same conditions. The mixed culture was grown in 200 mL of TSBNR to obtain a larger volume of bacterial inoculum.

The bacterial cocktail was transferred into sterile 50-mL centrifuge tubes and centrifuged at $4,122 \times g$ for 5 min using a 5810 R centrifuge (Eppendorf, Hamburg, Germany). The supernatant was discarded, and the bacterial pellets were washed twice with phosphate-buffered saline (PBS) prepared in the laboratory using 8.0 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 , and 0.24 g KH_2PO_4 per liter of deionized water. The washed pellets were resuspended in 10 mL of sterile PBS and mixed using a VX100 vortex mixer (Labnet International, Woodbridge, NJ, USA). The bacterial cocktail was adjusted to a 0.5 McFarland standard using sterile PBS to obtain an approximate concentration of $1.5 \times 10^8 \text{ CFU} \cdot \text{mL}^{-1}$.

2.4 Fertilizer Inoculum Preparation

A volume of 160 mL of the bacterial cocktail was mixed with 1,440 mL of each fertilizer solution to obtain a final bacterial concentration of approximately $10^7 \text{ CFU} \cdot \text{mL}^{-1}$. The inoculated fertilizer solutions were homogenized by stirring for 2 min before application. Following the 3-day germination period, the growing trays were uncovered and exposed to LED lighting conditions. Then, 1600 mL of the inoculated fertilizer solution was applied to each holding tray and supplied to the growing tray by subirrigation. After the initial inoculation, the holding trays received 200 mL of the corresponding non-inoculated fertilizer solution every other day to maintain moisture throughout the growth period.

2.5 Experimental Design

The experiment was conducted using a completely randomized design (CRD) with three independent replications per treatment ($n = 3$). To evaluate *S. enterica*

survival during both production and postharvest phases, two trays were assigned per replication and fertilizer treatment ($n = 24$). One tray was designated for destructive sampling during the production phase, including microgreen and grow mat collection at each timepoint. The second tray was reserved for postharvest evaluation and remained undisturbed until harvest, after which microgreens were collected and monitored during refrigerated storage.

For the production phase, microgreens and grow mats were sampled at days 1, 3, 5 and 8 after application of the inoculated fertilizer solution to evaluate the transfer and survival of *S. enterica* ($n = 96$). For the postharvest phase, microgreens were harvested at the commercial stage (8 days after germination), placed in plastic clamshells and stored at 4 °C. Microgreen samples were collected on Day 1 and 7 after harvest ($n = 24$). At each sampling timepoint, samples were aseptically collected from each replicate for microbiological analysis.

2.6 Sample Collection and Microbial Analysis

At each sampling timepoint, a 76.7 cm² section of the pre-cut grow mat was lifted using sterile forceps. Following this, 5 to 9 g of edible portions of kale microgreens were harvested from the same grow mat using the sterile surgical scissors. The grow mat and microgreen samples were placed in separate pre-labeled Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA) and weighed.

For grow mat samples, buffered peptone water (BPW; Becton, Dickinson and Company, Sparks, MD, USA) was added at a sample-to-solution ratio of 1:4 (w/v). For microgreens, PBS supplemented with 0.2 % Tween 80 was added at a sample-to-

solution ratio of 1:5 (w/v). The volume of each solution was adjusted according to the exact weight of the sample.

The samples were homogenized using a stomacher (Stomacher® 400 Circulator, Seward Inc., Bohemia, NY, USA) at 300 rpm for 30 s and then manually massaged for an additional 30 s. Serial tenfold dilutions of the homogenates were prepared in BPW. A 100- μ L aliquot of each dilution was spread-plated onto TSANR, and the plates were incubated at 37 °C for 24 h. Following incubation, colonies were counted, and bacterial populations were expressed as log CFU·g⁻¹.

Postharvest microgreen samples collected on days 1 and 7 of refrigerated storage were analyzed using the same procedure described for microgreen samples collected during production.

2.7 Statistical Analysis

Data were analyzed using JMP Version 18.1 (SAS Institute, Cary, NC, USA). Colony counts were converted to log CFU·g⁻¹ for both grow mats and microgreens. Data collected during production were analyzed separately for grow mats and microgreens using a mixed-effects model to account for repeated measurements over time. Fertilizer type, sampling time, and their interaction were included as fixed effects, and tray (replication) was included as a random effect. Sampling time was treated as a repeated measure within each tray, and an appropriate covariance structure was selected based on model fit.

For postharvest storage, microgreen data were analyzed using a similar mixed-effects model with fertilizer type, storage time, and their interaction as fixed effects, and tray as a random effect with repeated measures over storage time. Statistical

significance was determined at $\alpha = 0.05$. When significant effects were detected ($p < 0.05$), mean separations were conducted using Tukey's honestly significant difference (HSD) test.

3. Results

3.1 Survival of *S. enterica* in Grow Mats During Production

The *Salmonella* populations in grow mats were significantly affected by fertilizer treatment and sampling time, whereas fertilizer x sample time interaction was not significant (**Table 3.2**). Among the fertilizer treatments, the highest populations were recovered from grow mats treated with organic plant-based fertilizer Earth Juice Grow (7.28 log CFU·g⁻¹). In contrast, the lowest were observed in grow mats treated with synthetic fertilizer Miracle-Gro (6.17 log CFU·g⁻¹) (**Fig. 3.1a**). Following application of inoculated fertilizer solutions, populations were 6.9 log CFU·g⁻¹ on Day 1 and remained relatively stable through Day 5 (6.83 log CFU·g⁻¹). By the end of the production period (Day 8), populations decreased to 6.05 log CFU·g⁻¹, with a reduction of 0.85 log CFU·g⁻¹ compared to Day 1 (**Fig. 3.1b**).

3.2 Survival of *S. enterica* in Microgreens During Production

Sampling time had a significant effect on *Salmonella* populations in microgreens, while no differences were found among fertilizer type or the interaction of fertilizer x sample time (**Table 3.2**). *Salmonella* populations remained relatively consistent across plant-based Earth Juice Grow (5.18 log CFU·g⁻¹), animal-based Fish Emulsion (4.95 log CFU·g⁻¹) and synthetic Miracle-Gro (4.64 log CFU·g⁻¹), respectively (**Fig. 3.2a**).

Conversely, during the production period *Salmonella* populations were highest on Day 1 (5.63 log CFU·g⁻¹) and remained relatively stable through Day 5 (4.94 CFU·g⁻¹). By Day 8, populations had decreased to 3.74 CFU·g⁻¹, indicating an overall reduction of 1.89 log CFU·g⁻¹ (**Fig. 3.2b**).

3.3 Survival of *S. enterica* in Microgreens During Postharvest

During postharvest storage, *Salmonella* populations were not significantly affected by fertilizer treatment and storage timepoint. A significant interaction of fertilizer x storage timepoint was observed (**Table 3.2**). A numerical decrease of 2.17 log CFU·g⁻¹ and 0.35 log CFU·g⁻¹ was observed in microgreens treated with organic plant-based Earth Juice Grow and organic animal-based Fish Emulsion fertilizers, respectively. While a slight numerical increase of 1.13 log CFU·g⁻¹ *Salmonella* was observed in samples treated with synthetic Miracle-Gro. Although numerical differences were observed among fertilizer x storage timepoint combinations, Tukey's HSD test did not show significant pairwise differences (**Fig. 3.3**).

4. Discussion

In the present study, fertilizer treatment significantly affected *S. enterica* populations in grow mats, indicating that fertilizer composition influenced pathogen persistence during microgreen production. Both organic plant-based and animal fertilizers contain materials that increase nutrient availability and provide carbon for microorganisms, which supported higher *S. enterica* compared to synthetic fertilizer (Holley et al., 2006). Among the treatments, *S. enterica* populations were highest in

plant-based fertilizer, possibly due to the presence of molasses as a readily available carbon source for microbial growth. This is consistent with Duffy et al. (2004), who reported increased *Salmonella* regrowth with higher molasses concentrations in compost teas. Similarly, Raad et al. (2025) observed greater *Salmonella* populations in organic fish emulsion compared to synthetic fertilizers.

Regardless of fertilizer treatment, *S. enterica* populations remained relatively stable during early stages of production before gradually declining over time in both grow mats and microgreens. Consistent with our findings, Isik et al. (2024) also reported *Salmonella* persisted throughout microgreen production but gradually decreased over time as the crop developed. The initial stability may be associated with the nutrients released during seed germination and early plant growth, which support the pathogen survival (Wright and Holden, 2018). As production progressed, reduced nutrient availability, along with increasing competition from the background microbial communities may have limited pathogen persistence (Shaw et al., 2016).

In the present study, *S. enterica* populations were consistently higher in grow mats than in microgreens throughout the production period. This is likely because the grow mats were in direct contact with the inoculated fertilizer solution, whereas the edible tissues had less direct exposure to the contamination source. Previous studies have also reported greater *Salmonella* persistence in soilless growing media than in harvested plant tissues (Rao et al., 2025). Misra and Gibson (2020) suggested the physical properties of growing media, particularly greater water-holding capacity and organic carbon availability, create a more favorable environment for pathogen survival by reducing desiccation stress. These findings indicated organic growing substrate

served as an important reservoir for *S. enterica* during production and contributed to contamination of the edible portions of the crop.

Following production, persistence of *S. enterica* during postharvest storage was also evaluated. Fertilizer treatment and storage time did not significantly affect *Salmonella* populations, but a significant fertilizer x storage timepoint interaction was observed during the postharvest storage of microgreens. Interestingly, *Salmonella* remained detectable throughout refrigerated storage. Our results were consistent with previous studies by Tomás-Callejas et al. (2011), who reported that *E. coli* and *E. coli* O157:H7 were detectable on pre-inoculated mizuna, tatsoi and red chard during 7 days of storage at 5 °C, with only limited reductions in bacterial populations. Similarly, Xavier et al. (2026) reported the persistence of *Salmonella Enteritidis* in mustard and sunflower microgreens during refrigerated storage at 5 °C after inoculation into the growing substrate. Comparable findings were reported by Gandhi et al. (2001), who observed a small reduction in *Salmonella Stanley* populations on alfalfa sprouts during 4 days of storage at 4 °C. Refrigeration limits bacterial growth and extends product shelf life but does not eliminate *Salmonella*, allowing the pathogen to remain viable on fresh produce during storage (Delbeke et al., 2015). Therefore, preventing contamination during production and harvest is critical because refrigerated storage alone is insufficient to eliminate *Salmonella* from contaminated microgreens.

5. Conclusion

Fertilizer type significantly influenced the survival of *S. enterica* in grow mats during microgreen production, with plant-based fertilizer supporting the highest

pathogen populations. Although *S. enterica* populations declined during production, the pathogen remained detectable throughout production and postharvest storage. The consistently higher population observed in grow mats suggested that the growing substrate serves as an important reservoir for pathogen persistence. These findings further indicated contamination introduced through fertilizer can persist in the growing system and can be transferred from the contaminated substrate to microgreens during production. Therefore, commercial growers should carefully consider fertilizer source and microbial quality when developing production practices to minimize pathogen contamination and improve product safety.

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Tables

Table 3.1. The pH, electrical conductivity (EC), and macronutrient and micronutrient composition of fertilizer solutions used during kale microgreen.

Treatment	pH	EC	Nitrate	P	K	Mg	Ca	Na	Fe	Zn	B	Mn	Cu	Ni
Organic Fish Emulsion	3.93	0.76	BDL	95.97	16.62	2.87	39.97	9.94	0.10	0.19	BDL	BDL	0.06	BDL
Organic Earth Juice	3.45	0.36	BDL	28.04	7.19	1.67	26.77	9.85	0.12	0.06	BDL	BDL	0.05	BDL
Synthetic Miracle Grow	6.19	0.30	0.90	8.17	39.40	0.10	0.16	1.91	0.53	0.14	BDL	0.14	0.29	BDL

BDL = Below detection limit. Electrical conductivity values are expressed as $\text{dS} \cdot \text{m}^{-1}$. Macronutrients (Total N, Nitrate, P, K, Mg, and Ca) and micronutrients (Na, Fe, Zn, B, Mn, Cu, and Ni) concentrations are expressed as ppm.

Table 3.2. P-values for the effects of fertilizer type, sampling time, and their interaction on *Salmonella enterica* populations in grow mats and microgreens during production and in microgreens during postharvest storage.

Independent Variables	Dependent Variables		
	Production	Production	Postharvest
	Grow mats	Microgreens	Microgreens
Fertilizer Type	0.0007	0.3964	0.6999
Sample Time	<.0001	<.0001	0.2875
Fertilizer x Sample Time	0.2908	0.2114	0.0397

P-values were obtained from a two-way analysis of variance (ANOVA) conducted using JMP Pro 18.1 (SAS Institute Inc., Cary, NC, USA). Significance was defined as $P < 0.05$ ($\alpha = 0.05$).

Figures

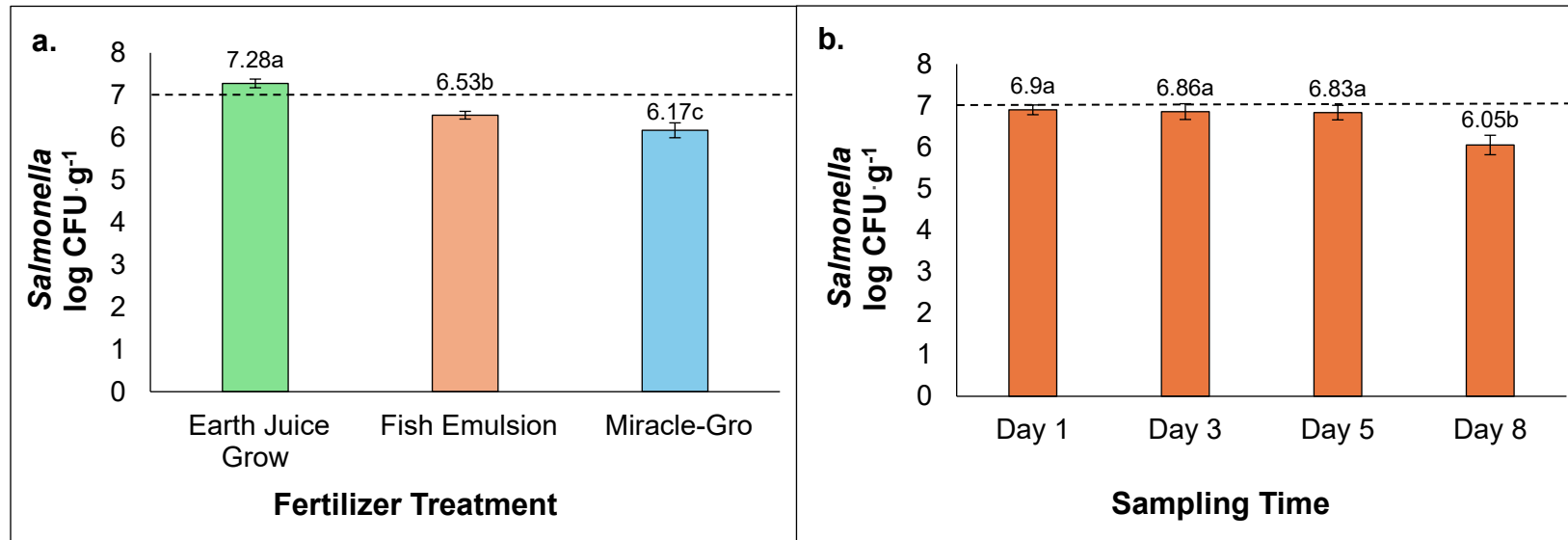


Fig. 3.1 *Salmonella enterica* populations on grow mats during production, affected by fertilizer treatment (a) and over the production period across sampling days (b), as affected by fertilizer treatment. Bars represent mean *Salmonella* populations (log CFU·g⁻¹) ± standard error (SE). Different letters indicate significant differences among means according to Tukey's HSD test ($p < 0.05$). The dashed horizontal line represents the initial inoculum level (7 log CFU·mL⁻¹).

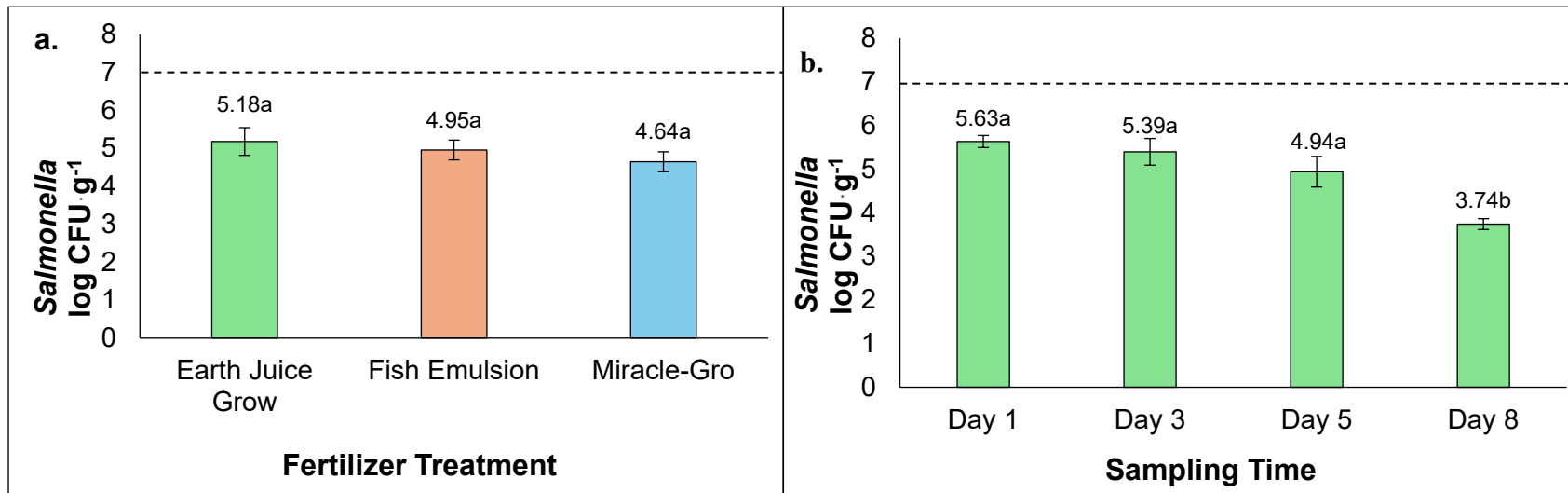


Fig. 3.2 *Salmonella enterica* populations on kale microgreens during production, shown by fertilizer treatment (a) and over the production period across sampling days (b). Bars represent mean populations (log CFU·g⁻¹) ± standard error (SE). Different letters indicate significant differences among means according to Tukey's HSD test ($p < 0.05$). The dashed horizontal line represents the initial inoculum level (7 log CFU·mL⁻¹).

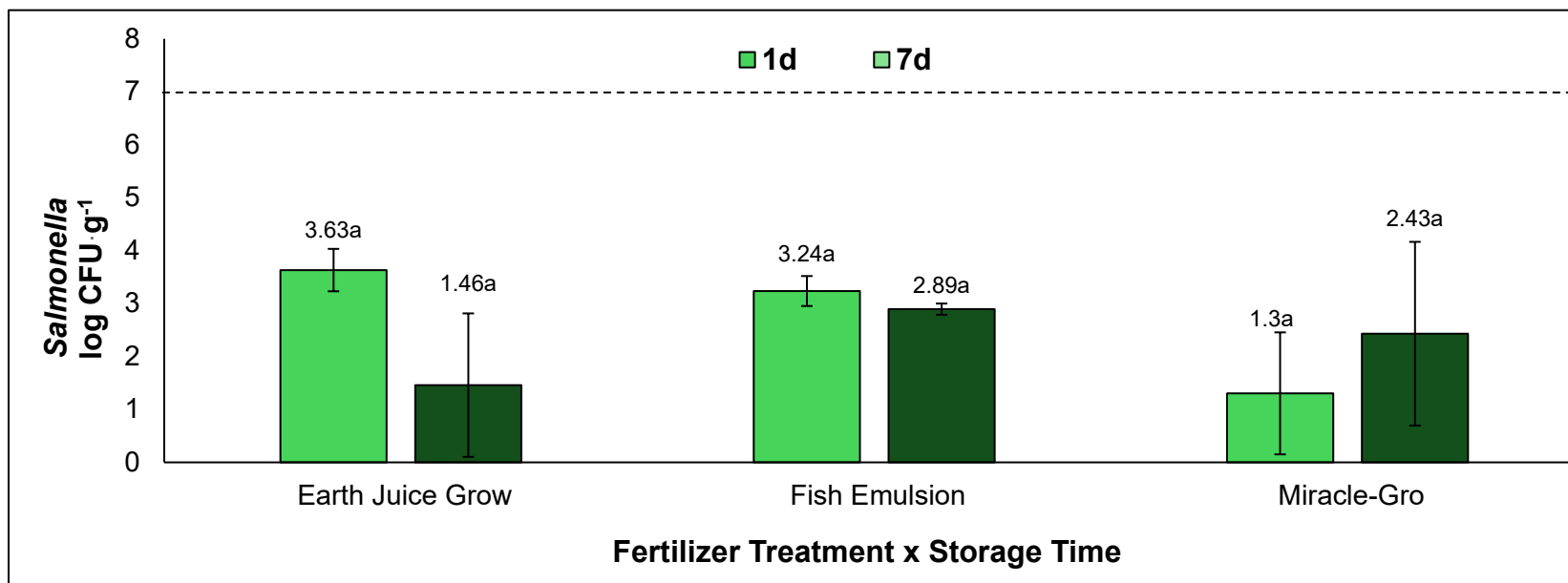


Fig. 3.3 Mean *Salmonella enterica* populations in kale microgreens during postharvest storage. Bars represent mean populations (log CFU·g⁻¹) ± standard error (SE). Means were separated using Student's *t*-test ($p < 0.05$). The dashed horizontal line represents the initial inoculum level (7 log CFU·mL⁻¹). Days (d) in cold storage are expressed as 1d and 7d, respectively.