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Food Science and Technology Department
B. Sc Food Science and Technology Major



Special Graduation Project

**Survival of *E. coli* O157:H7 on hydroponically grown traditional and
microgreen Dark Opal and Cinnamon basil (*Ocimum basilicum*) roots
and the water under home scale conditions**

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Honduras, november 2024

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Resumen

La albahaca es una de las hierbas culinarias más populares por su diversidad en aromas y sabores. Sin embargo, en sistemas hidropónicos, es susceptible a la contaminación, lo que puede provocar brotes de enfermedades si no se maneja adecuadamente la producción. En este estudio, se utilizaron dos métodos de crecimiento: tradicional y microplantas, con cuatro unidades cada variedad, tres inoculadas con *E. coli* O157 (GFP35150) y una como control. Para mantener un ambiente adecuado, se ajustó diariamente el pH (6.0 a 6.4) y la electroconductividad (1.0 a 1.40 mS/cm). El objetivo fue evaluar la supervivencia de *E. coli* O157 H7 en cultivos de albahaca morada y canela. Se inoculó *E. coli* O157 H7 en tres unidades hidropónicas y microplantas de cada cultivar. Se tomaron muestras en los días 0, 3, 6, 9, 12, 15, 18, 21, 24 y 27 para unidades hidropónicas y en los días 0, 3, 6, 9 y 14 para microplantas. Los resultados mostraron que *E. coli* O157 H7 estaba presente en la solución de nutrientes y raíces el día 0. Sin embargo, la cantidad de bacterias disminuyó a lo largo del estudio. Para el día 18, no se detectaron conteos de *E. coli* O157 H7 en la solución de nutrientes, y a partir del día 21, ya no se encontraron en las raíces en el sistema hidropónico. En las microplantas, la presencia de *E. coli* O157 H7 en las raíces y en la manta de coco se observó hasta el último día de muestreo (día 14), aunque en menor cantidad comparado con las plantas tradicionales.

Palabras clave: Albahaca morada, canela , *E. coli* O157 H7, microplantas, tradicional

Abstract

Basil is one of the most popular culinary herbs due to its diverse aromas and flavors. However, in hydroponic systems, it is susceptible to contamination, which can lead to disease outbreaks if production is not managed properly. This study used two growth methods: traditional and microgreens, with four units each variety, three inoculated with *E. coli O157 H7* (GFP35150), and one as a control. To maintain an adequate environment, the pH (6.0 to 6.4) and electrical conductivity (1.0 to 1.40 mS/cm) were adjusted daily. The objective was to evaluate the survival of *E. coli O157 H7* in Dark opal basil and cinnamon basil crops. The *E. coli O157 H7* was inoculated in three hydroponic and microgreen units of each cultivar. Samples were taken on days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 27 for hydroponic units and on days 0, 3, 6, 9, and 14 for microgreens. The results showed that *E. coli O157* was present in the nutrient solution and roots on day 0. However, the bacteria count decreased throughout the study. By day 18, no *E. coli O157 H7* counts were detected in the nutrient solution, and from day 21, none were found in the roots in the hydroponic system. In the microgreen, the presence of *E. coli O157 H7* in the roots and mats was observed until the last sampling day (day 14), although in smaller quantities compared to traditional plants. Moreover, with no significant differences for both basil varieties in traditional and microgreens.

Keywords: Cinnamon, Dark Opal, *E. coli O157 H7*, microgreens, traditional

Introduction

Basil (*Ocimum basilicum L.*) is an important essential oil plant, medicinal herb, and culinary spice from the Lamiaceae family, thriving in tropical and subtropical regions. Moreover, its essential oil is utilized in oral health and dental products and has applications in the fragrance and food industries (Shahrajabian et al., 2020). Given its diverse applications and increasing demand, the fresh herbs industry is projected to grow from USD 165.2 Billion in 2024 to USD 407.9 Billion by 2032, exhibiting a compound annual growth rate (CAGR) of 16.26% during the forecast period (2024 - 2032). Essential oils (EO), commonly volatile oils, are complex mixtures of low molecular weight compounds extracted from aromatic plants (Singh Chouhan et al., 2019). The essential oils derived from basil possess significant antimicrobial properties, making them valuable in various applications, including food preservation and healthcare. Furthermore, basil synthesizes various polyphenolic compounds, including rosmarinic acid, a derivative of cinnamic acid known for its strong antioxidant properties (Nguyen y Niemeyer, 2008). However, microgreens basil have been shown to contain higher concentrations of active compounds (phenolics, vitamins and minerals, etc.), compared to those found in mature plants or seeds (Mir et al., 2017).

According to Nguyen y Niemeyer (2008), polyphenolic compounds are produced by plants throughout their development for various purposes, including defense against microorganisms, insects, or herbivores; enhancing nutrient availability; adapting to exposure to ultraviolet radiation; and mediating allelopathic interactions. The primary constituents of basil essential oil are produced through two distinct biochemical pathways: phenylpropanoids, which include compounds like methyl chavicol (estragole), eugenol, methyl eugenol, and methyl cinnamate, are synthesized via the shikimic acid pathway; and terpenoids, such as linalool and geraniol, are synthesized through the mevalonic acid pathway (Stanojevic et al., 2017). Sinha, G. K y Gulati, B. C. (1990), discovered that each of these basil essential oils was effective against several bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella boydii*, and *Proteus vulgaris*.

This broad-spectrum antimicrobial activity demonstrates the significant potential of basil essential oils in various applications, particularly in enhancing food safety and preservation. Additionally, due to the growing consumer preference for natural over synthetic food additives, naturally derived antimicrobial agents such as basil are becoming increasingly significant in antimicrobial packaging. These natural agents are perceived to present a lower risk to consumers (Suppakul et al., 2003).

As the demand for sustainable agricultural practices increases, hydroponics has emerged as a promising solution. In addition, Hydroponics is a type of agriculture that does not require soil for farming and has gained popularity due to its ability to reduce dependence on agricultural land and pesticides. It can also be used on poor soil, which can help mitigate air pollution (Rajendran et al., 2024). One of the hydroponic systems used by the community is Deep Water Culture (DWC). DWC is a hydroponic system that supplies nutrient solution directly to the roots of the plants (Nursyahid et al., 2021). This method ensures efficient nutrient uptake and optimal growth conditions for the plants.

In recent years, fresh produce has been increasingly associated with outbreaks of foodborne illnesses caused by *Escherichia coli* O157 and *Salmonella* spp linked to the consumption of leafy greens have increased (Deering et al., 2012). Contamination of fresh produce poses a public health risk because there is no terminal kill step during harvest or at the processing facility to eliminate pathogens (Macarisin et al., 2014). Furthermore, *E. coli* O157 is responsible for approximately 63,000 cases of hemorrhagic colitis annually in the United States, and global incidence data from 10 of 14 World Health Organization subregions estimate 2.8 million cases each year (Ameer et al., 2024).

According to the Food and Drug Administration (2024), Infinite Herbs, LLC extended its recall to cover Melissa 's-brand organic basil, with the CDC reporting 36 cases across 14 states, resulting in four hospitalizations but no deaths associated with this incident. However, it is important to mention

that no such outbreak has been reported so far but still, the food safety of microgreens is the top priority concerning the health of the consumers (Bunning, 2019).

First to evaluate the survival of the *E. coli* 0157:H7 in the traditional Dark Opal and Cinnamon cultivars.

Second, to examine the survival of the *E. coli* 0157:H7 in the microgreens Dark opal and Cinnamon cultivars.

Last to compare the survival of the *E. Coli* 0157:H7 of the nutrient solution and roots for the varieties Dark Opal and Cinnamon.

Materials and Methods

Study Site

The study took place at the Experimental Sciences Building within the International Center for Food Industry Excellence (ICFIE) at Texas Tech University, located in Lubbock, Texas, USA.

Inoculum Preparation

Re-activation Procedure: The strains were first resuscitated by incubating them in 10 mL of Brain Heart Infusion (BHI) broth overnight. This step aimed to bring the bacterial strains back from cryogenic storage, ensuring their viability and metabolic activity for future experiments.

Following resuscitation, the concentration of the bacterial strains was determined utilizing plating techniques to calculate viable cell counts. The resuscitated strains were then centrifuged at $4,000 \times g$ for 10 minutes to pellet the cells. The resulting cell pellet was washed with 200 mL of sterile distilled water to remove any remaining media and concentrate the bacterial cells. The cell pellet was washed and suspended in sterile distilled water to achieve the desired inoculum concentration of 10^6 colony-forming units per milliliter (CFU/mL). This meticulously prepared inoculum solution was then used for standardized inoculation across experimental data units, ensuring consistency and reliability to get accurate results. Four milliliters of the 10^6 CFU/mL inoculum were transferred and introduced into the four-liter nutrient solution of each hydroponic unit. For the microgreens, we used 500 ml of distilled water and introduced four milliliters of 10^5 CFU/mL of the inoculum for each tray.

Media Preparation

To prepare MacConkey agar with 200 $\mu\text{g}/\text{mL}$ ampicillin to reduce background microflora, first dissolve the appropriate amount of MacConkey agar powder in distilled water and heat the mixture until fully dissolved. Sterilize the solution by autoclaving it at 121 °C for 15-20 minutes. After sterilization, cool the agar to approximately 50 °C to prevent antibiotic degradation. Prepare a filter-sterilized stock solution of ampicillin at 0.2 mg/mL, then add the necessary volume to the cooled agar to achieve a final concentration of 200 $\mu\text{g}/\text{mL}$. Mix thoroughly and pour the agar into sterile Petri

dishes, allowing it to solidify at room temperature. Once solidified, store the plates in a refrigerator at 2-8 °C until use, ensuring they are sealed to prevent contamination and drying. This process ensures selective growth of target microorganisms while reducing the presence of background microflora.

Methodology of Traditional Method- Germination

Traditional Hydroponic method growing: The experiment began with meticulous sanitization and disinfection procedures for the growth chambers, trays, and hydroponic units. Therefore this step established a sterile environment conducive to accurate experimentation. Basil seeds were then germinated in a controlled environment with a constant temperature of 24 °C and 90% relative humidity provided by a growth chamber from Percival Scientific with Controlled Environment Agriculture, using Rockwool plugs to optimize seedling development. A 100-ppm nutrient solution was meticulously prepared to meet Basil's optimal growth requirements using two formulations denoted as Part A and Part B. This solution aimed to replicate the essential nutrients required for basil plant development. Each hydroponic unit was formed by adding four liters of the nutrient solution. Healthy seedlings, typically displaying three to four true leaves, were carefully selected and transplanted into their respective hydroponic units to ensure uniformity throughout the experiment.

Inoculation in the Traditional

For inoculation using the traditional method, the plants were allowed to acclimate for 2 days in the hydroponic system. Subsequently, 4 mL of the solution with a concentration of 10^6 CFU/mL was transferred to the 4-liter nutrient solution tank of the hydroponic unit (through one of the holes on the top of the unit), achieving a final concentration of 10^5 CFU/mL. The pump was then run for 30 minutes to circulate the inoculum within the nutrient solution. Finally, 10 mL of the nutrient solution was sampled into a sterile empty tube, marking this as Day 0 for sampling.

Sampling Methodology

Nutrient solution samples were collected on days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 27. Similarly, root samples were taken on the same days. For the root samples, a rockwool cube containing the

respective plant was carefully pulled up, and the roots were cut off and placed into a stomacher bag. The roots were then weighed, and the appropriate amount of Buffer Peptone Water (BPW) was added by multiplying the weight by 9 to dilute the sample in a relation of 1:10. The sampling of the plants was evenly distributed over the specified time periods. For the nutrient solution samples, 10 mL were extracted from each of the 4 hydroponic units of Dark Opal and 4 units of cinnamon basil into sterile empty tubes. These samples were taken on the designated sampling days and directly plated onto MacConkey agar using the spread plating method.

Methodology of Microgreens Method-Germination

The growing process involves placing jute mats on the growing trays and then feeding 5 g of seeds onto these mat surfaces. The growth mats are then sprayed daily with 100 mL of deionized sterile water from a spray bottle. The prepared grow tray, covered with domes is then transferred to a growth chamber with a constant temperature of 24 °C and 90% relative humidity, kept in complete darkness. These trays remain undisturbed until day 5 of the culture period.

Inoculation in the microgreen

One milliliter from the 10^6 CFU/mL tube was transferred to the 100 ml nutrient solution, achieving a final concentration of 10^4 CFU/mL. The plants were then irrigated with this inoculated solution by placing it in the tray beneath the draining tray.

Sampling Methodology

A one-inch square sample of the growing mat was weighed, and the appropriate amount of Buffer Peptone Water (BPW) was added by multiplying the weight by 9. The sample was then homogenized in a stomacher for 2 minutes, designating this as Day 0. This sampling was conducted on Dark Opal and Cinnamon basil plants. There were four units for each basil variety, and in each unit, samples were taken from the grow mats and the roots.

Experimental Design

This project research used a Completely Randomized Design with measures repeated over time, performing four replicates for each treatment. Additionally, we used the t-test to compare the varieties and the samplings on each sampling day. This statistical test was chosen because it is effective for comparing the means of two independent groups to determine if there are significant differences between them.

Table 1 shows the sampling days for each unit in a traditional setup. Each units have sampling days scheduled every three days, starting from day 0 and continuing until day 27. The specific sampling days are days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 27.

Table 1

Sampling days for each unit in the traditional method.

	Unit 1	Unit 2	Unit 3	Unit 4
Sampling Days	0,3,6,9,12,15,18,21,24,27			

Table 2 presents the sampling days for each unit in a microgreens setup units 1, 2, 3, and 4 also have sampling days scheduled at regular intervals, but with a different frequency, covering days 0, 3, 6, 9, and 14.

Table 2

Sampling days for each unit in the Microgreens method.

	Unit 1	Unit 2	Unit 3	Unit 4
Sampling Days	0,3,6,9,14			

Results and Discussion

Basil is a popular choice for both container and hydroponic culture due to its high market demand, ease of cultivation, and rapid development cycle, which normally takes only about 28 days to reach maturity under excellent hydroponic conditions. In the presence of a source with nutrients, such as a hydroponic fertilizer solution, bacteria such as *E. coli* O157 H7 and *Salmonella* can survive and grow to levels that could cause serious illness in humans (Shaw et al., 2016). Table 3 shows that there are no noticeable differences between the nutrient solutions of Dark Opals and Cinnamon cultivars. Initially, both solutions had a significantly high log value, indicating a significant presence of microorganisms. However, as the observation period progressed, the logarithms of both solutions gradually decreased. This decrease indicates a decrease in the concentration of microorganisms over time, which can be influenced by factors such as nutrient depletion or environmental conditions.

Table 3

Counts of colony-forming units per milliliter (LogCFU/mL ± SD) of Dark Opal and Cinnamon nutrient solutions at different time intervals.

Time (Day)	Variety		C. V.%
	Dark Opal Nutrient Solution LogCFU/mL ± S. D.	Cinnamon Nutrient Solution LogCFU/mL ± S. D.	
0	5.72±0.14 ^a	5.63±0.18 ^a	2.92
3	3.63±0.52 ^b	3.91±0.38 ^b	12.04
6	1.06±0.09 ^c	1.49±0.42 ^c	18.94
9	1±0 ^d	1±0 ^d	0.00
12	1±0 ^d	1±0 ^d	0.00
15	0.33±0.57 ^e	0.67±0.57 ^e	43.30
18	0±0 ^e	0.33±0.57 ^e	0.00
21	0±0 ^{ef}	0±0 ^{ef}	0.00
24	0±0 ^{ef}	0±0 ^{ef}	0.00
27	0±0 ^{ef}	0±0 ^{ef}	0.00

Note. a-a Same letters represent that there are no differences. C. V. (%)= Coefficient of variation. CFU= Colony Forming Units. Log= Logarithms. S. D. = Standard Deviation.

In table 4 the data represent the number of colony-forming units per milliliter in roots treated with Dark Opal and Cinnamon solutions over a 27-day observation period. Both Dark Opal and cinnamon-treated roots showed significantly high colony-forming units, indicating a significant presence of microorganisms. However, by day 27, after the enrichment it can be observed that no colony-forming units were detected in the roots of both varieties. This absence indicates a significant reduction or destruction of microorganisms from root systems treated with both Dark Opal and cinnamon solutions.

Table 4

Counts of colony-forming units per milliliter (LogCFU/mL \pm SD) of Dark Opal and Cinnamon in the roots system at different time intervals.

Time (Day)	Variety		C. V. %
	Dark Opal Roots LogCFU/mL \pm S. D.	Cinnamon Roots LogCFU/mL \pm S. D.	
0	5.21 \pm 0.16 ^a	4.92 \pm 0.24 ^a	3.97
3	4.15 \pm 0.26 ^b	3.89 \pm 0.08 ^b	4.15
6	2.54 \pm 0.18 ^{bc}	2.33 \pm 0.41 ^{bc}	12.33
9	2.25 \pm 0.33 ^{cd}	2.09 \pm 0.30 ^{cd}	14.50
12	1.89 \pm 0.51 ^d	2.58 \pm 0.28 ^d	18.91
15	0.73 \pm 0.63 ^e	1.4 \pm 0.69 ^e	67.79
18	0.67 \pm 0.57 ^f	1.23 \pm 0.39 ^f	58.38
21	0.8 \pm 0.80 ^f	0.67 \pm 0.57 ^f	92.53
24	0.67 \pm 0.57 ^f	0 \pm 0 ^f	42.53
27	0 \pm 0 ^d	0 \pm 0 ^d	0.00

Notes. a-a Same letters represent that there are no differences, C. V.= Percentage Coefficient of variation . CFU: Colony Forming Units.

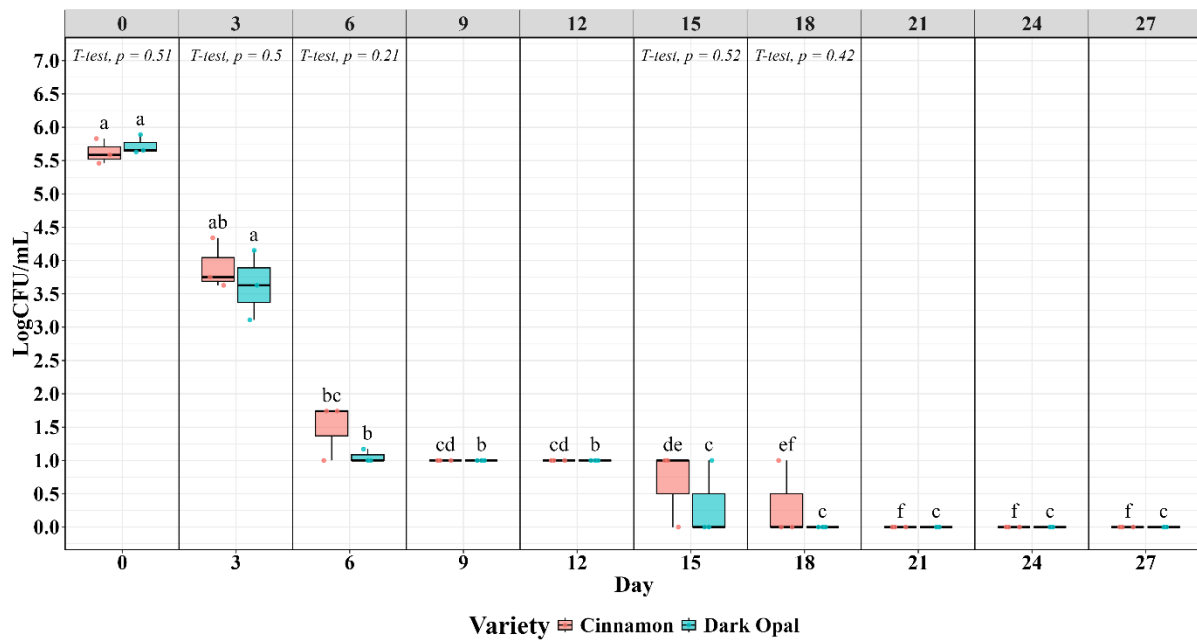
Log: Logarithms. S. D.= Standard Deviation.

The Figure 1 demonstrates the survival of *E. coli O157 H7* in the roots of two plant varieties, Cinnamon and Dark Opal, over 27 days in a nutrient solution. According to (Resh, 2016) the remaining six major elements are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) are particularly crucial for hydroponic growers. Like plants, bacteria also need a variety of nutrients for survival and growth. Shaw et al. (2016) noted that bacteria need a mix of elements such as carbon, oxygen, nitrogen, hydrogen, phosphorus, sulfur, potassium, magnesium, calcium, and iron. These nutrients are essential for bacterial metabolic processes and cellular functions. However, the consistent decline in *E. coli O157:H7* concentrations over time suggests that the nutrient solution, while initially supportive, does not sustain the bacteria in the long term. According to Sawyer (2021) the hydroponic system is challenged if the nutrients used were synthetic, as *E.coli* would face challenges to exist without a biological host or constant contamination. If an accidental exposure occurred in the hydroponics system, the pathogen would have become diluted or died off, making it undetected.

Over the entire 27-day observation period, the *E. coli O157 H7* concentrations in the nutrient solution of both plant varieties showed a consistent decline. A lack of organic matter in hydroponic systems can be the reason for the decrease in the presence of bacteria, as noted by (Dankwa, 2019). The lack of organic compounds creates an unfavorable environment for the strong growth and reproduction of *E. coli O157 H7* bacteria. Nevertheless, no statistically significant differences in *E. coli O157 H7* survival were detected between Cinnamon and Dark Opal at any measured time.

Figure 1

Comparative Survival of *E. coli* O157:H7 in Cinnamon and Dark Opal Over time in a Nutrient Solution.



Notes. (P value > 0.05) indicates no statistical differences, (P value < 0.05) indicates statistical significance.

Figure 2. illustrates the survival of *E. coli* O157 H7 in the roots of two plant varieties, Cinnamon and Dark Opal, over a period of 27 days. The concentration of *E. coli* O157 H7 is expressed in LogCFU/mL and is presented through box plots for each time interval measured (0, 3, 6, 9, 12, 15, 18, 21, 24, and 27 days). At each time interval, a T-test was conducted to compare the *E. coli* O157 H7 concentration between the two varieties. The p-values from these tests are displayed at the top of each time interval. A p-value less than 0.05 would indicate a statistically significant difference between the varieties.

On day 0, no significant difference was observed in the initial *E. coli* O157 H7 concentration between Cinnamon and Dark Opal ($p = 0.17$). By day 3, although the concentration decreased in both varieties, no significant difference was noted ($p = 0.23$). This trend continued day 6 ($p = 0.49$), day 9 ($p = 0.56$), day 12 ($p = 0.13$), day 15 ($p = 0.28$), day 18 ($p = 0.24$), day 21 ($p = 0.83$), day 24 ($p = 0.42$), and day 27, where the concentrations were nearly identical. Throughout the 27 days, the concentration of *E. coli* O157 H7 in the roots of both plant varieties progressively decreased.

According to Baetz (2016), root exudates in the rhizosphere act as chemical mediators in the interactions between plants and soil organisms, promoting positive interactions and serving as defense compounds in negative interactions. When a plant is under attack, root exudates are involved in two types of defense: direct and indirect. Exudates with direct defense properties repel, inhibit, or kill plant aggressors such as pathogens and herbivores. In contrast, exudates involved in indirect plant defense help initiate interactions with beneficial organisms that counteract the aggressors. These substances are released directly into the water surrounding the roots. In hydroponics, water serves as the primary medium where these substances dissolve and spread.

According to Bais et al. (2002), rosmarinic acid (RA) builds up in the roots of *Ocimum basilicum* (basil) as the plant grows. When the plant is attacked by pathogens, the roots release Rosmarinic Acid as part of their defense. This compound is very effective at inhibiting the growth of various soil microorganisms including potential human pathogens. As stated by Naylor y Coleman-Derr (2017), gram-negative bacteria are characterized by a thinner peptidoglycan cell wall layer than that of Gram-positive bacteria, which may render them less resistant to drought.

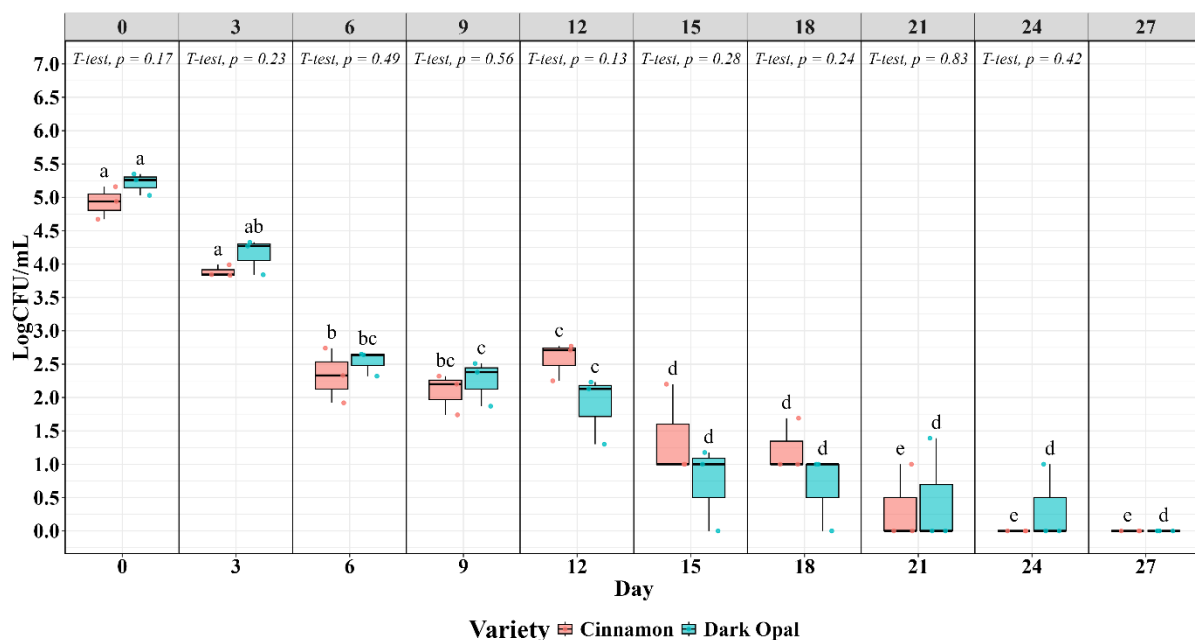
According to research by Herman et al. (2016), linalool, a monoterpene alcohol and a key component in many essential oils, exhibits significant antibacterial and antifungal properties. Their study also observed that with the application of 10 μ L of essential oil on *E. coli* at an approximate cell density of 1.5×10^8 CFU/mL, linalool demonstrated the highest antimicrobial activity.

Corresponding to Riggio et al. (2019), Water-grown (soil-less) plants such as in hydroponics/aquaponics may potentially be at a higher risk for pathogen internalization because they are continuously suspended in a liquid nutrient solution. Therefore, foodborne pathogens may have a greater chance of initial contact with the plant root system and subsequent attachment. Therefore, the structural integrity of the root system of plants grown in hydroponic systems seems critical for the ability of foodborne pathogens to internalize within these plants. However, at no point during the

study was there a statistically significant difference in the survival of *E. coli* 0157:H7 between the Cinnamon and Dark Opal varieties.

Figure 2

Comparative Survival of E. coli 0157:H7 in Cinnamon and Dark opal Over time in the roots.



Notes. (P value < 0.05) indicates statistical significance.

The data presented in Table 5 illustrate the level of microbial activity over time in two plant varieties, Dark Opal and Cinnamon, focusing on the roots and Mats. Initially (day 0), both cultivars had high microbial activity, as indicated by LogCFU values. As time progressed, the microbial activity in both cultivars decreased significantly, which can be seen as a decrease in LogCFU values. By day 14, microbial activity appears to be significantly reduced, with both cultivars having similar low LogCFU values of 1 (indicating minimal or no detectable microbial activity) and consistent standard deviations.

Table 5

The following table presents the counts of coly-forming units per milliliter (LogCFU/mL \pm SD) of Dark Opal and Cinnamon in mats and root systems at different time intervals.

Time (Days)	Variety					
	Dark Opal	Cinnamon	C. V. %	Dark Opal	Cinnamon	C. V. %
	Roots LogCFU \pm SD	Roots LogCFU \pm SD		Mats LogCFU \pm SD	Mats LogCFU \pm SD	
0	5.13 \pm 0.13 ^a	5.09 \pm 0.19 ^a	3.13	5.24 \pm 0.25 ^a	5.12 \pm 0.15 ^a	3.845
3	2.61 \pm 0.53 ^b	3.55 \pm 0.47 ^b	16.775	3.39 \pm 0.47 ^b	4.07 \pm 0.25 ^b	10.00
6	2.29 \pm 0.30 ^c	1.43 \pm 0.59 ^c	27.18	1.86 \pm 0.50 ^c	1.88 \pm 0.63 ^c	30.19
9	2.17 \pm 0.67 ^c	1.56 \pm 0.54 ^c	32.75	1.8 \pm 0.51 ^{cd}	1.42 \pm 0.73 ^{cd}	39.86
14	1 \pm 0 ^d	1 \pm 0 ^d	0	1.79 \pm 0.44 ^e	1.21 \pm 0.23 ^e	21.79

Notes. a-a Same letters represent that there are no differences. CV (%): Coefficient of variation. CFU: Colony Forming Units. Log. Logarithms.

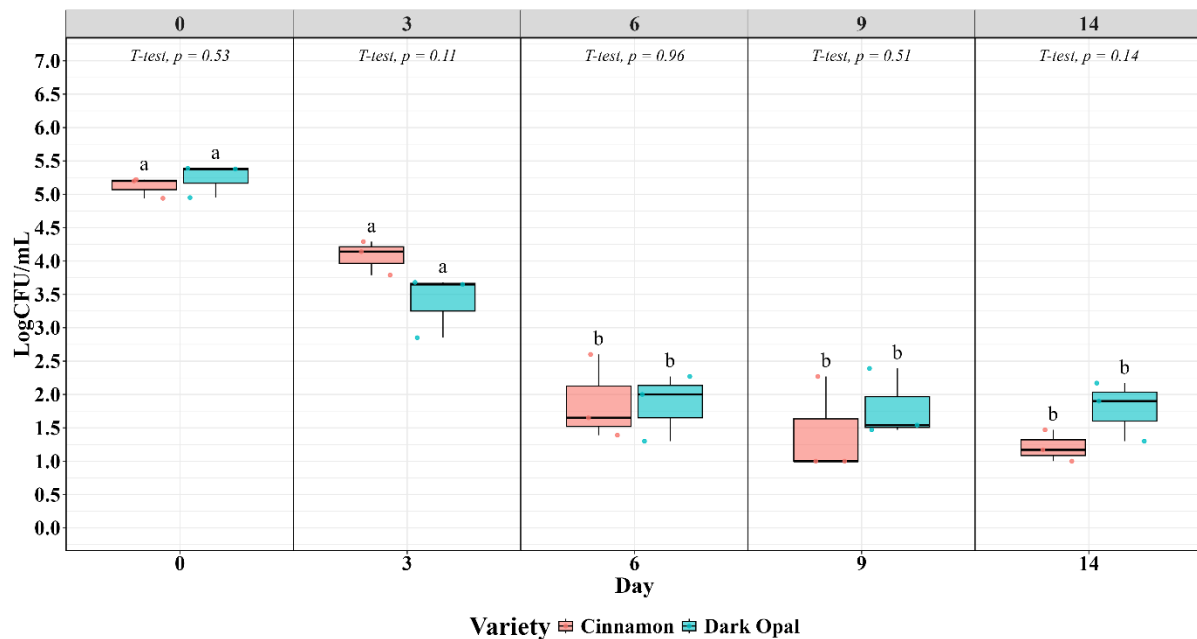
SD: Standard Deviation.

Figure 3. presents the survival of *E. coli* O157 H7 in microgreens-mat samples of the two plant varieties, Cinnamon and Dark Opal, across a 14-day period. On day 0, the initial concentrations of *E. coli* O157 H7 in Cinnamon and Dark Opal showed no significant difference ($p = 0.53$). By day 3, even though the concentrations had decreased in both varieties, the difference was still not significant ($p = 0.11$). This pattern persisted on day 6 ($p = 0.96$), day 9 ($p = 0.51$), and day 14 ($p = 0.14$), with no significant differences noted. Nevertheless, at no time during the study was a statistically significant difference observed in the survival of *E. coli* O157 H7 between the Cinnamon and Dark Opal varieties. This implies that on day 14, the *E. coli* O157 H7 bacteria in the root samples decayed more than those in the mats. As this research project focuses on *E. coli* O157, a Gram-negative bacterium, Hartmann et al. (2017), indicates that *E. coli* is considered a copiotrophic bacterium, meaning it thrives in environments where nutrients and water are abundant. In other words, due to their adaptation to these conditions, they often struggle to survive in dry places or areas with low nutrient availability. The mats can absorb up to 9% of the water. Microgreens are maintained by the moisture content of water, which can absorb their weight up to five times. The increased water absorption in the mats causes the bacteria in the trays to be more mobile. According to a study by Semenov et al. (2007), temperature fluctuations had a more pronounced effect at an average temperature of 23 °C compared

to lower temperatures. Additionally, at 33°C, *E. coli* O157 declined so rapidly that it became undetectable by plate counting after just one week.

Figure 3

Comparative Survival of E. coli O157:H7 in Microgreens-mats Samples of Cinnamon and Dark Opal Over time.



Notes. (P value > 0.05) indicates no statistical differences, (P value < 0.05) indicates statistical significance.

Figure 4 shows the survival of *E. coli* O157 H7 in the roots of microgreens for the two plant varieties, for 14 days. The corresponding p-values are displayed at the top of each interval. A p-value less than 0.05 would indicate a statistically significant difference between the varieties. On day 0, the initial concentrations of *E. coli* O157 H7 in Cinnamon and Dark Opal showed no significant difference ($p = 0.45$). By day 3, although the concentrations had decreased in both varieties, the difference was still not significant ($p = 0.687$). This pattern continued on day 6 ($p = 0.11$), day 9 ($p = 0.75$), and day 14, with no significant differences observed. Xiao, Nou, et al. (2014) state that while *E. coli* levels in microgreens are typically lower than in sprouts, the bacteria can grow and persist in microgreens during production. This is relevant considering that the graph shows the persistence of *E. coli* O157 in microgreens over 14 days. Wright y Holden (2018) conducted a comparison of nine microgreen

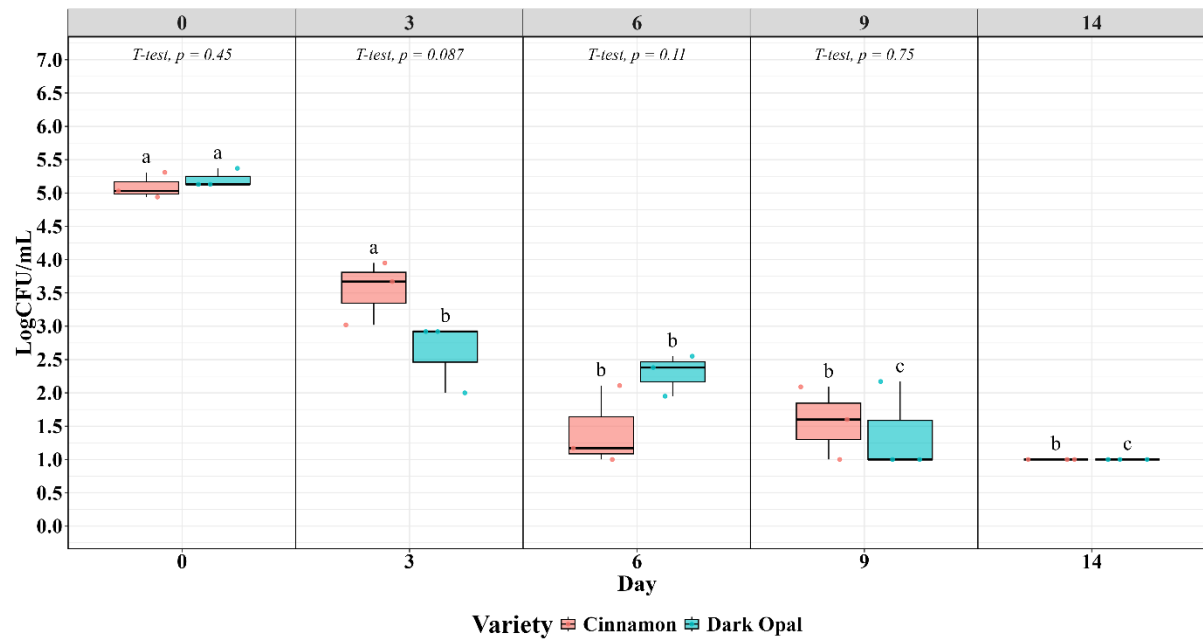
species—Amaranth, Broccoli, Kale, Mustard Red Frill, Coriander, Rocket, Basil variety Purple Dark Opal, and Radish. Their study revealed that basil plants exhibited the lowest levels of colonization by STEC (Shiga Toxin Escherichia Coli). Chitarra et al. (2014) observed that *E. coli* appeared unable to colonize more mature basil plants aged 15 to 25 days. This suggests that as basil plants mature, they may develop mechanisms or conditions that make them less susceptible to colonization by *E. coli*.

However, microgreens basils have been shown to contain higher concentrations of active compounds (phenolics, vitamins and minerals, etc.), compared to those found in mature plants or seeds (Mir et al., 2017). Moreover, during the early growth stages, plants focus on producing a high amount of nutrients and secondary metabolites essential for rapid development and protection against environmental stress. Additionally, since microgreens are harvested quickly, they retain more of these concentrated nutrients before they are dispersed throughout the plant as it matures.

Additionally, Xiao, Luo, et al. (2014) indicate that the time needed between planting and harvesting ranges from one to three weeks (21 days), which aligns with the 14-day study period shown in the graph, providing context on the typical growth and harvest duration for microgreens.

Figure 4

Comparative Survival of *E. coli* 0157:H7 in Microgreens-Roots of Cinnamon and Dark Opal Over Time.



Notes. (P value < 0.05) indicates statistical significance.

Conclusions

The study showed that *E. coli O157 H7* declined more effectively in the nutrient solution than in the roots of the Dark Opal and Cinnamon basil cultivars. As a result, *E. coli O157 H7* adherence was found to be higher in the roots than nutrient solution using the traditional method.

The Dark Opal and Cinnamon cultivars experienced a decline in bacteria at the same time in the traditional and microgreens methods.

It was observed that in microgreens, the both Dark Opal and Cinnamon cultivars showed similar resistance to the *E. coli O157:H7*. However, a greater decrease was obtained in the roots compared to the mats.

Recommendations

Employ other test to analyze the essential oils of Dark Opal and Cinnamon basil. This technique will enable the quantification of key compounds such as estragole and eugenol, providing insights into their potential antimicrobial properties.

Extend the study to include an additional type of bacteria. Comparing the survival rates of *E. coli O157* with another bacterium will offer a broader understanding of microbial behavior.

Conduct studies with different plant varieties beyond basil. This will help determine whether the survival dynamics of *E. coli O157* observed in basil are consistent across other plant species, or if there are unique interactions specific to basil.

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Appendices

Appendix A

Data on the Cinnamon nutrient solution variety in the traditional growth method for 27 days.

Repetition (BLk)	Day (Time)	System	Variety	Samples	Log CFU
1	0	Traditional	Cinnamon	NS	5.59
2	0	Traditional	Cinnamon	NS	5.83
3	0	Traditional	Cinnamon	NS	5.46
1	3	Traditional	Cinnamon	NS	3.63
2	3	Traditional	Cinnamon	NS	3.75
3	3	Traditional	Cinnamon	NS	4.34
1	6	Traditional	Cinnamon	NS	1.74
2	6	Traditional	Cinnamon	NS	1.00
3	6	Traditional	Cinnamon	NS	1.74
1	9	Traditional	Cinnamon	NS	1.00
2	9	Traditional	Cinnamon	NS	1.00
3	9	Traditional	Cinnamon	NS	1.00
1	12	Traditional	Cinnamon	NS	1.00
2	12	Traditional	Cinnamon	NS	1.00
3	12	Traditional	Cinnamon	NS	1.00
1	15	Traditional	Cinnamon	NS	0.00
2	15	Traditional	Cinnamon	NS	1.00
3	15	Traditional	Cinnamon	NS	1.00
1	18	Traditional	Cinnamon	NS	0.00
2	18	Traditional	Cinnamon	NS	1.00
3	18	Traditional	Cinnamon	NS	0.00
1	21	Traditional	Cinnamon	NS	0.00
2	21	Traditional	Cinnamon	NS	0.00
3	21	Traditional	Cinnamon	NS	0.00
1	24	Traditional	Cinnamon	NS	0.00
2	24	Traditional	Cinnamon	NS	0.00
3	24	Traditional	Cinnamon	NS	0.00
1	27	Traditional	Cinnamon	NS	0.00
2	27	Traditional	Cinnamon	NS	0.00
3	27	Traditional	Cinnamon	NS	0.00

Appendix B

Data on the Dark Opal nutrient solution variety in the traditional growth method for 27 days.

Repetition (BLk)	Day (Time)	System	Variety	Samples	Log CFU
1	0	Traditional	Dark Opal	NS	5.65
2	0	Traditional	Dark Opal	NS	5.89
3	0	Traditional	Dark Opal	NS	5.63
1	3	Traditional	Dark Opal	NS	3.63
2	3	Traditional	Dark Opal	NS	4.15
3	3	Traditional	Dark Opal	NS	3.11
1	6	Traditional	Dark Opal	NS	1.00
2	6	Traditional	Dark Opal	NS	1.00
3	6	Traditional	Dark Opal	NS	1.17
1	9	Traditional	Dark Opal	NS	1.00
2	9	Traditional	Dark Opal	NS	1.00
3	9	Traditional	Dark Opal	NS	1.00
1	12	Traditional	Dark Opal	NS	1.00
2	12	Traditional	Dark Opal	NS	1.00
3	12	Traditional	Dark Opal	NS	1.00
1	15	Traditional	Dark Opal	NS	1.00
2	15	Traditional	Dark Opal	NS	0.00
3	15	Traditional	Dark Opal	NS	0.00
1	18	Traditional	Dark Opal	NS	0.00
2	18	Traditional	Dark Opal	NS	0.00
3	18	Traditional	Dark Opal	NS	0.00
1	21	Traditional	Dark Opal	NS	0.00
2	21	Traditional	Dark Opal	NS	0.00
3	21	Traditional	Dark Opal	NS	0.00
1	24	Traditional	Dark Opal	NS	0.00
2	24	Traditional	Dark Opal	NS	0.00
3	24	Traditional	Dark Opal	NS	0.00
1	27	Traditional	Dark Opal	NS	0.00
2	27	Traditional	Dark Opal	NS	0.00
3	27	Traditional	Dark Opal	NS	0.00

Appendix C

Data on the Dark Opal and Cinnamon variety for mats in the microgreen growth method for 14 days

Repetition (BLk)	Day (Time)	System	Variety	Samples	Log CFU
1	0	Microgreens	Dark Opal	Mats	5.38
1	3	Microgreens	Dark Opal	Mats	3.65
1	6	Microgreens	Dark Opal	Mats	2.27
1	9	Microgreens	Dark Opal	Mats	1.54
1	14	Microgreens	Dark Opal	Mats	1.30
1	0	Microgreens	Cinnamon	Mats	5.20
1	3	Microgreens	Cinnamon	Mats	4.14
1	6	Microgreens	Cinnamon	Mats	1.65
1	9	Microgreens	Cinnamon	Mats	1
1	14	Microgreens	Cinnamon	Mats	1.17
2	0	Microgreens	Dark Opal	Mats	5.39
2	3	Microgreens	Dark Opal	Mats	3.68
2	6	Microgreens	Dark Opal	Mats	2.00
2	9	Microgreens	Dark Opal	Mats	2.39
2	14	Microgreens	Dark Opal	Mats	2.17
2	0	Microgreens	Cinnamon	Mats	5.22
2	3	Microgreens	Cinnamon	Mats	4.29
2	6	Microgreens	Cinnamon	Mats	2.60
2	9	Microgreens	Cinnamon	Mats	2.27
2	14	Microgreens	Cinnamon	Mats	1.47
3	0	Microgreens	Dark Opal	Mats	4.95
3	3	Microgreens	Dark Opal	Mats	2.85
3	6	Microgreens	Dark Opal	Mats	1.30
3	9	Microgreens	Dark Opal	Mats	1.47
3	14	Microgreens	Dark Opal	Mats	1.90
3	0	Microgreens	Cinnamon	Mats	4.94
3	3	Microgreens	Cinnamon	Mats	3.79
3	6	Microgreens	Cinnamon	Mats	1.39
3	9	Microgreens	Cinnamon	Mats	1.00
3	14	Microgreens	Cinnamon	Mats	1.00

Appendix D

Data on the Dark Opal and Cinnamon variety for roots in the microgreen growth method for 14 days

Repetition (BLk)	Day (Time)	System	Variety	Samples	log UCF
1	0	Microgreens	Dark Opal	Roots	5.13
1	3	Microgreens	Dark Opal	Roots	2.92
1	6	Microgreens	Dark Opal	Roots	2.38
1	9	Microgreens	Dark Opal	Roots	2.17
1	14	Microgreens	Dark Opal	Roots	1.00
1	0	Microgreens	Cinnamon	Roots	4.94
1	3	Microgreens	Cinnamon	Roots	3.95
1	6	Microgreens	Cinnamon	Roots	1.00
1	9	Microgreens	Cinnamon	Roots	1.00
1	14	Microgreens	Cinnamon	Roots	1.00
2	0	Microgreens	Dark Opal	Roots	5.13
2	3	Microgreens	Dark Opal	Roots	2.92
2	6	Microgreens	Dark Opal	Roots	1.95
2	9	Microgreens	Dark Opal	Roots	1.00
2	14	Microgreens	Dark Opal	Roots	1.00
2	0	Microgreens	Cinnamon	Roots	5.31
2	3	Microgreens	Cinnamon	Roots	3.67
2	6	Microgreens	Cinnamon	Roots	2.11
2	9	Microgreens	Cinnamon	Roots	1.60
2	14	Microgreens	Cinnamon	Roots	1.00
3	0	Microgreens	Dark Opal	Roots	5.37
3	3	Microgreens	Dark Opal	Roots	2.00
3	6	Microgreens	Dark Opal	Roots	2.55
3	9	Microgreens	Dark Opal	Roots	1.00
3	14	Microgreens	Dark Opal	Roots	1.00
3	0	Microgreens	Cinnamon	Roots	5.03
3	3	Microgreens	Cinnamon	Roots	3.02
3	6	Microgreens	Cinnamon	Roots	1.17
3	9	Microgreens	Cinnamon	Roots	2.09
3	14	Microgreens	Cinnamon	Roots	1.00