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Survival of *E. coli* O157:H7 on hydroponically grown traditional and microgreen Genovese and Lemon basil (*Ocimum basilicum)* under home-scale conditions

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Content

List of Tables

List of Figures

List of Appendices

Abstract

The consumption of fresh culinary herbs, especially basil, has increased due to their health benefits. Consequently, their production has surged, particularly in hydroponic systems at home. Since culinary herbs are often consumed raw, they pose a food safety risk if contaminated. Recent outbreaks in hydroponic production have shown that foodborne pathogens can survive in these systems, including components such as nutrient solutions, growth mats, and roots. For this reason, this study determined the survival rates of *E. coli* O157:H7 within hydroponic units of Genovese and Lemon basil cultivars under home-scale conditions. The study compared the survival of *E. coli* O157:H7 over 27 days and 14 days in the traditional and microgreen systems, respectively. Bacterial counts were monitored in the nutrient solution, growth mat, and root samples to evaluate each variety's microbial interactions and possible inhibitory effects. Results indicated a significant reduction in bacterial counts over time ($p < 0.05$). In the traditional system, *E. coli* O157:H7 decreased from 6 Log CFU/mL to 0 Log CFU/mL over 27 days, while in the microgreens system, it reduced from 5 Log CFU/mL to 1 Log CFU/mL over 14 days. No significant differences were observed between the two basil varieties within each system ($p > 0.05$). Both varieties showed similar bacterial reduction in all samples, suggesting that bacterial persistence may depend more on factors other than variety influence in bacterial survival. These findings highlight the potential of growing basil in traditional and microgreen systems to mitigate bacterial contamination, with implications for ensuring food safety and improving agricultural practices.

Keywords: cultivars, food safety, hydroponic system, traditional system

Resumen

El consumo de hierbas culinarias frescas, como la albahaca, ha aumentado debido a sus beneficios para la salud, impulsando su producción en sistemas hidropónicos caseros. Sin embargo, dado que se consumen crudas, representan un riesgo de seguridad alimentaria si están contaminadas. Brotes recientes han demostrado que patógenos como *E. coli* O157:H7 pueden sobrevivir en sistemas hidropónicos, incluidos componentes como soluciones nutritivas, substratos y raíces. Este estudio evaluó la supervivencia de *E. coli* O157:H7 en unidades hidropónicas de cultivares albahaca genovesa y albahaca limón bajo condiciones a pequeña escala. Se comparó la supervivencia de *E. coli* O157:H7 durante 27 días en el sistema tradicional y 14 días en el sistema de microvegetales. Se monitorearon los conteos bacterianos en la solución nutritiva, substrato de crecimiento y muestras de raíces para analizar las interacciones microbianas de cada variedad y posibles efectos inhibitorios. Los resultados mostraron una reducción significativa en los conteos bacterianos ($p < 0.05$). En el sistema tradicional, *E. coli* O157:H7 disminuyó de 6 Log UFC/mL a 0 Log UFC/mL en 27 días, y en microvegetales de 5 Log UFC/mL a 1 Log UFC/mL en 14 días. No se observaron diferencias significativas entre las variedades en cada sistema ($p > 0.05$). Ambas variedades mostraron reducciones bacterianas similares, sugiriendo que la persistencia bacteriana depende más de otros factores que de la variedad. Estos resultados destacan el potencial de cultivar albahaca en sistemas tradicionales y de microvegetales para mitigar la contaminación bacteriana, con implicaciones para la seguridad alimentaria y la mejora de prácticas agrícolas.

Palabras clave: cultivares, seguridad alimentaria, sistema hidropónico, sistema tradicional

Introduction

Culinary herbs have long been characterized by their aromatic flavors and medicinal properties, tracing back to ancient civilizations where they were vital for their culinary and therapeutic benefits. Nowadays, the cultivation and consumption of culinary herbs have evolved into a global phenomenon, with the United States and Italy standing as significant players in both production and consumption as part of their cultures (Spence, 2024). Among the myriad herbs, basil holds a prominent place in American cuisine, adding depth and freshness to various dishes. In addition, due to its typical pungent and composite fragrance, it is employed in Mediterranean cuisine and is commonly used as a food garnish and preservative, being considered a valuable source of antioxidants (Corrado et al., 2020).

While traditional cultivation methods have been the norm for centuries, the emergence of microgreen varietals has introduced new dimensions to herb cultivation, offering intensified flavors and enhanced nutritional profiles. Concurrently, there is a growing trend toward home cultivation of fresh culinary herbs, driven by a desire for sustainability, convenience, and flavor due to the high concentration of nutrients mostly found in leaves. According to recent studies, while herb production in the United States is primarily conducted within controlled environment agriculture systems, including greenhouses, there is also a growing trend of consumers cultivating fresh culinary herbs at home (Dohlman et al., 2024).

Home hydroponic production, although efficient and space-saving, can inadvertently predispose to microbial contamination and growth if proper precautions are not observed, and if humid conditions favor microbial growth (Dankwa et al., 2020). Given the recent outbreaks and recalls related to foodborne pathogens in fresh vegetables and culinary herbs, such as *E. coli* O157:H7, there is a pressing need to address the safety concerns surrounding home hydroponic production. Despite multiple efforts and measures to prevent and reduce contamination, most Shiga toxin-producing *E. coli* (STEC) cases have been linked to the consumption of leafy greens, particularly lettuce, with 40

outbreaks reported in the United States and Canada between 2009 and 2018 (Marshall et al., 2020). Although basil has had fewer outbreaks compared to lettuce, recent cases have still been reported. Between 1996 and 2015, nine outbreaks involving basil, parsley, and cilantro were documented, resulting in 2,699 illnesses. Specifically, four outbreaks were related to basil, three to cilantro, and two to parsley where *Cyclospora cayetanensis* caused seven outbreaks, while *E. coli* O157:H7 and *Shigella sonnei* each caused one (Food and Drug Administration [FDA], 2022).

Also, more recently, epidemiological and tracing investigations have identified additional outbreaks related to basil. According to the Centers for Disease Control and Prevention (2024), an outbreak that concluded on June 18th, 2024, was linked to Salmonella contamination in organic basil from the Infinite Herbs brand, affecting 36 people and resulting in four hospitalizations across 14 states.

E. coli O157:H7, a particularly virulent strain of *Escherichia coli*, poses significant risks to human health when present in fresh vegetables due to its potential to cause severe illness, including hemolytic uremic syndrome. Understanding the factors contributing to its proliferation in fresh produce, including herbs, is paramount to ensuring food safety. Every year, it is estimated by the U.S. Centers for Disease Control and Prevention (CDC) that 48 million Americans are affected by foodborne illnesses annually, resulting, unfortunately, in 128,000 hospitalizations and 3,000 deaths (K. Turner et al., 2019). Recent literature has demonstrated extensive production and consumption of herbs, with the United States producing around 200 billion pounds of herbs and spices annually (Sanchez, 2023). These quantities are closely related to the healthy and cultural consumption trend worldwide. Regarding basil, global production is estimated at 42.7 million tons, with China covering the highest production rate at 48% (Yitbarek & Wendimu, 2023).

Although cultivating plants in the hydroponic system represents a solution for increasing food production and reducing the environmental footprint, there are still aspects that need to be studied since the survival of pathogens changes (Dobrin et al., 2018). In particular, the varieties of basil play

an important role in bacterial survival. In general, basil plants can be classified into four major essential oil (EO) chemotypes based on their EO composition: those rich in methyl chavicol, linalool, methyl eugenol, and methyl cinnamate, along with numerous additional subtypes (Verrillo et al., 2021). In addition, it is important to highlight that understanding basil's compound profiles and the influence of different varieties on their concentration is crucial for determining effective methods of bacterial inhibition. Specifically, the presence of phenolic components in the essential oil can contribute to antimicrobial activity by causing the leakage of intracellular ATP and potassium ions, which leads to cell death (Joshi, 2014). Understanding the mechanism of action of basil's compounds against different bacteria is vital, as is studying the various production systems in which basil is grown, in developing bacterial inhibition strategies to improve food safety practices. Therefore, studying the different dynamics of pathogens has become vital in understanding the extent to which a production system can be exposed depending on the conditions in which it is found.

Therefore, this study aims to determine the survival rates of *E. coli* O157:H7 in traditional and microgreen Genovese and Lemon basil. Secondly, to compare the survival of *E. coli* O157:H7 in nutrient solution and roots of Lemon and Genovese cultivars. Lastly, aims to investigate where within the hydroponic system *E. coli* O157:H7 survives best, including nutrient solution, substrate, water, and roots.

Materials and Methods

Location

The entire experiment was conducted at the International Center for Food Industry Excellence (ICFIE), located in the Experimental Sciences Building at Texas Tech University in Lubbock, Texas, United States of America.

Inoculum Preparation

For this process, to resuscitate *E. coli* O157:H7 35150 GFP, isolates were picked from one vial of the ampicillin-resistant bacteria stored in a -80 °C freezer. One loop was transferred into four 10 mL of Brain Heart Infusion (BHI) broth and incubated at 37 °C overnight. After confirming the starting inoculum concentration, the four 10 mL BHI tubes were poured into two sterile 30 mL high-strength centrifuge tubes, balancing 20 mL per tube in the centrifuge. The tubes containing the inoculum were centrifuged at 4000 rpm for a total of 10 minutes. Subsequently, 20 mL of sterile water/PBS was added to each centrifuge tube. The contents of both centrifuge tubes were then thoroughly mixed by vortexing. The final inoculum concentration was plated to confirm it had reached 10^7 CFU/mL.

Traditional Varieties

Phase 1 – Germination Stage

Basil was produced using the Deep-Water Culture (DWC) method for the traditional method. Two varieties were used: Genovese basil and Lemon basil, each with three replicates and one control per variety. Genovese (Lot: 80185) and Lemon (Lot: 74089) basil seeds were obtained from Johnny's Selected Seeds supplier, and the nutrient solution was acquired from JR Peters Inc. (Jack’s Nutrients) using two formulations denominated as Part A and Part B. For the germination process, since it was needed to work with basil under homegrown conditions, basil seeds germinated for fourteen days in a plant growth chamber from Percival Scientific with Controlled Environment Agriculture (CEA). First, the growth chamber trays, and hydroponic units were disinfected to ensure aseptic conditions for the start of germination. A volume of 4 liters of nutrient solution per unit was prepared to meet the

optimum requirements for basil (100 to 150 ppm N), and the rockwool cubes with the seeds were saturated. The temperature was set at 20 °C (68 °F) with 70% relative humidity and a constant CO2 concentration of 1000 ppm. Once the units were ready, the healthiest-looking seedlings that showed three true leaves were transplanted into the Deep-Water Culture (DWC) hydroponic system, and the units were programmed to run in vegetative mode. In this mode, a cycle of 16 hours of light and 8 hours of darkness is run with color LED lights, including red, white, and blue lights.

Inoculation of Nutrient Solution

Once the desired concentration was obtained, and after two days of transplanting and acclimatizing the plants, 4 mL of the inoculum was transferred from the tube containing 10^7 CFU/mL. This inoculum was then introduced into the 4-liter nutrient solution of the Deep-Water Culture (DWC) hydroponic unit for the traditional system through one of the holes on the top of the unit. This process resulted in a final concentration of 10^4 CFU/mL, as one log reduction was expected. To sample, the inoculum was circulated by the internal pump for 30 minutes in the nutrient solution; this first sampling was Day 0, and 10 mL of the nutrient solution was taken into a sterile empty tube.

Phase 2 – Sampling process

For 45 days, the Electrical Conductivity (EC) and pH were adjusted daily according to the plant requirements to maintain ideal conditions for each cultivar, within the range of 5.8-6.2 for pH and 1.0-1.4 mS/cm for EC. During this period, samples were taken every three days, including both nutrient solution and root samples, totaling 24 samples across both types.

Nutrient Solution Samples.

Samples of the nutrient solution were collected by extracting 10 mL from the reservoir into a sterile empty tube. Depending on the sampling day, these samples were diluted with 9 mL of Buffered Peptone Water (BPW) and directly plated onto MacConkey agar with 200 µg/mL ampicillin using the spread plating method. Several dilutions were performed to ensure countable plates and allow for reporting of colony-forming units (CFU) within the range of 25-250 colonies.

Root Samples.

Root samples were obtained by removing the rock wool with the respective plant and cutting the roots into a stomacher bag at 230 rpm. Additionally, the roots were weighed, and the corresponding amount of Buffered Peptone Water (BPW) was added by multiplying the weight by 9 to dilute the sample in a relation of 1:10. The samples were homogenized for 1 minute using a stomacher at 230 rpm before being directly plated onto MacConkey agar with 200 µg/mL ampicillin using the spread plating method.

Microgreens Varieties

Phase 1 – Germination and Growth Process

For the microgreen method, two varieties were used: Genovese basil and Lemon basil, each with three replicates and one control per variety. Genovese (Lot: 100147) and Lemon (Lot: 61205) basil seeds were obtained from Johnny's Selected Seeds supplier, and the nutrient solution was acquired from JR Peters Inc. (Jack's Nutrients) using two formulations denoted as Part A and Part B. The basil seeds were germinated for seven days, as their germination period is shorter compared to traditional methods. Initially, the plant growth chamber from Percival Scientific with Controlled Environment Agriculture (CEA) and the growing trays were disinfected to maintain aseptic conditions for germination. To begin the germination process, 5 g of seeds were placed on grow mats and sprayed with 100 mL of deionized sterile water and nutrient solution. To ensure optimal germination, the grow trays were covered with domes and placed in a growth chamber set at a constant temperature of 20 °C (68 °F) with 70% relative humidity, complete darkness, and a constant CO2 concentration of 1000 ppm. The trays with the seeds were kept in darkness until day 5 to simulate industry practices.

Inoculation of Microgreens

Once the desired concentration of 10^6 CFU/mL was achieved, on Day 6, 1 mL was transferred from the 10^6 CFU/mL tube and inoculated into 100 mL of water used to submerge the microgreen growth mat placed above the draining tray. This resulted in a concentration of 10^4 CFU/mL, as one

log reduction was expected. To sample the inoculum, a 1-inch square of the grow mat was placed into a stomacher bag at 230 rpm for both mat and root samples; this initial sampling occurred on Day 0.

Phase 2

For 21 days, the microgreens were grown in the plant growth chamber from Percival Scientific with Controlled Environment Agriculture (CEA), under ideal plant conditions. Each day, every grow mat was irrigated with 50 mL of deionized water and nutrient solution. Throughout this period, samples were collected on predetermined days for both the growth mat (media) and root samples, totaling 8 samples for each. Samples were collected by cutting a 1-inch square of the growth mat into a stomacher bag at 230 rpm for both the media and root samples. It was necessary to weigh the grow mat cubes for both sample types, and for the root samples, only the roots were weighed after cutting the plants. After sampling, the respective amount of Buffered Peptone Water (BPW) was added by multiplying the weight by 9 to dilute the sample. The samples were homogenized for one minute using a stomacher at 230 rpm before being directly plated onto MacConkey agar with 200 µg/mL ampicillin using the spread plating method.

Microbial Enumeration.

To enumerate bacterial colonies, the selected dilutions were plated in duplicate on MacConkey agar supplemented with 200 µg/mL ampicillin to reduce background microflora. Plates were then incubated at 37 °C for 24 hours. After incubation, colonies were counted within the range of 25-250 colonies per plate. In cases where plates showed an excessive number of colonies, additional dilutions were plated to achieve counts within the manageable range.

Colonies were counted based on the characteristics exhibited by the bacteria on the medium. The selected medium, MacConkey agar with 200 µg/mL ampicillin, resulted in pink colonies because *E. coli* O157:H7 ferments lactose, which decreases the pH of the agar. Additionally, in cases where no bacterial colonies were observed, an enrichment with Brain Heart Infusion (BHI) broth was performed to verify the presence of the bacteria in the system.

Experimental Design

A 2x2 factorial Randomized Complete Block design (Factorial RBD) was employed to assess measures over time. The experiment comprised four distinct treatment combinations, each defined by the interaction between two factors: type of cultivation system (traditional and microgreen) and variety of basil (Genovese and Lemon). For each treatment combination, there were three replicates, resulting in a total of 16 experimental units. For the microgreen system, the samplings were carried out over a period of 14 days with five sampling points on days 0, 3, 6, 9, and 14 (Table 1). For the traditional system, the samplings were carried out for 27 days with 10 sampling points on days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 27 (Table 2). Data analysis was conducted using the statistical software R Studio 4.3.1, utilizing ANOVA alongside the non-parametric Pairwise Wilcoxon Test to identify significant differences between treatments ($p < 0.05$).

Table 1

Sampling design for microgreen cultivar: roots and nutrient solution across timepoints (days).

Replicate	Variety	Treatment	Sample type	Timepoint (Days)/ Sample number				
				0	3	6	9	14
Control	Genovese	Control	Roots	1	2	3	4	5
			Nutrient solution	6	7	8	9	10
1	Genovese	*E. coli* O157:H7	Roots	11	12	13	14	15
			Nutrient solution	16	17	18	19	20
2	Genovese	*E. coli* O157:H7	Roots	21	22	23	24	25
			Nutrient solution	26	27	28	29	30
3	Genovese	*E. coli* O157:H7	Roots	31	32	33	34	35
			Nutrient solution	36	37	38	39	40
Control	Lemon	Control	Roots	41	42	43	44	45
			Nutrient solution	46	47	48	49	50
1	Lemon	*E. coli* O157:H7	Roots	51	52	53	54	55
			Nutrient solution	56	57	58	59	60
2	Lemon	*E. coli* O157:H7	Roots	61	62	63	64	65
			Nutrient solution	66	67	68	69	70
3	Lemon	*E. coli* O157:H7	Roots	71	72	73	74	75
			Nutrient solution	76	77	78	79	80

Note. Treatments applied in 3 replicates for Genovese and Lemon basil varieties. Control: non-inoculated hydroponic units. E. coli O157:H7: inoculated units. Timepoint indicates sampling days (0, 3, 6, 9, 14).

Table 2

Sampling design for traditional cultivar: roots and nutrient solution across timepoints (days).

Replicate	Variety	Treatment	Sample type	Timepoint (Days)/ Sample number									
				0	3	6	9	12	15	18	21	24	27
Control	Genovese	Control	Roots	1	2	3	4	5	6	7	8	9	10
			Nutrient solution	11	12	13	14	15	16	17	18	19	20
1	Genovese	*E. coli* O157:H7	Roots	21	22	23	24	25	26	27	28	29	30
			Nutrient solution	31	32	33	34	35	36	37	38	38	40
2	Genovese	*E. coli* O157:H7	Roots	41	42	43	44	45	46	47	48	49	50
			Nutrient solution	51	52	53	54	55	56	57	58	59	60
3	Genovese	*E. coli* O157:H7	Roots	61	62	63	64	65	66	67	68	69	70
			Nutrient solution	71	72	73	74	75	76	77	78	79	80
Control	Lemon	Control	Roots	81	82	83	84	85	86	87	88	89	90
			Nutrient solution	91	92	93	94	95	96	97	98	99	100
1	Lemon	*E. coli* O157:H7	Roots	101	102	103	104	105	106	107	108	109	110
			Nutrient solution	111	112	113	114	115	116	117	118	119	120
2	Lemon	*E. coli* O157:H7	Roots	121	122	123	124	125	126	127	128	129	130
			Nutrient solution	131	132	133	134	135	136	137	138	139	140
3	Lemon	*E. coli* O157:H7	Roots	141	142	143	144	145	146	147	148	149	150
			Nutrient solution	151	152	153	154	155	156	157	158	159	160

Note. Treatments applied in 3 replicates for Genovese and Lemon basil varieties. Control: non-inoculated hydroponic units. E. coli O157:H7: inoculated units. Timepoint indicates sampling days (0, 3, 6, 9, 15, 18, 21, 24, 27).

Results and Discussion

Traditional System

According to Dhulappanavar and Gibson (2023), one of the largest sources of contamination in hydroponic systems is the water used in the preparation of nutrient solutions. They found that water is the entry route for human pathogens through the roots of plants submerged in a nutrient solution within the hydroponic system (Dhulappanavar & Gibson, 2023). For this reason, this section focuses on first determining the growth of *E. coli* O157:H7 in the nutrient solution for each of the two varieties in the study (Table 3).

Table 1 provides data on the differences in the growth of *E. coli* O157:H7 in nutrient solution for the Genovese and Lemon varieties over a 27-day sampling period. Both varieties showed a decrease in *E. coli* O157:H7 counts, indicating reduced bacterial growth. Notably, on Day 3, both varieties experienced a significant decrease in *E. coli* counts compared to Day 0, with a reduction of 2.6 Log CFU/mL for Genovese and 2.19 Log CFU/mL for Lemon. However, the decrease was slightly more pronounced in the Genovese variety compared to the Lemon variety. Despite higher counts on other sampling days, it was determined that there was no statistically significant difference between the two varieties ($p > 0.05$).

The behavior of bacterial growth in the nutrient solution could have been influenced by the compounds of the two varieties of basil. The basil varieties in this study, Genovese and Lemon share many of the same essential oils; however, the concentration of these oils varies significantly between the two varieties. Lemon basil is distinguished by its dominant aromatic profile of α-citral (33.34%) and β-citral (27.29%), compounds responsible for its strong citrus aroma, while Genovese basil has a markedly different aromatic composition, with high levels of linalool (40.77%), eucalyptol (29.37%), and trans-α-bergamotene (4.27%) (Ciriello et al., 2023). It is important to emphasize that the concentration of basil compounds is influenced not only by the variety and the plant's genetics but also by the cultivation methods employed. This is particularly relevant in the context of hydroponic

systems. In addition, there are exudates released by the plants, which may inhibit bacterial growth, such as various organic acids, phenolic compounds, and flavonoids. This is supported by the results of a study by Shaw et al. (2016), which found that although *E. coli* O157: H7 was able to outcompete the background flora in the hydroponic system, the absorption of nutrients by plants and the release of ions from the roots could affect the survival and growth of pathogens at a lower rate. Despite the composition of the compounds for each variety in the nutrient solution, a notable decrease in bacterial counts was observed, though there were no significant differences between the varieties evaluated.

Table 3

Differences between the growth of E. coli O157:H7 in nutrient solution between the Genovese and Lemon varieties in the traditional system over twenty-seven days.

Time (Day)	Variety		
	Genovese	Lemon	
	Nutrient solution Log CFU/mL ± SD	Nutrient solution Log CFU/mL ± SD	CV (%)
0	6.17 ± 0.05[a]	6.00 ± 0.06[a]	0.92
3	3.57 ± 0.12[a]	3.81 ± 0.31[a]	5.78
6	2.15 ± 0.32[a]	2.54 ± 0.94[a]	25.91
9	1.43 ± 0.74[a]	1.97 ± 0.91[a]	49.12
12	1.18 ± 0.31[a]	1.00 ± 0.00[a]	13.29
15	1.13 ± 0.23[a]	1.00 ± 0.00[a]	10.14
18	1.00 ± 0.00[a]	1.00 ± 0.00[a]	0.00
21	1.00 ± 0.00[a]	1.00 ± 0.00[a]	0.00
24	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00
27	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00

Note. SD: Standard deviation. CV (%): Coefficient of variation. [a-b] Means with the same lowercase letters for *E. Coli* log CFU/mL indicate no significant differences ($p > 0.05$) in each column. Each Nutrient Solution Log CFU/mL microbiological count per variety represents the mean of 3 repetitions.

Table 4 shows the bacterial counts in Log CFU/mL for both Genovese and Lemon varieties, where no significant differences were observed in the root system of the plants for each variety ($p > 0.05$). Comparing the groups between varieties, the bacterial reduction for each sampling day was statistically the same, so the same letter was attributed to each mean. Although no significant differences were found between varieties, there was a logarithmic reduction over time. Unlike in the

nutrient solution, bacterial counts were no longer reported until day 24 of sampling, and the presence of bacteria was still detected in roots on day 27 of sampling.

Table 4

Differences between the growth of E. coli O157:H7 in roots between the Genovese and Lemon varieties in the traditional system over twenty-seven days.

Time (Day)	Variety		
	Genovese	Lemon	
	Roots Log CFU/mL ± SD	Roots Log CFU/mL ± SD	CV (%)
0	3.64 ± 0.66[a]	3.59 ± 0.23[a]	12.22
3	3.01 ± 0.72[a]	3.23 ± 0.40[a]	18.09
6	1.35 ± 0.16[a]	2.43 ± 0.90[a]	24.32
9	1.55 ± 0.64[a]	2.30 ± 0.50[a]	33.51
12	1.38 ± 0.34[a]	1.39 ± 0.39[a]	26.25
15	1.10 ± 0.17[a]	1.06 ± 0.10[a]	12.70
18	1.00 ± 0.00[a]	1.00 ± 0.00[a]	0.00
21	1.00 ± 0.00[a]	1.00 ± 0.00[a]	0.00
24	1.00 ± 0.00[a]	1.00 ± 0.00[a]	0.00
27	1.00 ± 0.00[a]	1.00 ± 0.00[a]	0.00

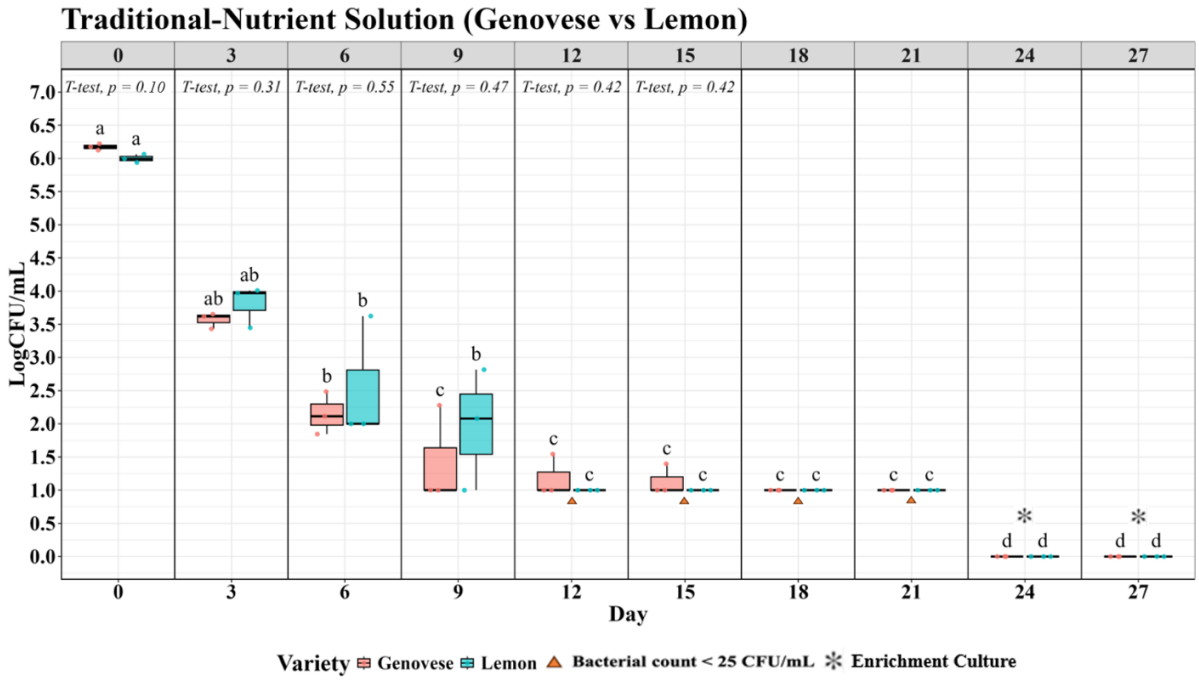
Note. SD: Standard deviation. CV (%): Coefficient of variation. [a-b] Means with the same lowercase letters for *E. Coli* log CFU/mL indicate no significant differences ($p > 0.05$) in each column. Each microbiological count of Roots Log CFU/mL per variety represents the mean of 3 repetitions.

Figures 1 and 2 reveal the growth of *E. coli* O157:H7 in the traditional system for both nutrient solution and roots, considering both varieties of basil. It can be seen how, over time, for a sampling period of 27 days, the effect of the variety variable was not significant since the Wilcoxon test and T-test analysis in the comparison of the two paired variety samples did not reveal a significant difference ($p > 0.05$) between treatments in both nutrient solution and roots samples. It should be noted that in both varieties, a similar survival curve of *E. coli* O157:H7 was obtained without statistical differences in terms of the data. This similarity may be attributed to both varieties having similar physiological characteristics, such as similar leaf morphology and chemical profiles. Both varieties possess essential oils like eugenol and linalool, and comparable levels of flavonoids and phenolic compounds that

conferred comparable resistance mechanisms causing them to respond similarly to the bacteria in the experimental hydroponic environment with uniform crop conditions (Zote et al., 2024).

Figure 1

Growth of E. coli O157:H7 in nutrient solution samples between the Genovese and Lemon varieties in the traditional system over twenty-seven days.



The data obtained showed that no significant differences were observed between both varieties in the nutrient solution and root samples. This finding may be attributed to the type of sampling conducted and the specific part of the plant analyzed, as each part contains varying amounts of essential oils that inhibit bacteria based on their content. This observation is supported by a study conducted by Kamelnia et al. (2023), which determined that basil leaves and stems predominantly contain essential oil constituents such as Eugenol (42.74%), Linalool (20.54%), and Eucalyptol (15.27%) of the overall essential oil composition of the basil plant, reflecting these percentages in different proportions. Additionally, many of these compounds are released through root exudates, which can lead to reduced bacterial presence in the rhizosphere due to their antimicrobial properties. These

constituents are known for their biological activities, including the antifungal and anti-aflatoxin properties of the plant. Basil's properties disrupt ergosterol biosynthesis in fungal plasma membranes and alter mitochondrial membrane potential, impeding in that way fungal growth and inhibiting aflatoxin B1 production (Kumar et al., 2020).

Figure 2

Growth of E. coli O157:H7 in roots samples between the Genovese and Lemon varieties in the traditional system over twenty-seven days.

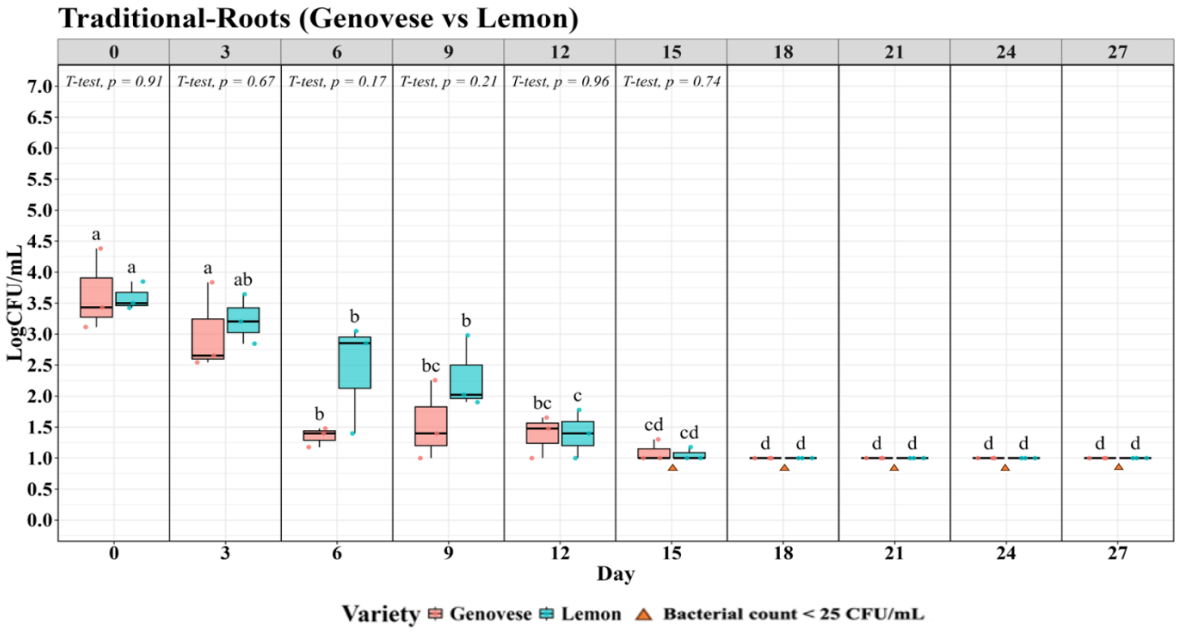


Table 5 shows the growth of O157:H7 in nutrient solution and roots of the two varieties in the traditional system, with sampling carried out over twenty-seven days. At first, on day 0, a difference was detected between nutrient solution and roots (a-b), which indicated that despite the system having been inoculated at the same time and with the same logarithmic concentration (6 Log CFU/mL), a lower percentage of bacteria was attached to the roots compared to nutrient, which can be supported by the counts obtained of 6 Log CFU/mL in nutrient solution and 3.60 Log CFU/mL in roots. Additionally, the data reflect significant differences ($p < 0.05$) both in the comparison of nutrient solution and roots and for the comparison of each case throughout the sampling time with significant differences in each of the days.

Table 5

Growth of E. coli O157:H7 in nutrient solution and roots between the Genovese and Lemon varieties in the traditional system over twenty-seven days.

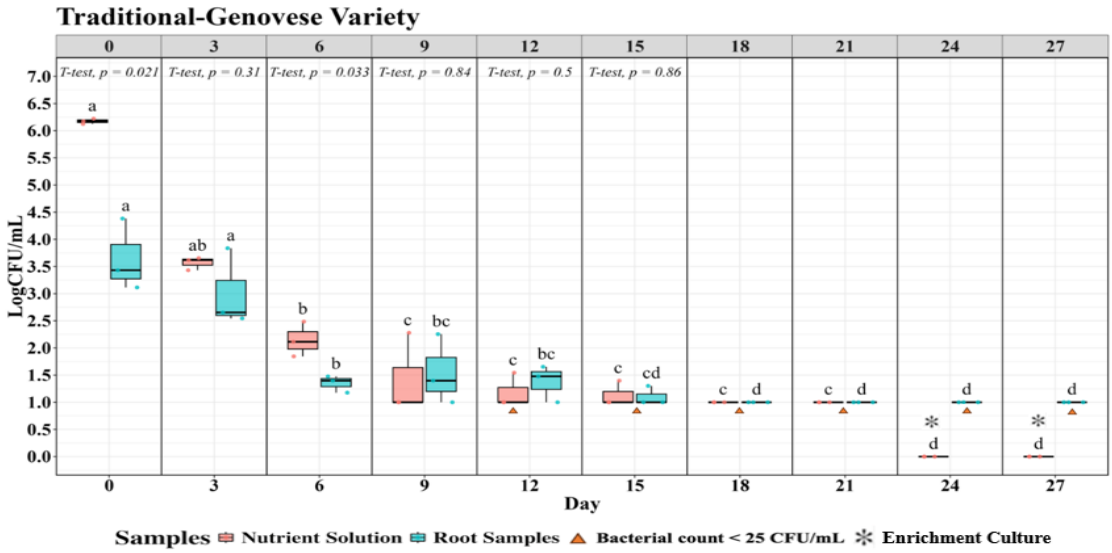
Time (Day)	Variety				
	Genovese		Lemon		
	Nutrient solution Log CFU/mL ± SD	Roots Log CFU/mL ± SD	Nutrient solution Log CFU/mL ± SD	Roots Log CFU/mL ± SD	CV (%)
0	6.17 ± 0.05^{au}	3.64 ± 0.66^{bu}	6.00 ± 0.06^{au}	3.59 ± 0.23^{bu}	6.57
3	3.57 ± 0.12^{av}	3.01 ± 0.72^{au}	3.81 ± 0.31^{av}	3.23 ± 0.40^{au}	11.94
6	2.15 ± 0.32^{aw}	1.35 ± 0.16^{av}	2.54 ± 0.94^{aw}	2.43 ± 0.90^{av}	25.12
9	1.43 ± 0.74^{ax}	1.55 ± 0.64^{avw}	1.97 ± 0.91^{ax}	2.30 ± 0.50^{avw}	41.32
12	1.18 ± 0.31^{ay}	1.38 ± 0.34^{awx}	1.00 ± 0.00^{ay}	1.39 ± 0.39^{awx}	19.77
15	1.13 ± 0.23^{ay}	1.10 ± 0.17^{ax}	1.00 ± 0.00^{ay}	1.06 ± 0.10^{ax}	11.42
18	1.00 ± 0.00^{ay}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ay}	1.00 ± 0.00^{ax}	0.00
21	1.00 ± 0.00^{ay}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ay}	1.00 ± 0.00^{ax}	0.00
24	0.00 ± 0.00^{bz}	1.00 ± 0.00^{ax}	0.00 ± 0.00^{bz}	1.00 ± 0.00^{ax}	0.00
27	0.00 ± 0.00^{bz}	1.00 ± 0.00^{ax}	0.00 ± 0.00^{bz}	1.00 ± 0.00^{ax}	0.00

Note. SD: Standard deviation. CV (%): Coefficient of variation. $^{a-b}$ Means with different lowercase letters for *E. Coli* log CFU/mL indicate significant differences between treatments in each variety ($p < 0.05$) in each column; $^{u-z}$ Means with different lowercase letters for *E. Coli* log CFU/mL indicate significant differences between the evaluated times ($p < 0.05$) in each row. Each microbiological count of Nutrient Solution and Roots Log CFU/mL per variety represents the mean of 3 repetitions.

The growth behavior of *E. coli* O157:H7 in relation to the type of sampling of the two varieties is displayed in Figure 3 and Figure 4. After analyzing the incorporation of the two varieties with each type of sample and finding no differences between them, it was shown that the variable of the sample type presented significant differences ($p < 0.05$) between nutrient solution and roots. These differences were observed on days 0, 6, 24, and 27 for the Genovese variety analysis (a-d). Subsequently, days with the same letter designation (a-a) showed no significant difference ($p > 0.05$) for the other sampling days. This explains the growth behavior of the bacteria in the traditional system over time. Despite an initial inoculation concentration of 10^7 CFU/mL, the nutrient solution exhibited a concentration of 6 Log CFU/mL on day 0 of sampling, while root samples showed 3.60 Log CFU/mL, indicating a higher concentration in the nutrient solution initially. However, an increase in the bacteria was not observed, as both concentrations exhibited an exponential decrease over the sampling period, attributed to the essential oils of basil.

Figure 3

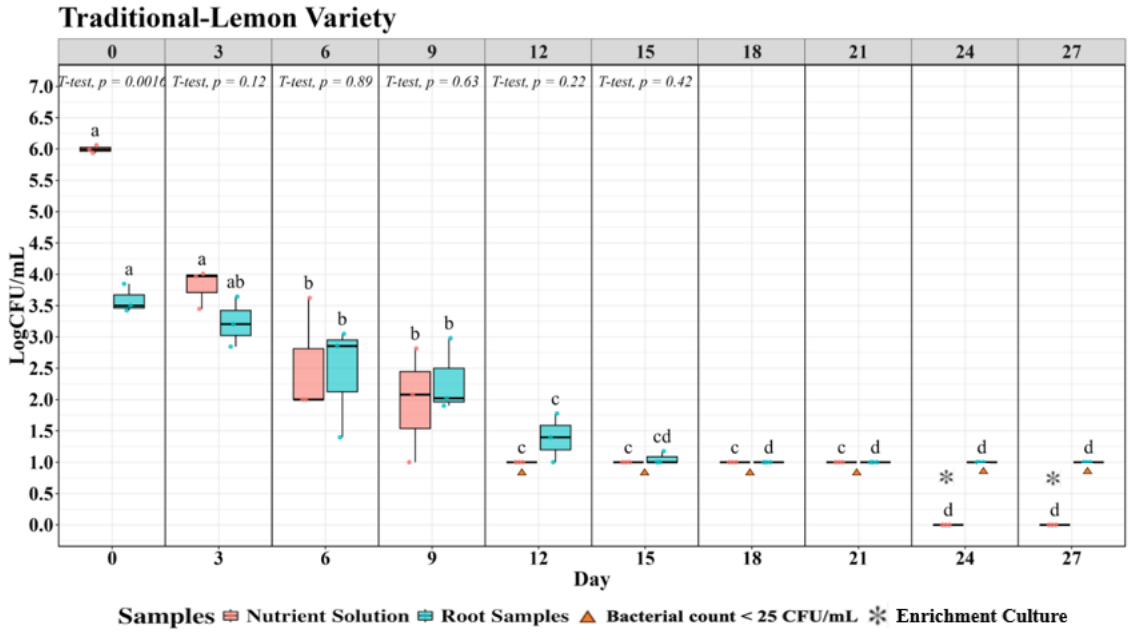
Growth behavior of E. coli O157:H7 in relation to the type of samples in nutrient solution and roots of Genovese basil in the traditional system over twenty-seven days.



For the Lemon variety analysis (Figure 4), significant differences ($p < 0.05$) in the growth behavior of E. coli O157 were observed on days 0, 24, and 27 (a-d). Subsequently, days with the same letter designation (a-a) showed no significant differences ($p > 0.05$) for the other sampling days. Although the specific days of bacterial reduction varied between the Genovese and Lemon varieties, the overall trend remained consistent. This illustrates the similar growth behavior of the bacteria when comparing nutrient solution and root samples.

Figure 4

Growth behavior of E. coli O157:H7 in relation to the type of samples in nutrient solution and roots of Lemon basil in the traditional system over twenty-seven days.

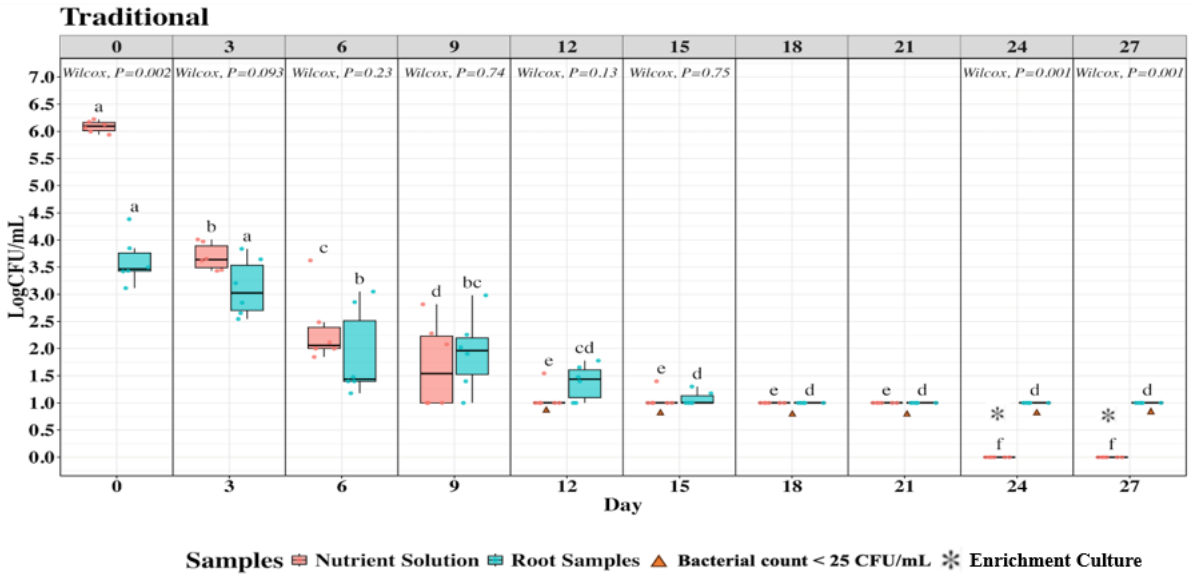


In Figure 5, the growth behavior of *E. coli* O157:H7 is presented as a function of time. The Wilcoxon Test was utilized to compare two groups, nutrient solution and roots, in relation to the dependent variable of bacterial counts in Log CFU/mL. This test revealed significant differences ($p < 0.05$) in bacterial counts between these two sample types. Additionally, the Kruskal-Wallis Test was applied to compare independent samples across different sampling days, demonstrating significant differences ($P < 0.05$) in bacterial counts over time. Each letter (a-f) in the figure denoted differences between sampling days. The results showed that, although the Nutrient Solution initially had a higher concentration of bacteria, the rate of bacterial decrease over time was greater compared to the Roots. Specifically, by day 24 in the traditional system, *E. coli* O157:H7 was no longer detectable in the Roots samples. This absence was further confirmed through an enrichment process, which also indicated no

presence of the bacteria. These findings highlight the differential impact of the sample type on the survival and reduction of *E. coli* O157:H7 over the study period.

Figure 5

Growth pattern of E. coli O157:H7 in nutrient solution and roots of traditional cultivars of Genovese and Lemon basil over a 27-day production cycle.



The behavior of bacterial growth in aquatic environments is influenced by various factors, from the system's temperature to the nutrient solution used. In aquatic environments, bacterial survival is strongly dependent on the source and presence of nutrients in the system since they allow bacterial cells to accumulate essential material for motility functions, energy production, and cellular biomass, allowing bacteria to reduce their disappearance rate (Ozkanca, 1993). This explains why, in the traditional system in nutrient solution, a higher concentration of *E. coli* O157:H7 was observed due to the nutrient-rich medium. Likewise, despite being in a rich medium, the bacteria would be exposed to certain components of the plant that inhibit bacterial growth. On the other hand, although bacteria can lose the ability to grow in an environment when they enter a viable but non-culturable state, they can continue absorbing nutrients and maintaining a reserve of metabolites (Dhulappanavar & Gibson, 2023).

In the case of roots samples in the system during the sampling days, the bacteria prevailed differently than in nutrient solutions, which suggests that the bacteria attached to the roots and presented reduced but longer survival rates because it was able to provide an environment most favorable for survival such as an anaerobic environment with less exposure to environmental stressors. These findings are similar to a study conducted by Dankwa et al., (2020) which found that in hydroponic systems the highest bacterial counts were found in the substrate for coliforms associated with the fact that some substrates retain nutrients on their surfaces serving as a nutrient reservoir for plant use and providing a favorable environment for microorganisms to thrive. Likewise, the bacteria decrease in root samples over time could have been due to the internalization of the bacteria in other parts of the plant not sampled. Studies have shown that *E. coli* O157:H7 can internalize hydroponically grown plants through the root system's injury, allowing the bacteria to disperse to the stem and leaves (Moriarty et al., 2019).

The reduction of *E. coli* O157:H7 counts in the traditional system from 6 to 1 Log CFU/mL in the roots over a 27-day period demonstrates that, although *E. coli* O157:H7 was present, no bacterial counts were reported in the nutrient solution by day 24. This reduction could be attributed to compounds released by basil through its roots, specifically the plant's exudates. Root exudates released in the rhizosphere primarily consist of carbon-based compounds but also include released ions (such as H+), inorganic acids, oxygen, and water; among these compounds are secondary metabolites and phenols, which have been shown to have antibacterial mechanisms (Badri y Vivanco, 2009). Similarly, in the traditional system, the reduction in bacterial counts in the roots could be attributed to the concentration of exudates in the system, influenced by environmental factors and light intensity. In the traditional system, a 16-hour light cycle with red, white, and blue lights was used. Studies have shown that light intensity affects the exudation of secondary metabolites due to changes in photosynthesis, as the root exudation process follows diurnal rhythms, increasing during light periods (Badri & Vivanco, 2009).

The reduction of bacteria in the roots of both varieties is attributed to a compound present in both, specifically rosmarinic acid (RA). Research has shown that rosmarinic acid (RA) is one of the major compounds exuded by basil roots in response to pathogen attack or stress conditions. This compound has been demonstrated to cause morphological changes in the cells of the affected organisms (Bais et al., 2002).

The reduction of *E. coli* O157:H7 observed over the 27 days of sampling demonstrated the efficacy of different compounds in both Genovese and Lemon basil varieties. Lemon basil contains 60.63% citral, with α-citral (33.34%) and β-citral (27.29%), whereas Genovese basil has high levels of linalool (40.77%), eucalyptol (29.37%), and trans-α-bergamotene (4.27%), with eugenol (2.63%) present in lower concentrations compared to Lemon basil (Ciriello et al., 2023). Essential oils (EOs) from each variety contain various active compounds that affect bacterial cells. For instance, eugenol and linalool increase cell membrane permeability, leading to cell viability loss by disrupting ionic homeostasis in the electron transport chain, decreasing intracellular potassium levels while increasing extracellular levels, ultimately resulting in a loss of vital functions and cell death (Stan et al., 2022). The mechanism of action against bacteria is determined by the active molecules of each compound and their structures, such as the phenolic and aldehyde compounds in basil, which are responsible for the cytotoxicity of essential oils. These compounds lead to the denaturation of cytoplasmic proteins and the inactivation of cellular enzymes, resulting in bacterial cell death (Raut & Karuppayil, 2014).

Microgreens System

The production method of microgreens is similar to that used for sprouted seeds, creating favorable conditions for the growth of microbial pathogens since they are typically grown in closed facilities. Due to this reason, this section will focus on determining the growth of *E. coli* O157:H7 in Mat Samples and Roots Samples over a shorter period, considering two varieties of basil in the study.

Table 6 shows the results for the growth of *E. coli* O157:H7 for mat samples, where no significant differences ($p>0.05$) were observed in the concentration of the bacteria between the

Genovese and Lemon varieties in mat samples throughout the fourteen days of study. This could suggest that, in this specific system, the two plant varieties did not significantly influence the growth of the bacteria in the microgreen system, in which the variety variable did not significantly affect the dependent variable of the study.

Table 6

Differences between the growth of E. coli O157:H7 in mat between the Genovese and Lemon varieties in the microgreens system over fourteen days.

Time (Day)	Variety		CV (%)
	Genovese	Lemon	
	Mat Log CFU/mL ± SD	Mat Log CFU/mL ± SD	
0	5.08 ± 0.16[a]	5.26 ± 0.06[a]	2.19
3	3.39 ± 0.36[a]	3.61 ± 0.37[a]	10.35
6	2.65 ± 0.44[a]	2.57 ± 0.40[a]	16.16
9	2.17 ± 0.42[a]	1.83 ± 0.26[a]	16.67
14	1.00 ± 0.00[a]	1.59 ± 0.36[a]	11.27

Note. SD: Standard deviation. CV (%): Coefficient of variation. [a-b] Means with the same lowercase letters for *E. Coli* log CFU/mL indicate no significant differences ($p > 0.05$) in each column. Each Mat Log CFU/mL microbiological count per variety represents the mean of 3 repetitions.

The growth of *E. coli* O157:H7 in the roots of both basil varieties within the microgreens system is presented in Table 7. The data indicates that there are no significant differences ($p > 0.05$) in bacterial counts between the two varieties' roots, as the daily counts are statistically similar throughout the fourteen-day period. In both cases, the logarithmic bacterial counts remained comparable.

Table 7

Differences between the growth of E. coli O157:H7 in roots between the Genovese and Lemon varieties in the microgreens system over fourteen days.

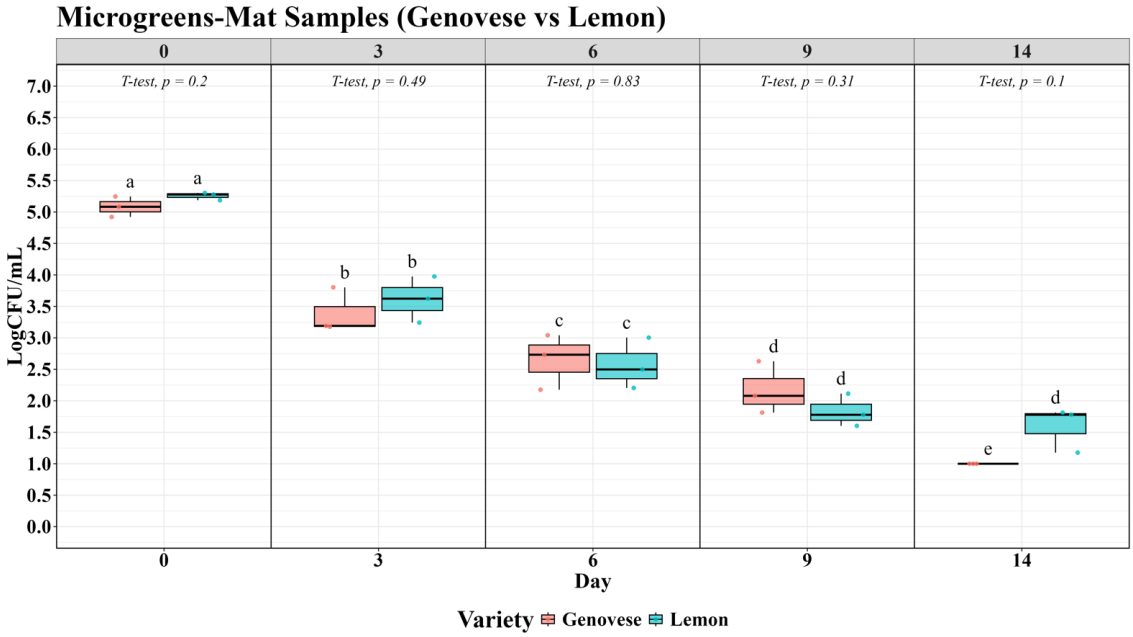
Time (Day)	Variety		CV (%)
	Genovese	Lemon	
	Roots Log CFU/mL ± SD	Roots Log CFU/mL ± SD	
0	5.07 ± 0.18[a]	5.18 ± 0.24[a]	4.12
3	3.12 ± 0.19[a]	3.05 ± 0.11[a]	4.72
6	2.61 ± 0.23[a]	2.34 ± 0.29[a]	10.47
9	1.30 ± 0.52[a]	1.74 ± 0.23[a]	26.79
14	1.06 ± 0.10[a]	1.43 ± 0.15[a]	10.16

Note. SD: Standard deviation. CV (%): Coefficient of variation. [a-b] Means with different lowercase letters for *E. Coli* log CFU/mL indicate significant differences ($p < 0.05$) in each column. Each microbiological count of Roots Log CFU/mL per variety represents the mean of 3 repetitions.

Figures 6 and 7 show how the variety variable did not have a significant effect after the analysis with the Wilcoxon test and T-test ($p > 0.05$) in the comparison of the Genovese and Lemon varieties. The reduction of the bacteria decreased in the sampling period similarly, which is why the same letters (a-a) are assigned between groups. In relation to the data obtained, the effect of both varieties did not show differences, although antimicrobial compounds are attributed to them. The Genovese and Lemon varieties belong to the wide range of basil that is produced by the properties and preferences of the market; however, for microgreens both varieties have a similar concentration of polyphenols, flavonoids, anthocyanins, and nitrates in which their antioxidant capacity is the one that differs, being 19.6% for Genovese variety and 11.8% for Lemon Variety (Fayezizadeh et al., 2023).

Figure 6

Growth of E. coli O157:H7 in mat samples between the Genovese and Lemon varieties in the microgreens system over fourteen days.



The data obtained showed that no significant differences ($p > 0.05$) were observed between the two varieties (Genovese and Lemon basil) in the mat and roots samples on each of the sampling days. A reduction in bacterial count was observed over time for both the mat and roots samples. Although this reduction was not statistically significant as indicated by the different letters (a-e) denoting time points, there was an evident decline in bacterial concentration from approximately 5 Log CFU/mL to 1 Log CFU/mL. This reduction was more pronounced in the root samples over time, which can be attributed to the components present in the roots.

Figure 7

Growth of E. coli O157:H7 in roots samples between the Genovese and Lemon varieties in the microgreens system over fourteen days.

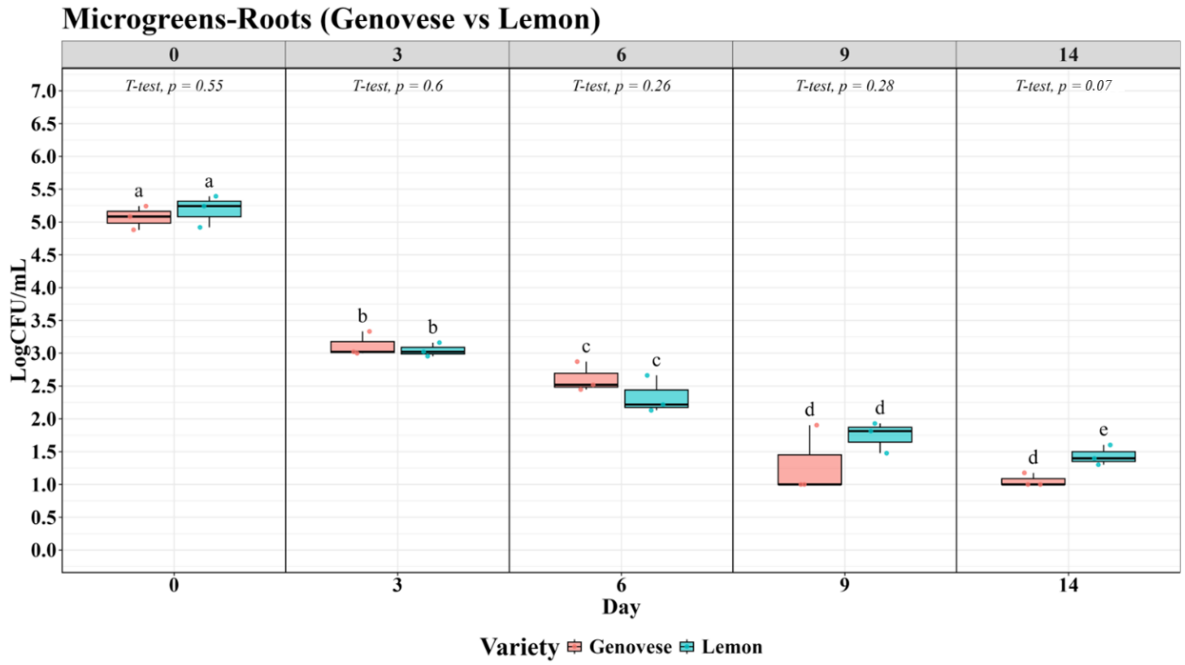


Table 8 presents the bacterial counts of *E. coli* O157:H7 for both basil varieties, categorized by sample type and time over the fourteen-day sampling period. The data shows a logarithmic reduction over time, with significant differences ($p < 0.05$) observed on day 3, where counts were 3.50 Log CFU/mL for mat samples and 3.00 Log CFU/mL for roots samples, as indicated by different letters (a-b) between samplings. No statistical differences ($p > 0.05$) were observed on days 0, 6, 9, and 14, which can be attributed to the bacterial inhibition properties of the microgreen compounds. However, significant differences ($p < 0.05$) were noted across different sampling days, with each day being statistically distinct, as represented by the letters (u-y).

Table 8

Growth of E. coli O157:H7 in mat and Roots between the Genovese and Lemon varieties in the microgreens system over fourteen days.

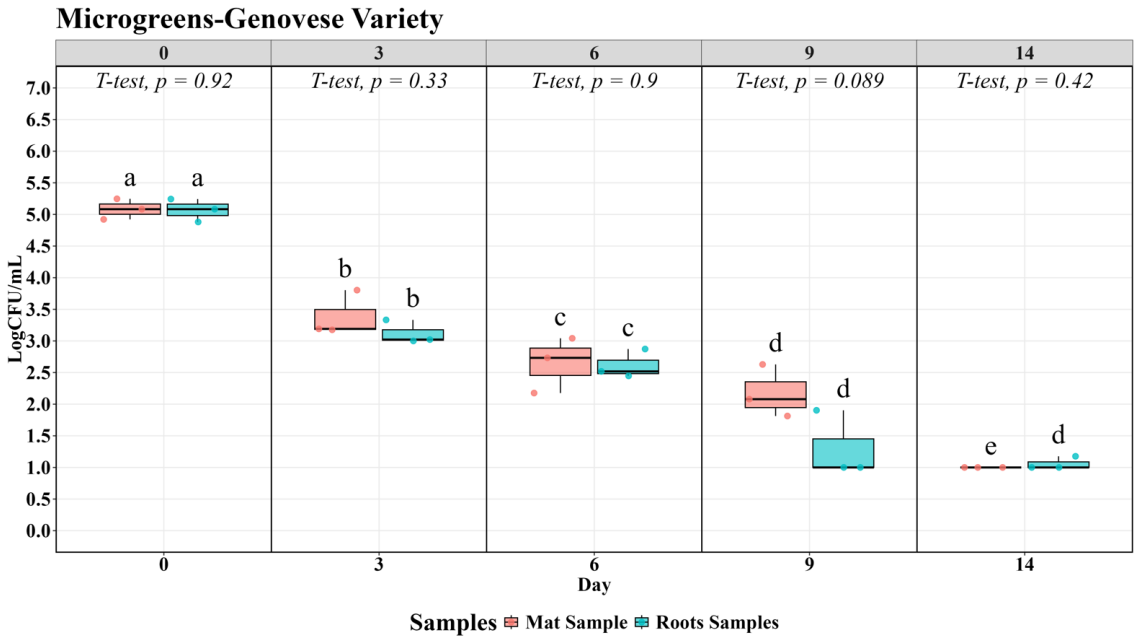
Time (Day)	Variety				
	Genovese		Lemon		
	Mat Log CFU/mL ± SD	Roots Log CFU/mL ± SD	Mat Log CFU/mL ± SD	Roots Log CFU/mL ± SD	CV (%)
0	5.08 ± 0.16^{au}	5.07 ± 0.18^{au}	5.26 ± 0.06^{au}	5.18 ± 0.24^{au}	3.15
3	3.39 ± 0.36^{av}	3.12 ± 0.19^{bv}	3.61 ± 0.37^{av}	3.05 ± 0.11^{bv}	7.53
6	2.65 ± 0.44^{aw}	2.61 ± 0.23^{aw}	2.57 ± 0.40^{aw}	2.34 ± 0.29^{aw}	13.31
9	2.17 ± 0.42^{ax}	1.3 ± 0.52^{ax}	1.83 ± 0.26^{ax}	1.74 ± 0.23^{ax}	21.73
14	1.00 ± 0.00^{ay}	1.06 ± 0.1^{ax}	1.59 ± 0.36^{ay}	1.43 ± 0.15^{ax}	10.71

Note. SD: Standard deviation. CV (%): Coefficient of variation. $^{a-b}$ Means with different lowercase letters for *E. Coli* log CFU/mL indicate significant differences between treatments in each variety ($p < 0.05$) in each column; $^{u-y}$ Means with different lowercase letters for *E. Coli* log CFU/mL indicate significant differences between the evaluated times ($p < 0.05$) in each row. Each Mat and Roots Log CFU/mL microbiological count per variety represents the mean of 3 repetitions.

The bacterial growth behavior of *E. coli* O157:H7 depending on the type of sampling over time, is shown in Figures 8 and 9. Over time, an exponential logarithmic reduction was observed. Although significant differences ($p < 0.05$) were observed on day 14, the bacterial reduction was similar in both mat samples and root samples at the beginning. However, by the last day of sampling, a difference was evident between mat Samples and roots samples. Additionally, in contrast to the traditional system, in microgreens, both samples after inoculation did not present significant differences on the first day, as was observed in the nutrient solution of the traditional system. This behavior may be due to the bacteria in the mat not taking advantage of a nutrient-rich environment since they are attached to a mat rather than being in a submerged water system. This finding is supported by an investigation conducted by Wright and Holden (2018), which found higher levels of bacteria in the mat samples in the absence of plants. Despite having an initial similar concentration of bacteria, the bacterial concentrations in the plants were lower due to the presence of plant compounds.

Figure 8

Growth behavior of E. coli O157:H7 in relation to the type of samples in mat and roots of Genovese basil in the microgreens system over fourteen days.



Similarly, the Lemon variety showed a significant reduction in bacterial counts to day 14, as did the Genovese variety, demonstrating the reduction effect these basil varieties possess due to their essential oils. Despite not showing significant differences between them, this effect can be attributed to both varieties sharing the same environmental conditions and being subject to the same changes. Additionally, for the Lemon variety, during the initial sampling days, the reduction in bacterial counts between mat samples and roots samples was not significantly different until day 9 ($p > 0.05$).

Figure 9

Growth behavior of E. coli O157:H7 in relation to the type of samples in mat and roots of Lemon basil in the microgreens system over fourteen days.

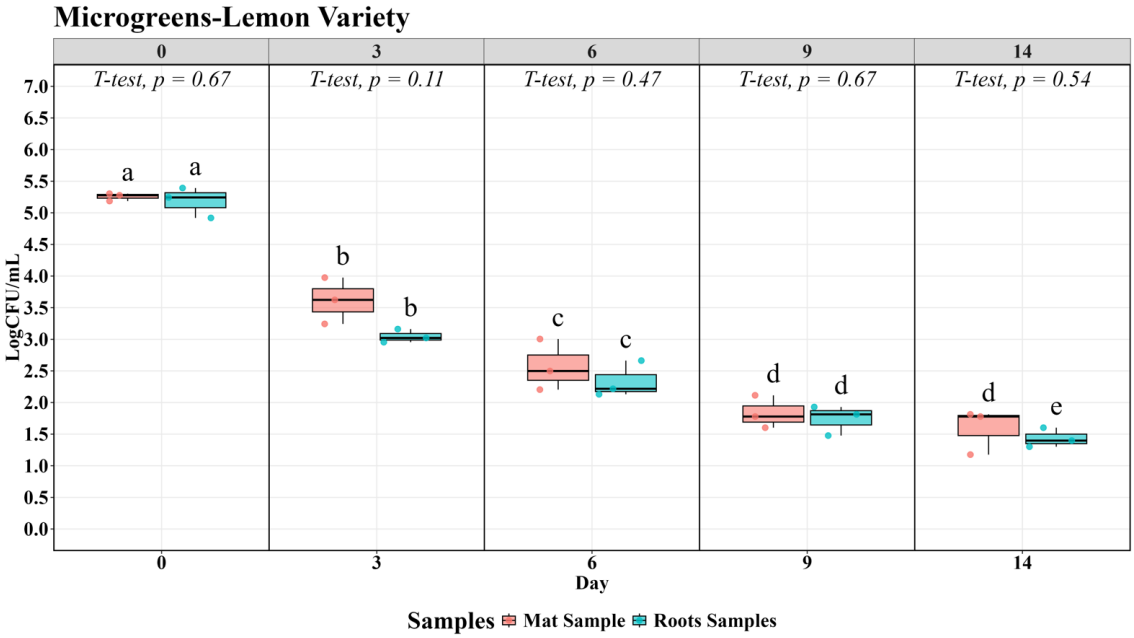
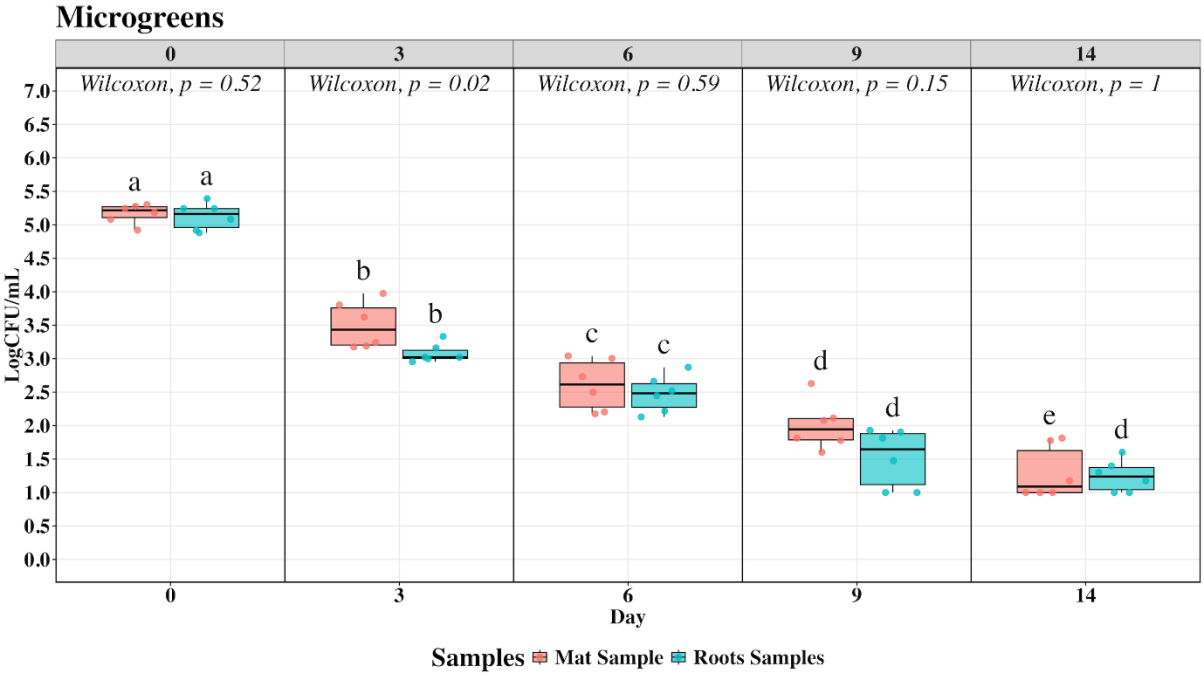


Figure 10 shows the growth behavior of *E. coli* O157:H7 as a function of time, where for the time variable, significant differences were reported ($p < 0.05$) using Krystal-Wallis and Wilcoxon test, having a significant and statistically different reduction as they progressed in the sampling days. The different letters symbolize differences between the days evaluated (a-e), where on day 9 for roots, the bacterial counts are reduced further until reaching day 14 with the same rate of reduction, so both days did not present differences. Statistics ($p > 0.05$).

Figure 10

Growth pattern of E. coli O157:H7 in media (mat) and roots of microgreen cultivars of Genovese and Lemon basil over a 14-day production cycle.



The growth behavior of *E. coli* O157:H7 in the microgreens system is directly related to the environmental conditions that affect the colonization of bacteria, one of the main factors being the availability of water and local humidity (Xiao et al., 2015). Environmental factors in the study could have impacted bacterial survival since the microgreens were grown in a controlled environment chamber. Temperature, along with pH influences *E. coli* enzyme activity where the ideal temperature for *E. coli* is 37 °C, with a minimum of 7 °C and a maximum of 46 °C (Bryant, 2017). Deviations from this range can cause enzyme denaturation and disrupt cellular functions. It is worth noting that the microgreens were maintained at a temperature of 20 °C within the controlled environment chamber, which is below the optimal temperature of 37 °C for the bacteria. Consequently, this lower temperature likely resulted in slower growth or could have been attributed to reduced metabolic activity.

Based on the results obtained, it was determined that both Genovese and Lemon varieties did not present significant differences in the logarithmic decrease of the bacteria; this could be because the properties of both varieties had a similar effect on bacterial inhibition. However, the effect of time in the study determined significant differences in the reduction of *E. coli* O157:H7 for both mat samples and roots samples, with a greater reduction in the last two days of sampling. This can be reinforced by research carried out by Wright and Holden (2018) where in a study they quantified bacterial colonies of *E. coli* O157:H7 in microgreens they determined that viable counts decreased on day 12. In addition, on days 9 and 14, no significant differences were shown, which may be due to a lower reduction in bacteria on day 14 compared to the other days.

The bacterial counts for the present system reduced throughout the 14 days of sampling, in which the initial inoculum concentration of 10^6 CFU/mL decreased to low logarithmic concentrations. It should be noted that microgreens are more vulnerable to bacterial internalization than mature plants since, in immature plants, the structures that protect the plant system are not completely formed, which allows bacteria to enter the Xylem, unlike mature plants that protect them with the Casparian strip: a cell wall made up of insoluble substances (E. R. Turner et al., 2020). In the case of mat samples, a reduction was also observed, though it was less pronounced compared to the roots. This could be attributed to the inert and porous substrate, which helps retain the nutrient solution for the bacteria without directly interacting with the inhibitory compounds emitted by the basil roots (Rusu et al., 2021).

The reduction in the concentration of *E. coli* O157:H7 in the microgreens system could be attributed to the essential properties of the basil varieties, as well as the different conditions of the system that impacted the development of the plants and facilitated the potential internalization of the bacteria. The roots of microgreens, being smaller and more exposed, present more damage compared to other systems; this allows pathogens to adhere to the roots and be internalized into the plant within approximately seven days (Wang et al., 2021).

In general basil microgreens contain volatile oils at concentrations ranging from 0.2% to 1.7% with the main compounds including estragole 15-80% linalool 35-50% and eugenol as well as flavonoids and phenolic acids such as chicoric acid (Rusu et al., 2021). Due to their rapid and active growth stage, microgreens typically have higher concentrations of nutrients and antioxidants compared to mature plants. The antioxidant capacity, as previously mentioned, being 19.6% for the Genovese variety and 11.8% for the Lemon Basil variety, in relation to the presence of linalool, estragole, and phenolic acids, leads to a decrease in pH, which inhibits bacterial growth (Fayezizadeh et al., 2023). Based on the findings, bacterial inhibition may be attributed not only to the structure of the compounds but also to stress factors affecting bacteria, such as pH. It is important to note that the optimal pH range for *E. coli* growth is between 6.0 and 7.0, with a minimum tolerance of 4.0 to 4.5 (Montville et al., 2012). Consequently, changes in pH can affect *E. coli* growth and survival, as low pH can lead to membrane denaturation and inhibit growth. Furthermore, organic acids such as phenolic acids, once dissociated inside the cell, can penetrate the cell membrane, causing the release of protons and subsequently reducing intracellular pH (Bryant, 2017).

Conclusions

The study revealed that survival rates of *E. coli* O157:H7 varied significantly between the traditional system and microgreens, indicating that the growth environment was influential in bacterial survival.

It was found that the selection of the basil variety was not a determining factor in the effectiveness of pathogen control, but rather the conditions in each system.

Genevese and Lemon basil varieties did not present significant differences in the inhibition of the bacteria, however, both varieties allowed *E coli* O157:H7 to attach to their roots, leading to longer persistence in the hydroponic system.

Recommendations

Increase the sample size, as well as the number of repetitions, and consider the different growing conditions to reduce variability in the data.

Perform an analysis of phenolic compounds, essential oils and nutrient solution in the plant of interest to identify components with antimicrobial properties.

Optimize crop conditions that influence the inhibition of bacterial growth, such as nutrient solution, pH, electrical conductivity, temperature, and light spectrum.

Explore and apply new strategies in the cultivation system, such as more controlled conditions that affect the production of bioactive compounds.

Designate ideal spaces during sampling to avoid risks and cross-contamination, ensuring safety and promoting an ideal work environment.

Work with different crops and varieties that are suitable for the hydroponic system to gather more information and determine differences for research improvement.

Perform a cost-benefit analysis to determine the profitability of scaling hydroponic units versus implementing alternative methods.

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Appendices

Appendix A

Ampicillin requirement

Ampicillin requirement

Stock Concentration ampicillin 50µg/mL

4mg/mL using sterile distilled water and stored at 4 °C.

Added 12.5 mL of stock to 1 L sterile distilled water to achieve a final concentration of 50µg/mL.

50µg/mL of ampicillin in 1 L of sterile distilled water

$$\frac{4mg}{ml} x\ 12.5\ mL = 50\ \mu g/mL$$

25 µg /mL of ampicillin in 1 L of sterile distilled water

$$12.5\ mL\ x\ \frac{250\mu g}{mL}\ x\ \frac{mL}{50\ \mu g} = 62.5$$

Appendix B

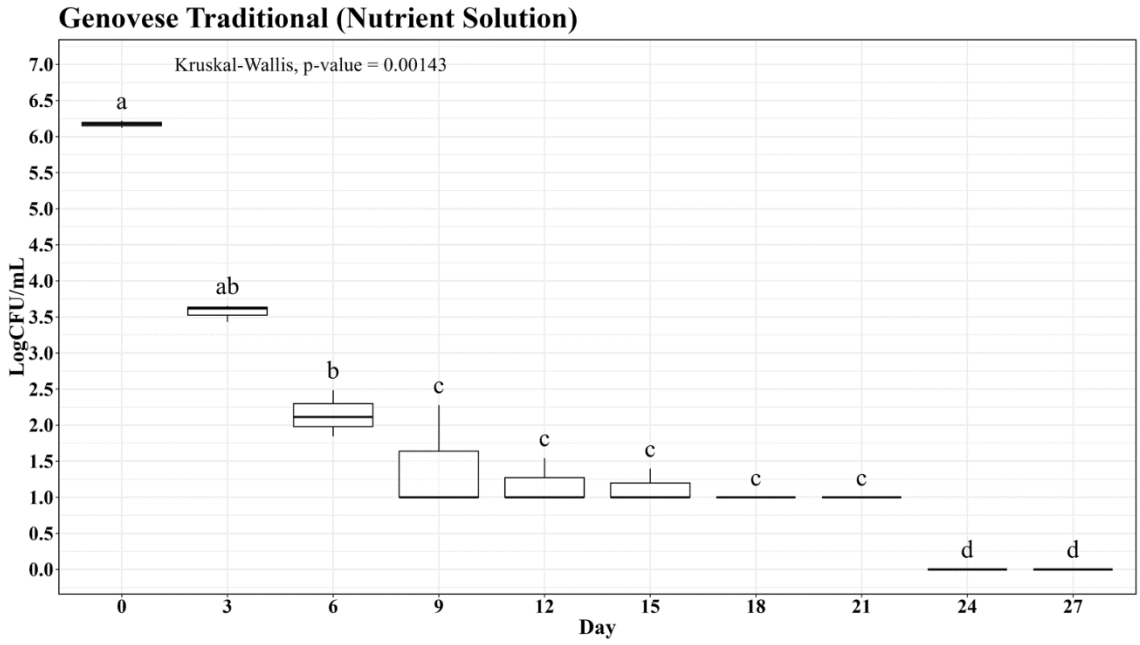
Sampling schedule for traditional system and microgreens

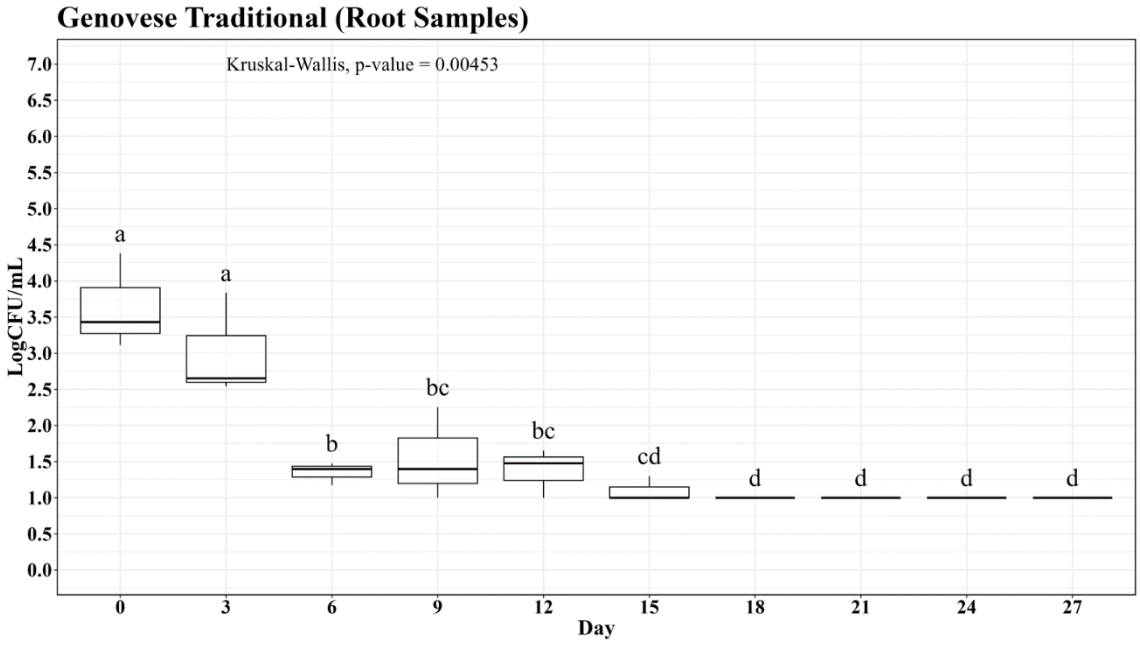
Traditional System				0	3	6	9	12	15	18	21	24	27
	Germination												
	24-Feb	10-Mar	11-Mar	12-Mar	15-Mar	18-Mar	21-Mar	24-Mar	27-Mar	30-Mar	2-Apr	5-Apr	8-Apr
Germination													
Transfer to units													
Prepare inoculum													
Inoculation													
Water sampling													
Plant sampling (Roots)													

Microgreens System	Germination		0	1	3	6	9	14
	18-Mar	22-Mar	23-Mar	24-Mar	26-Mar	29-Mar	1-Apr	6-Apr
Celanining and sanitizing of trays								
Sowed								
Expose the trays to light								
Prepare inocolum								
Inoculate								
Media sampling								
Plant sampling (Roots)								

Appendix C

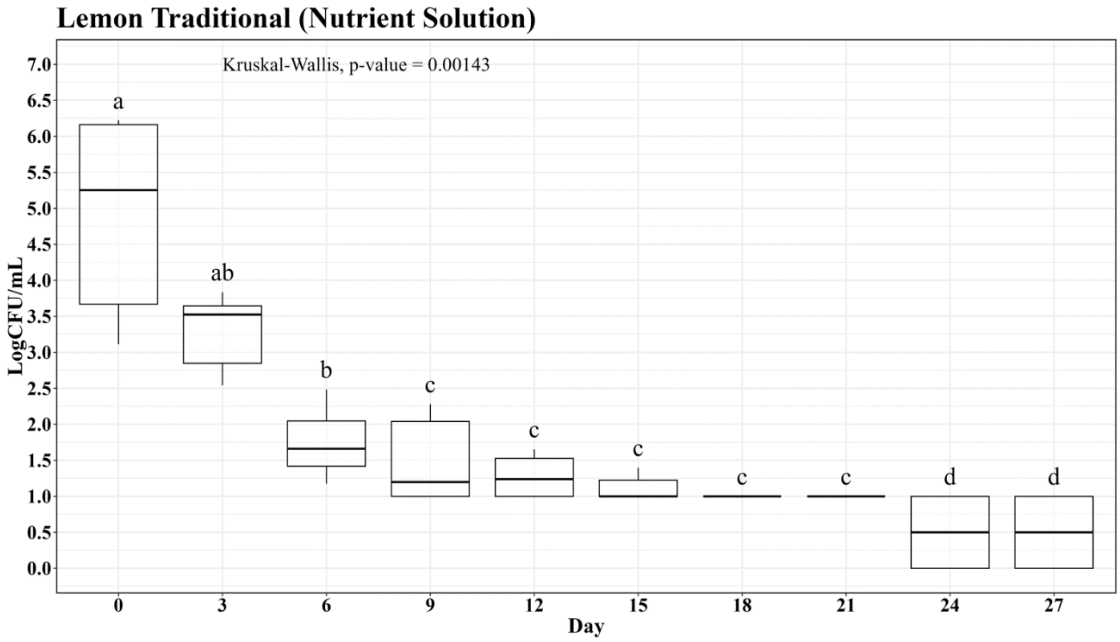
Box plot of E. coli O157:H7 growth pattern in nutrient solution and roots of the traditional Genovese basil cultivar over a 27-day production cycle

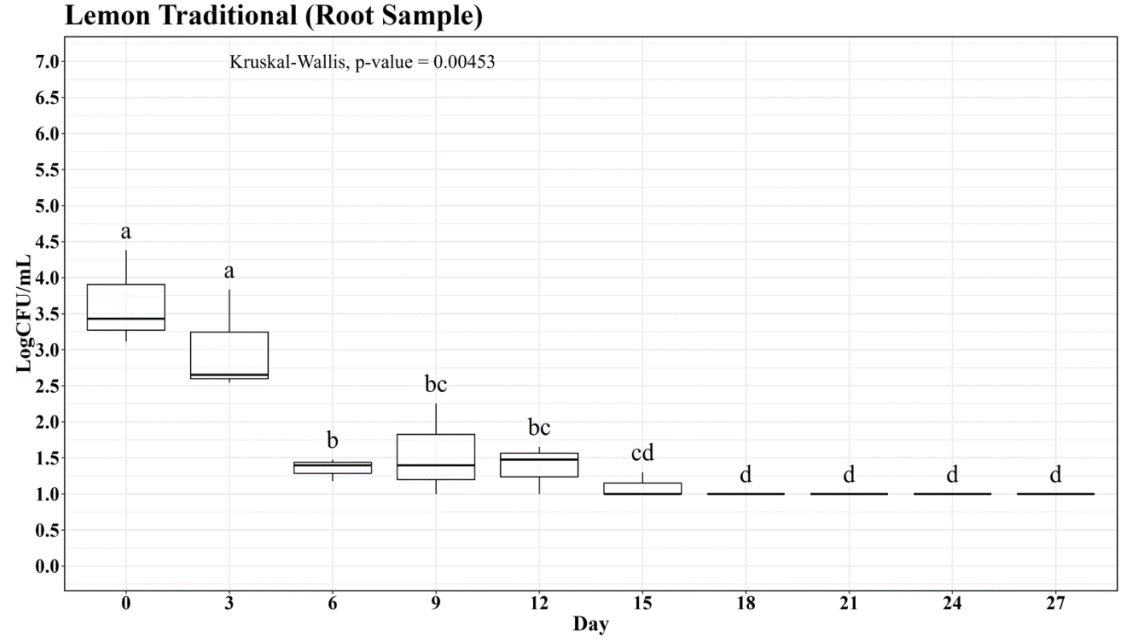




Appendix D

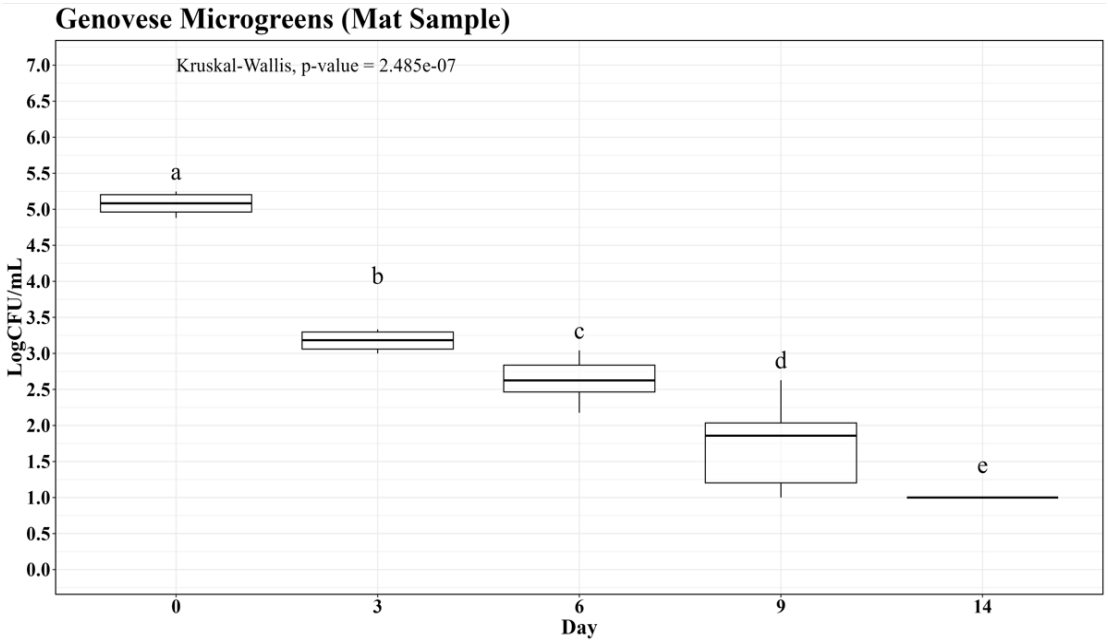
Box plot of E. coli O157:H7 growth pattern in nutrient solution and roots of the traditional Lemon basil cultivar over a 27-day production cycle

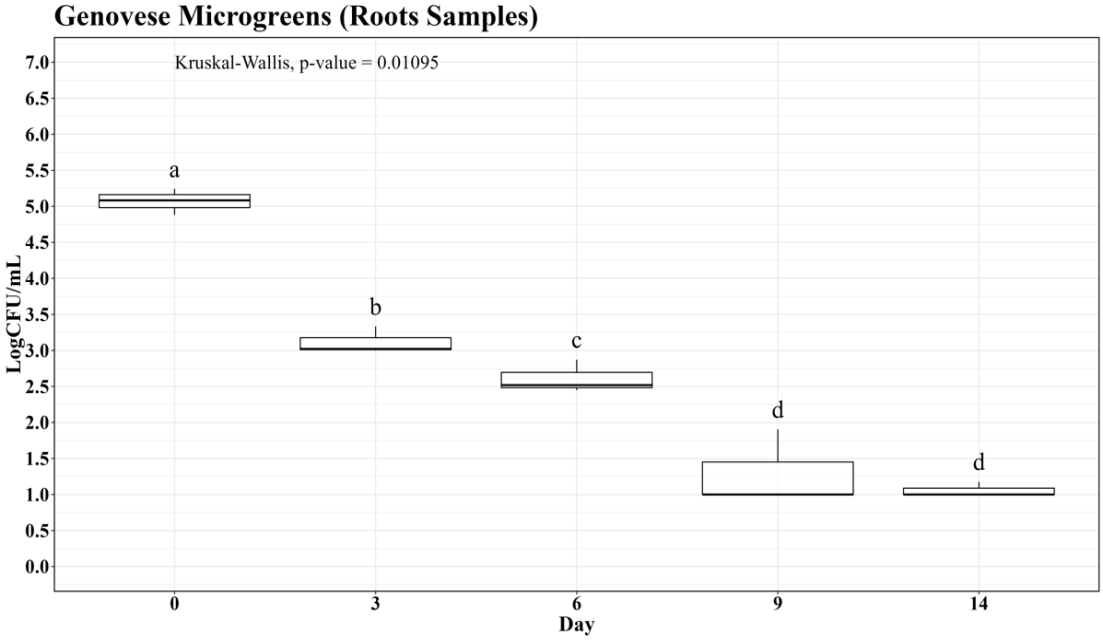




Appendix E

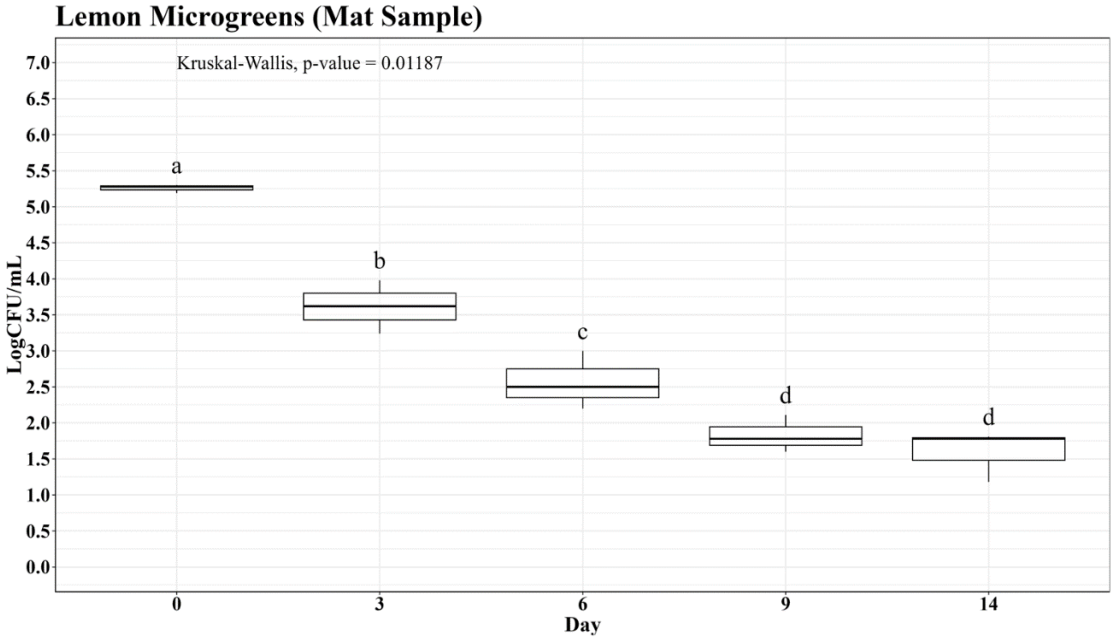
Box plot of E. coli O157:H7 growth pattern in nutrient mat and roots of the microgreen Genovese basil cultivar over a 27-day production cycle

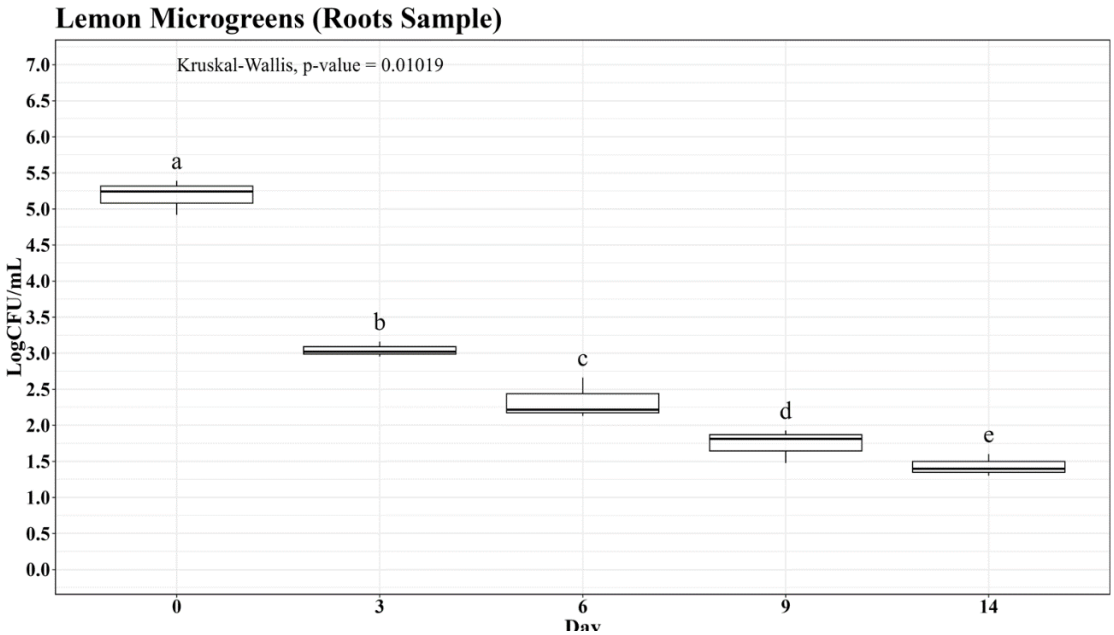




Appendix E

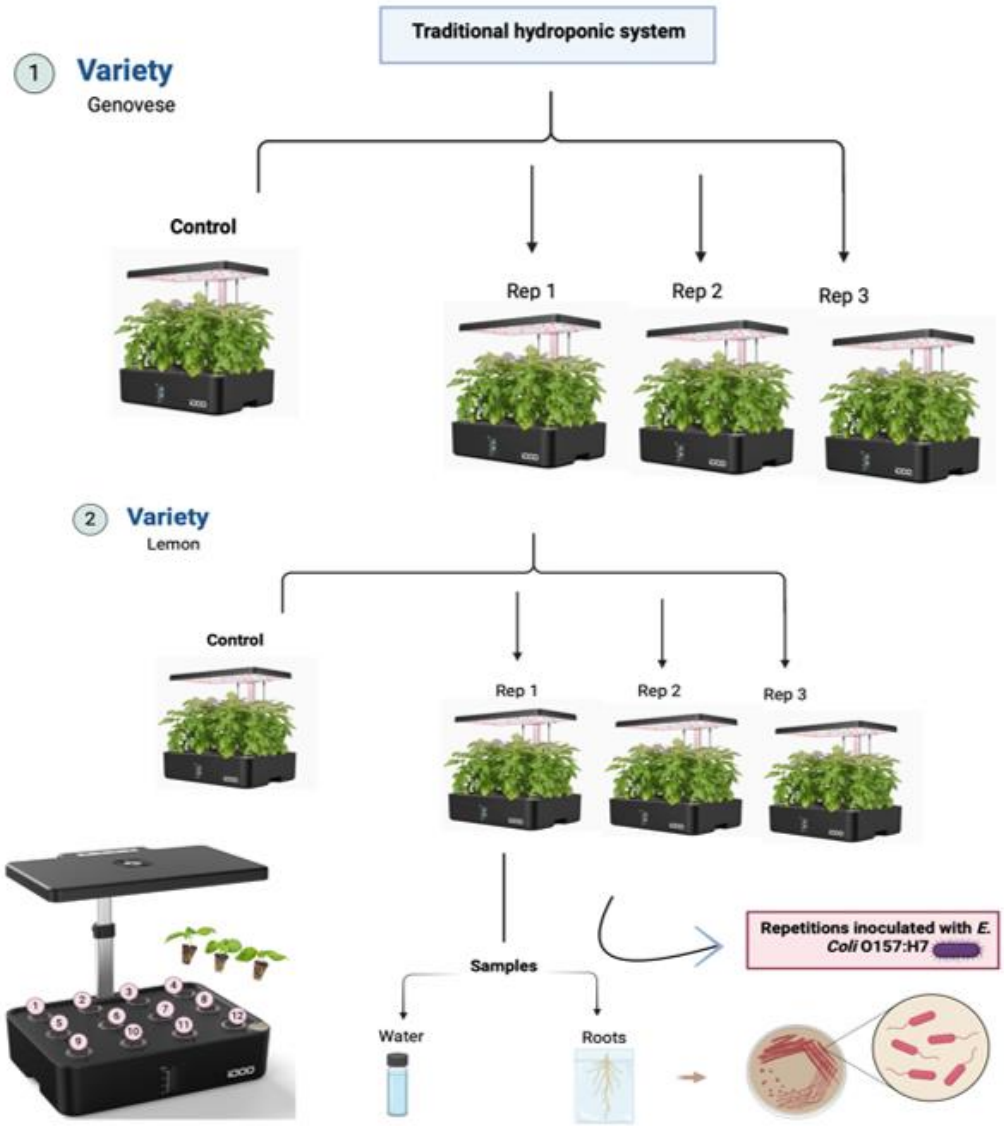
Box plot of E. coli O157:H7 growth pattern in nutrient mat and roots of the microgreen Lemon basil cultivar over a 27-day production cycle





Appendix F

Hydroponic units used for Genovese and Lemon basil varieties in the traditional system



Appendix G

Hydroponic units used for Genovese and Lemon basil varieties in the microgreens system