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Food Science and Technology Department

B.S. in Food Science and Technology

Special Graduation Project

Surveillance of *Salmonella* Typhimurium in Hydroponic Basil (*Ocimum basilicum*) Systems under Varying Nutrient Solution Electrical Conductivities

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Abstract

This study aimed to evaluate the influence of electrical conductivity (EC) levels of the nutrient solution on the survival of *Salmonella* Typhimurium in hydroponic basil (*Ocimum basilicum)* grown under the Nutrient Film Technique (NFT). Pathogen survival was analyzed in nutrient solution, roots, surfaces, and leaves under four EC levels (1.0, 1.3, 1.6, and 2.0 $mS \cdot cm^{-1}$) during a 28-day crop cycle. The system was inoculated with S. Typhimurium at ~8 log CFU/mL (2 mL added to 7.6 L per reservoir), and samples were collected at multiple time points. Data were analyzed using non-parametric tests (Shapiro–Wilk and Kruskal–Wallis, $p < 0.05$). Results showed that EC had no significant effect ($p > 0.05$), while time was highly significant ($p < 0.001$). A biphasic decline was observed: rapid from days 1–7 and slower until day 28. Nutrient solution showed the highest initial counts, roots and surfaces tested positive in early stages, and leaves remained consistently negative. No significant interaction between EC and time was detected. Overall, S. Typhimurium persisted at low levels in nutrient solution, roots, and surfaces until day 28, while leaves stayed free of contamination. These findings demonstrate that survival trends were driven by time rather than EC, and that persistence in hydroponic NFT systems represents a potential risk for cross-contamination.

Keywords: Cross-contamination risk, food safety, herbs, Nutrient Film Technique (NFT), pathogen persistence

Resumen

Este estudio tuvo como objetivo evaluar la influencia de diferentes niveles de conductividad eléctrica (CE) de la solución nutritiva sobre la supervivencia de *Salmonella* Typhimurium en albahaca (*Ocimum basilicum*) cultivada en un sistema hidropónico de Técnica de Película de Nutrientes (NFT). La supervivencia del patógeno se analizó en solución nutritiva, raíces, superficies y hojas bajo cuatro niveles de CE (1.0, 1.3, 1.6 y 2.0 $mS \cdot cm^{-1}$) durante un ciclo de cultivo de 28 días. El sistema fue inoculado con *S.* Typhimurium a ~8 log CFU/mL (2 mL añadidos a 7.6 L por reservorio), y se recolectaron muestras en varios tiempos. Los datos se analizaron mediante pruebas no paramétricas (Shapiro-Wilk y Kruskal–Wallis, $p < 0.05$). Los resultados mostraron que la CE no tuvo un efecto significativo ($p > 0.05$), mientras que el tiempo sí fue altamente significativo ($p < 0.001$). Se observó un patrón bifásico: rápido entre los días 1–7 y más lento hasta el día 28. La solución nutritiva presentó las concentraciones iniciales más altas, raíces y superficies dieron positivo en las etapas tempranas, y las hojas permanecieron negativas. No se detectó interacción significativa entre CE y tiempo. En general, *S.* Typhimurium persistió a bajos niveles en solución nutritiva, raíces y superficies hasta el día 28, mientras que las hojas se mantuvieron libres de contaminación. Estos hallazgos demuestran que las tendencias de supervivencia estuvieron determinadas por el tiempo más que por la CE, y que la persistencia en sistemas NFT representa un riesgo potencial de contaminación cruzada.

Palabras clave: Hierbas, inocuidad alimentaria, persistencia del patógeno, riesgo de contaminación cruzada, técnica de película nutritiva (NFT).

Introduction

Herbs such as basil (*Ocimum basilicum*) play a unique role in modern diets, serving not as staple foods but as flavor-enhancing and health-promoting ingredients. Widely used in culinary traditions around the world, basil is valued for its aromatic compounds, essential oils, and functional properties, including antimicrobial and antioxidant activity (Zdolec et al., 2024). Increasing consumer interest in natural ingredients, fresh herbs, and plant-based wellness products has driven growth in both production and international trade. Globally, countries like India, Egypt, and the United States lead basil cultivation, with production exceeding 500,000 metric tons annually (Food and Agriculture Organization [FAO], 2017). This growing demand has prompted producers to explore innovative cultivation methods, such as hydroponic systems, to ensure year-round availability and high product quality. Alongside production, the international trade of basil continues to expand. Kenya has emerged as a dominant exporter to Europe, while countries like Israel, Egypt, and Ethiopia also supply premium markets in Europe and North America (International Trade Centre [ITC], 2023).In the United States, basil is commercially cultivated in regions such as California and Arizona; however, local production satisfies only part of the country's demand (United States Department of Agriculture, Economic Research Service [USDA], 2023). Consequently, approximately 60% of fresh basil consumed in the U.S. is imported, primarily from Mexico, Colombia, and Peru (USDA, 2023). In contrast, U.S. exports remain relatively low, totaling fewer than 5,000 metric tons annually, mostly to Canada (ITC, 2023). Meanwhile, the European Union maintains a positive trade balance in herbs and spices, with exports consistently surpassing imports (Rokicki & Wiluk, 2016). This imbalance, especially notable in the U.S., highlights the need for sustainable, safe, and efficient production systems to meet rising demand across diverse markets (ITC, 2023; USDA, 2023).

Genovese basil, one of the most widely cultivated varieties, is particularly appreciated for its sensory attributes and nutritional value. The global basil market reached a valuation of $62.5 billion in 2023 and is projected to grow to $94.64 billion by 2031, driven by dietary trends and the pursuit of

functional foods (Market Research Trends & Forecast, 2024; United States Department of Agriculture [USDA], 2022).To maintain consistent quality and maximize resource efficiency, basil is increasingly grown using hydroponic systems, particularly the Nutrient Film Technique (NFT). This system delivers a thin film of nutrient-enriched water over the plant roots, enabling controlled environmental conditions and improved water and nutrient management (Ilic et al., 2022).

Nevertheless, hydroponic cultivation presents specific challenges to food safety. In closed-loop systems like NFT, the recirculation of water can facilitate the spread of pathogens such as *Salmonella* spp., which may colonize plant roots and persist on edible tissues (Ilic et al., 2022). Foodborne illness outbreaks in recent years have underscored this risk. For instance, in April 2024, a *Salmonella* outbreak linked to fresh basil affected 36 individuals across 14 U.S. states (Centers for Disease Control and Prevention [CDC], 2024). Abadias et al. (2008) also demonstrated that herbs can harbor pathogens, reinforcing the importance of hygiene protocols in soilless systems.

Moreover, *Salmonella* Typhimurium has shown the ability to survive in typical greenhouse conditions (24–29 °C, 80–90% relative humidity) and to contaminate key parts of the hydroponic setup, including reservoirs, channels, and plant tissues (Ilic et al., 2022). This is especially concerning since basil is often consumed raw, offering no post-harvest kill step. In 2021, another outbreak tied to hydroponic leafy greens led to 31 reported illnesses in four U.S. states (U.S. Food and Drug Administration [FDA], 2020). Overall, herbs and leafy greens have been linked to over 2,000 cases and several deaths in recent outbreaks (CDC, 2023). A separate 2024 basil-linked outbreak affected 12 individuals across 29 states (FDA, 2024), further illustrating the urgency of addressing food safety in hydroponic production.

Among the factors influencing pathogen behavior in hydroponic systems, electrical conductivity (EC), a measure of nutrient concentration in solutions, has emerged as particularly relevant. Optimal EC levels for basil typically range from 1.0 to 1.6 $mS \cdot cm^{-1}$ and have been associated with improved antioxidant content in plants (Ren et al., 2022). On the microbial side, high EC levels can cause osmotic stress that

disrupts bacterial membranes, reducing their viability (Keerthirathne et al., 2016). However, some studies suggest that specific pathogens may adapt or even benefit from certain EC thresholds (Kong et al., 2012), while EC levels around 3.5 $mS \cdot cm^{-1}$ have been shown to enhance enzymatic activity and reduce microbial populations (Khalil, 2011).

Therefore, this study aims to evaluate the influence of different electrical conductivity (EC) levels on the survival of *Salmonella* Typhimurium in NFT channels, basil roots, leaves, and nutrient solutions in hydroponic basil (*Ocimum basilicum*) production. The findings will contribute to a better understanding of how nutrient conditions affect microbial risks and will support the development of safer, more sustainable production strategies for culinary herbs like basil.

In this study, a general objective and three hypotheses were established.

Main Objective

To evaluate the influence of different electrical conductivity levels on the survival of *Salmonella* Typhimurium in NFT channels, basil roots, leaves, and nutrient solutions in hydroponic basil (*Ocimum basilicum*) production.

Hypothesis

At least one of the electrical conductivity levels (1.0 $mS \cdot cm^{-1}$, 1.3 $mS \cdot cm^{-1}$, 1.6 $mS \cdot cm^{-1}$, and 2.0 $mS \cdot cm^{-1}$) will affect the survival of *Salmonella* in the growth cycle of basil.

The survival and growth rates of *Salmonella* will differ significantly between basil roots, leaves, the nutrient solution, and the surfaces of hydroponic units.

Salmonella will survive in the hydroponic system for the entire growth cycle of 28 days.

Materials and Methods

Growing Procedure for Basil

The basil seeds used in this study were from Johnny's® Selected Seeds. Germination occurred in rockwool cubes that were thoroughly saturated with distilled water, with seeds carefully placed in the crevices at the apex of each cube. The cubes were subjected to continuous light exposure at an intensity of 50 µmol m^{-2} s^{-1} for a duration of 24 hours to promote uniform germination. Environmental parameters were maintained at 25 °C with a relative humidity of 70% to facilitate early growth. Seedlings were permitted to develop until they reached the 3–4 true leaf stage, approximately two weeks subsequent to sowing. The seedlings were cultivated using the NFT system within controlled-environment Percival Plant Growth Chambers (models E-41L2 and AR-41L3) located at the Science Experimental Building I at Texas Tech University.

These chambers allowed for the precise regulation of temperature, humidity, CO_2 levels, and light intensity. Each chamber accommodated four independent NFT systems, designated Treatment 1 through 4, with each unit supporting four basil plants. This configuration was replicated four times, culminating in a total of 128 plants across the two chambers.

Approximately three weeks after germination, the seedlings were transplanted into desktop NFT systems developed by Crop King Inc. (Lodi, Ohio), as this developmental stage is optimal for nutrient uptake in hydroponic systems (Schierstaedt et al., 2020). Each system measured 24 inches in length, 16 inches in width, and 10 inches in height, featuring two growing channels, with each channel accommodating four plants, thereby resulting in eight plant sites per unit. A 9.5-liter reservoir equipped with a 160 GPH pump ensured the continuous recirculation of the nutrient solution. This circulation provided consistent nutrient delivery and oxygenation to the root zones throughout the experiment.

The nutrient solution was formulated using Maxigro™ (10-5-14), a balanced hydroponic fertilizer containing 10% total nitrogen (1.5% ammoniacal and 8.5% nitrate), 5% phosphate, and 14% soluble

potash. It also incorporates essential secondary nutrients such as calcium (6%), magnesium (2%), and sulfur (3%), as well as trace elements including iron (0.12%) and manganese (0.05%) (General Hydroponics, 2021). Following transplantation, light conditions were adjusted to a 14-hour photoperiod at 25 ± 2 °C and 70% humidity, followed by a 10-hour dark period at 20 ± 2 °C and 80% humidity. The daily light intensity was calibrated to 250 μmol m^{-2} s^{-1}, resulting in a daily light integral (DLI) of 12.6 mol m^{-2} day^{-1} (Erickson et al., 2019).

The basil plants were exposed to four distinct electrical conductivity (EC) levels in the nutrient solution: 1.0, 1.3, 1.6, and 2.0 mS·cm^{-1}, corresponding to nitrogen concentrations of 99, 129, 150, and 182 ppm, respectively, as per International Organization for Standardization (2017) conversion standards. The pH of the solution was meticulously monitored and maintained at 5.8 ± 0.2 utilizing a digital potentiometer (HANNA Instruments). Daily adjustments were implemented using potassium hydroxide (20–25%) to elevate the pH and phosphoric acid (10–30%) to lower it (FDA, 2020). This comprehensive management of nutrient composition and pH levels ensured stable conditions conducive to optimal plant development throughout the cultivation period.

Preparation of Inoculum

The bacterial strain utilized in this experiment was procured from the American Type Culture Collection (ATCC). Specifically, the *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain G11013 (ATCC BAA-190) was chosen due to its relevance as a multidrug-resistant strain initially isolated from a clinical case in the United States. This DT104 strain has been shown to exhibit resistance to numerous antibiotics, including ampicillin, chloramphenicol, spectinomycin, streptomycin, sulfonamides, and tetracycline (ATCC, n.d.-b). Its selection was aligned with the objectives of this study, which aimed to evaluate pathogen behavior under realistic contamination scenarios involving high-risk microorganisms.

To prepare the bacterial cultures, isolates were retrieved from storage at -80 °C. A sterile loop was employed to scrape the surface of the frozen stocks gently, and the isolates were streaked onto brain

heart infusion (BHI) agar plates (BD, Franklin Lakes, NJ). These plates were incubated at 35 °C for a duration of 24 hours to facilitate colony development. From the resulting pure colonies, individual cultures were subsequently grown in 10 mL of BHI broth for an additional 24 hours under identical temperature conditions. This procedure ensured adequate bacterial biomass for the preparation of the inoculum.

The following day, 1 mL of each overnight culture was transferred into two separate 9 mL tubes of fresh BHI broth and further incubated at 35 °C for 24 hours. Serial dilutions were then performed, followed by spread plating to achieve the targeted bacterial concentration of 10^8 log CFU/mL. The process was repeated as necessary to confirm viable cell counts. Upon reaching the desired concentration, bacterial cells were harvested via centrifugation at 4000 rpm for 10 minutes at 4 °C, and the resulting pellets were washed twice with 10 mL of 1X phosphate-buffered saline (PBS, pH 7.4). Ultimately, the concentrated cells were resuspended in 10 mL of PBS to obtain a standardized suspension approximating 10^8 log CFU/mL.

Inoculation of Nutrient Solution

After preparing the bacterial suspensions, inoculation was conducted two days after basil seedlings were transplanted into the NFT hydroponic systems. An inoculum volume of 2 mL was introduced into 7.6 L of nutrient solution within each reservoir for each treatment, yielding an initial bacterial load of 8 log CFU/mL. After dilution within the system, the resulting concentration of *Salmonella* in the nutrient solution was approximately 5 log CFU/mL.

Sampling Collection, and Processing Procedure

A systematic sampling plan was implemented for the collection of nutrient solution samples. Initial samples were obtained within 24 hours post-inoculation, with specific short intervals of 10 minutes allocated for attachment studies. Subsequent samples were gathered at 0, 4, 8, and 12 hours, as well as on days 1, 7, 14, 21, and 28. At each time point, 10 mL of the nutrient solution was extracted using sterile

test tubes. Serial dilutions were then conducted with 9 mL tubes of Buffered Peptone Water (BPW) for microbial analysis.

Basil plant samples were collected on days 1, 7, 14, 21, and 28. During each sampling event, whole plants were meticulously removed from the hydroponic system. The roots and edible leaves were separated using sanitized scalpels and tweezers to prevent contamination. Each component was placed into distinct Whirl-Pak bags, weighed, and recorded. To prepare for a 1:10 dilution, the plant weight was multiplied by nine to determine the volume of Buffered Peptone Water (BPW) required. The resultant mixture was then homogenized for one minute utilizing a stomacher, followed by the preparation of serial dilutions in 9 mL tubes of BPW for subsequent microbial analysis

Surface swabbing was carried out to evaluate bacterial presence on specific structural parts of two NFT growing channels (Figure 1). Sponge-Stick Swabs with Neutralizing Buffer were used to collect samples on days 1, 7, 14, 21, and 28, each covering a surface area of 100 cm^2. On Day 1, the right wall at the beginning of the right growing channel was swabbed. On Day 7, the sample was taken from the top cover of the right channel, while Day 14 focused on the top cover of the left channel. Sampling continued at the end of the left growing channel, with the right wall sampled on Day 21. This methodology effectively facilitated the assessment of bacterial presence on surfaces where biofilms are likely to develop (Abigail Aba Mensah et al., 2022).

For the quantification of *Salmonella* populations, Xylose Lysine Deoxycholate (XLD) Agar plates were employed, utilizing an overlay of Tryptic Soy Agar (TSA, Merck KGaA, Darmstadt, Germany). This technique involved the application of a thin layer of TSA with antibiotic (50 µg/mL nalidixic acid) over the XLD agar post-inoculation, which enhanced the recovery of sub-lethally injured cells. *Salmonella* colonies were identified by their distinctive black or black-centered appearance and a yellow periphery (Corry et al., 2011). For samples below the limit of quantification (25 CFU/mL, an enrichment process was performed by transferring 1 mL of the sample into 9 mL of BHI broth, followed by incubation at 37 °C for

24 hours. After incubation, samples were streaked onto selective media to confirm the presence or absence of *Salmonella*.

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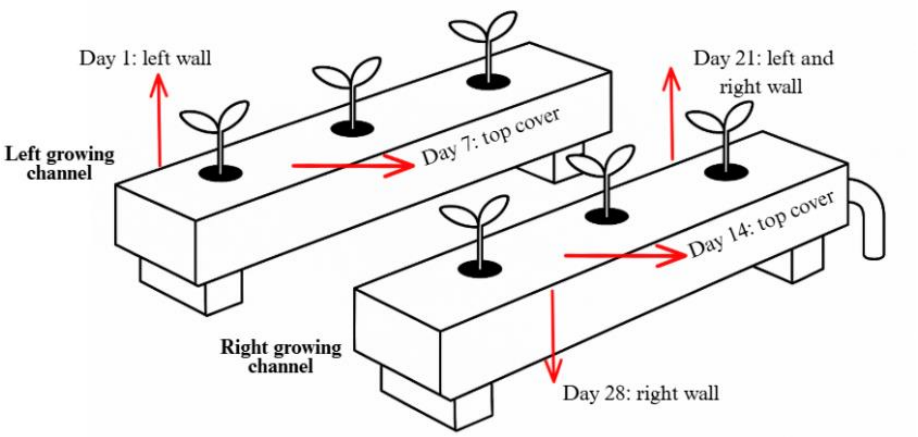
Figure 1

Sampling locations in the NFT hydroponic system. Surface swabbing with sponge-stick swabs was performed on specific areas of two growing channels (100 cm² each).



Day 1 and 2: right and left walls at the beginning of the right channel; Day 7 and 14: right top cover of the right channel and left top cover of the left channel; Day 21 and 28: right and left walls at the end of the left channel.

Experimental Design

In this study, four treatment groups were established to investigate the effects of varying electrical conductivity (EC) levels (1.0, 1.3, 1.6, and 2.0 $mS \cdot cm^{-1}$) on the survival of *Salmonella*. The experiment followed a randomized complete block design (RCBD), where the main factors were EC level and sampling time. The study was conducted inside three Percival growth chambers, and each NFT unit was randomly assigned to a treatment and labeled accordingly. A total of 128 experimental units (basil plants) from each treatment group were sampled, encompassing nutrient solutions, roots, leaves, and surface swabs. During the 28-day experiment, sampling was conducted at specific intervals to monitor microbial presence and system conditions. Nutrient solution samples were collected at ten time points: 0, 4, 8, and 12 hours, as well as on days 1, 7, 14, 21, and 28. Root and leaf samples were taken on days 1, 7, 14, 21, and 28. Additionally, surface swab samples were collected from six different locations along the NFT system channels on days 1, 7, 14, 21, and 28. Throughout the study, environmental parameters such as pH, electrical conductivity (EC), and temperature were continuously monitored to maintain controlled conditions.

To ensure the results were consistent and reliable, the experiment was repeated four times. Microbial counts were expressed as $\log_{10}$ CFU per sample types; nutrient solution (NS): CFU/mL, roots: CFU/g fresh weigh swabbed surfaces: CFU/cm^2. A sample was deemed positive when ≥1 CFU was detected after enrichment; values below that threshold were treated as non-detects. Zeros were handled with a $\log_{10}(x + 1)$ transform for visualization only; all inferential tests were performed on ranks. Normality was assessed with the Shapiro-Wilk test, and because counts were non-normal, non-parametric procedures were applied ($p < 0.05$). In this study, the Shapiro–Wilk test was used to assess normality. Because the data did not follow a normal distribution, the Kruskal–Wallis test was applied. When the Kruskal–Wallis test was significant, pairwise Wilcoxon post hoc tests were performed. All data analysis was evaluated using R statistical software.

To evaluate the Hypothesis H1 (whether at least one EC level 1.0, 1.3, 1.6, 2.0 $mS \cdot cm^{-1}$ affects *Salmonella* survival over the basil cycle), the study ran ART-ANOVA separately for each sample type (NS, roots, swabbed surfaces), with EC, time, and EC × time as factors. For H2 (differences among sample types), on each sampling day (pooling EC levels) a Kruskal–Wallis test compared NS, roots, and swabbed surfaces; leaves were excluded from inference when all observations were non-detects but were reported descriptively. When the test was significant, Wilcoxon post-hoc comparisons were performed. For H3 (survival through the 28-day cycle), persistence was defined as any detection ≥1 CFU per matrix unit on Day 28. For each matrix, the proportion detected was reported with exact binomial 95% confidence intervals, and Day-28 counts were summarized relative to the detection limit.

Results and Discussion

Normality was evaluated with the Shapiro–Wilk test the result was significant ($p < 0.05$), which means the data do not follow a normal distribution. This is also seen when comparing the two figures: Figure 3 shows the theoretical normal distribution (histogram with a normal curve), while Figure 2 shows the distribution of the data from this study, whose histogram and density curve deviate from the expected shape. Therefore, to compare differences between factors, it is appropriate to use non-parametric procedures, as recommended when the normality assumption is violated (Ghasemi and Zahediasl; Shapiro & WilkI, 1965)

Figure 2

Result of histogram.

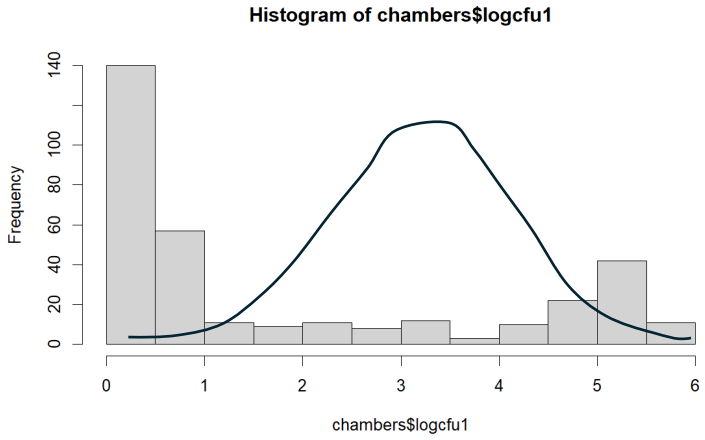
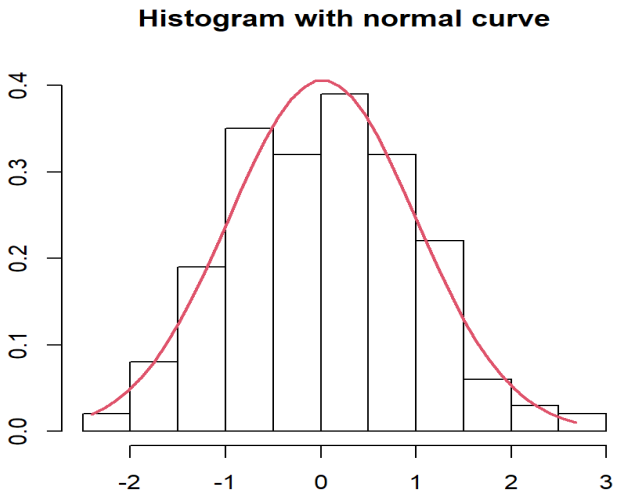


Figure 3

Normal distribution.



The Shapiro-test results were also compared with the visual distribution presented in a histogram (Figure 2). The data are not symmetrically distributed, and there is a clear deviation from the bell-shaped curve that would be expected under normal conditions.

Because the assumption of normality was not met, a nonparametric analysis method must be used. Therefore, the Aligned Rank Transform (ART) for two-way ANOVA is appropriate to evaluate the effects of the treatment and time, as well as their interaction.

Hypothesis 1 - At Least one of the Electrical Conductivity Levels (1.0 mS·cm^{-1}, 1.3 mS·cm^{-1}, 1.6 mS·cm^{-1}, and 2.0 mS·cm^{-1}) will Affect the Survival of *Salmonella* in the Growth Cycle of Basil

These findings likely indicate that, within the EC range tested, the salinity and nutrient concentrations of the nutrient solution did not measurably influence *Salmonella* survival or proliferation on basil (Table 1). This interpretation aligns with evidence that *Salmonella* tolerates a broad range of osmotic conditions and adapts to environmental stress by accumulating osmoprotectants and using specialized transport systems (Altendorf et al., 2009). Persistence may also be explained by the organism's

ability to form biofilms on nutrient film technique (NFT) surfaces and to adhere to plant roots, which protects cells from environmental stressors such as modest EC shifts (Giaouris et al., 2015). Similarly, Ivers et al. (2024) report that once established, *Salmonella* biofilms can be resistant to routine cleaning protocols, even when EC varies in hydroponic systems. Finally, the absence of a significant treatment × time interaction ($p = 0.9494$) indicates that changes in bacterial levels over the 28-day growth period were uniform across all EC levels, reinforcing that salinity or nutrient levels alone were not decisive drivers of *Salmonella* persistence in this system (Table 1).

In contrast, time had a clear effect on *Salmonella* survival ($p < 0.001$), as counts decreased markedly over the 28-day growth cycle. These findings agree with previous hydroponic studies showing that, although *Salmonella* can persist in NFT, its populations usually decline as the crop cycle progresses (Guillén et al., 2021; Ilic et al., 2022). Several mechanisms especially integrated stress responses and the system's microbial dynamics may explain this temporal reduction (Dong & Feng, 2022; Guillén et al., 2021). First, hydroponic systems are dynamic microbial environments in which shifts in community composition and nutrient availability can create unfavorable conditions for pathogen persistence. In NFT, a time dependent decline of *Salmonella* is expected because significant reductions occur in the nutrient solution throughout the crop cycle, while residual cells may persist on roots and inert media (Ilic et al., 2022). Moreover, high temperatures and constant water circulation can promote limited survival through starvation and microbiome changes (Dong & Feng, 2022). Similarly, Weller et al. (2020) note that hydroponic systems offer controlled conditions that can reduce contamination risks for *Salmonella*.

Second, plant-microbe interactions likely contribute to this decline. Hydroponic crops actively shape their microbiomes through root exudates, nutrient mixes, and chemical signals that vary with plant stage and stress; these exudates can attract or repel specific bacteria, limiting pathogen survival (Thomas et al., 2024). Third, basil provides an additional chemical barrier: its phenolic and terpenoid metabolites (e.g., linalool, eugenol, estragol) show antibacterial and antibiofilm activity against foodborne bacteria,

supporting the role of basil-derived chemistry in suppressing enteric pathogens over time (Azizah et al., 2023). In addition, one study reports that basil contains citral, which is effective against *Salmonella* enteritidis and reduces its viability (Gutiérrez-Pacheco et al., 2023; Wang et al., 2023). Likewise, water-soluble fractions of basil, rich in metaeugenol, have shown a reduction of bacterial load by destabilizing the *Salmonella* membrane, increasing permeability, and causing ATP leakage and depolarization, thus producing a bactericidal effect (Yadav et al., 2025)

Table 1

Statistical analysis of Salmonella Typhimurium counts regardless of the electrical conductivity treatments (1.0 mS·cm⁻¹, 1.3 mS·cm⁻¹, 1.6 mS·cm⁻¹, and 2.0 mS·cm⁻¹) and sample type (nutrient solution, roots, swabs) over 28 days of basil growth.

Effect type	P – value
Microbial counts	
Treatment	0.3255
Time	$p < 0.001$***
Treatment*Time	0.9494

Note. *** = highly significant at p < 0.001

These notes show how confident you can be in rejecting the null hypothesis. In this case, the effect of time was highly significant (p < 0.001).

Differences in the survival and growth rate of Salmonella on basil roots, basil leaves, the nutrient solution, and hydroponic unit surfaces

Hypothesis 2

The survival and growth rates of Salmonella will differ significantly between basil roots, leaves, the nutrient solution, and the surfaces of hydroponic units.

Hypothesis 3

Salmonella will survive in the hydroponic system for the entire growth cycle.

As shown in Table 2, time had a statistically significant effect (p < 0.001) on microbial counts in nutrient solutions, roots, and swabbed surfaces, with counts declining over the growth cycle regardless of

EC treatment. Overall, these results indicate that time was the primary factor driving the observed decrease in microbial counts, while the treatment had no detectable effect.

Table 2

*Effect of time and treatment on microbial counts in the Nutrient solution, roots, and surface swabs***.**

Microbial counts	
Effect type	P value
Nutrient Solution	
Time	$p < 0.001$ ***
Treatment	0.0997
Time*Treatment	0.9988
Roots	
Time	p < 0.001***
Treatment	0.2714
Time*Treatment	0.2996
Surface Swabs	
Time	p < 0.001***
Treatment	0.4307
Time*Treatment	0.4338

Note. *** = highly significant at p < 0.001

These notes show how confident you can be in rejecting the null hypothesis. In this case, the effect of time was highly significant (p < 0.001).

Nutrient solution.

Table 3 shows that microbial counts in nutrient solutions changed significantly over the 28-day basil growth period. Counts remained high and stable during the first 12 hours (5.09–5.35 log CFU/mL), peaking at 4 hours with no significant differences among early time points. From Day 1, microbial load declined sharply, dropping to 2.37 log CFU/mL, halving by Day 2 (1.28 log CFU/mL), and falling below the quantifiable limit (1.4 log CFU/mL, ~25 cells) by Day 7, representing a 97% reduction. Counts remained detectable until harvest, reaching a 99% reduction by day 28.

Table 3

Microbial counts by sampling point of the nutrient solution, regardless of treatments.

Time	Unit	Log CFU/mL ± SD
0	Hour	5.20 ± 0.40^{ab}
4	Hour	5.35 ± 0.24^{a}
8	Hour	5.15 ± 0.41^{ab}
12	Hour	5.09 ± 0.46^{b}
1	Day	2.37 ± 0.66^{c}
7	Day	0.14 ± 0.07^{d}
14	Day	0.09 ± 0.09^{e}
21	Day	0.03 ± 0.07^{ef}
28	Day	0.02 ± 0.06^{f}

Note. Microbial counts (log CFU/mL ± SD) from the nutrient solution sampling point over time, regardless of treatment. Values represent the mean of four replicates per sampling point, $^{a, b, c, d, e, f, g}$. Different lowercase letters indicate statistically significant differences between time points ($P < 0.05$), SD. standard deviation.

The observed temporal decline in microbial counts in the nutrient solution (Table 3) indicates that *Salmonella* populations cannot sustain high levels in hydroponic systems over prolonged periods. In hydroponic systems, nutrient solution characteristics influence *Salmonella* Typhimurium survival, with the exception of EC as earlier presented (Table 1). The initial stability during the first 12 hours suggests that the bacteria were able to survive briefly in the nutrient-rich environment, likely due to favorable conditions such as adequate nutrients and minimal stress (Thapa et al., 2024). However, the rapid decline from Day 1 onwards suggests that the nutrient solution became increasingly inhospitable over time (Ilic et al., 2022). This decrease may be attributed to factors such as natural inhibition, including nutrient depletion, competition with other microorganisms, and accumulation of waste metabolites (Gonzalez & Aranda, 2023). Environmental stressors such as temperature fluctuations, pH shifts, and oxygen exposure in the recirculating water could also reduce bacterial viability (Liu et al., 2018). Although pathogen concentrations declined over time, *Salmonella* remained detectable throughout the study, indicating persistence under hydroponic conditions (Dhulappanavar & Gibson, 2023). Experimental work shows that

Salmonella can survive for several weeks in hydroponic nutrient solutions across a range of temperatures, and in NFT systems can persist on roots and inert media even as waterborne counts fall (Ilic et al., 2022).

The persistence of *Salmonella* in the nutrient solution underscores the need to treat recirculating water as a potential contamination vector (Abigail A. Mensah et al., 2024). While counts tend to decrease with time, understanding the drivers of this decline can guide early interventions especially during the first day, when concentrations are highest and inform sanitation of wetted surfaces and equipment. Reviews and intervention studies in hydroponic NFT systems indicate that targeted water and surface treatments are essential; for example, certain peroxyacetic-acid and QAC sanitizers can eliminate S. Typhimurium from NFT surfaces, whereas chlorine at typical rates may be less effective (Abigail A. Mensah et al., 2024; Sela Saldinger et al., 2023).

Roots.

Table 4 shows that microbial counts on the roots changed significantly over the 28-day basil growth period. The highest load occurred on Day 1 (4.36 ± 0.32 log CFU/mL), indicating strong initial colonization. Counts declined sharply below quantifiable limits by Day 7 (0.90 ± 0.52 log CFU/mL) and continued to decrease over time till Day 28, showing a 98% decline, reflecting a gradual reduction in *Salmonella* persistence on roots. Statistical analysis confirmed significant differences between most sampling days ($P < 0.05$).

Table 4

Microbial counts of root samples regardless of treatments.

Time	Unit	Log CFU/grams ± SD
1	Day	4.85 ± 0.32^{a}
7	Day	0.91 ± 0.52^{b}
14	Day	0.39 ± 0.51^{c}
21	Day	0.24 ± 0.37^{cd}
28	Day	0.09 ± 0.26^{d}

Note. Microbial counts (log CFU/mL ± SD) from root samples, averaged across all treatments. Values represent the mean of four replicates per sampling point. ^{a,b,c,d}, different lowercase letters indicate statistically significant differences between time points ($P < 0.05$). SD. standard deviation.

While the time factor was statistically significant ($p < 0.05$), the EC treatment did not have a significant impact on microbial counts. This suggests that factors other than the electrical conductivity treatments, such as natural processes or environmental conditions, were more influential in shaping the microbial dynamics within the root zone (Ivey et al., 2025). The observed decline in *Salmonella* counts on basil roots over the 28-day period suggests that while the roots supported strong initial colonization, conditions became progressively unfavorable for long-term persistence. The sharp reduction by Day 7 may reflect limited nutrient availability at the root surface, increased competition with native microbiota, or the activation of plant defense mechanisms, such as antimicrobial compounds in root exudates. Similar reductions in root-associated pathogens have been reported in hydroponic and soil systems, where root exudates and microbial community dynamics influence pathogen survival (Olanrewaju et al., 2019).

Several factors can explain the observed changes in *Salmonella* Typhimurium counts. First, root exudates mixtures of sugars, amino acids, and organic acids provide readily utilizable substrates that can support survival and initial growth of enteric bacteria near roots (Kwan et al., 2015). These compounds help create a favorable rhizosphere microhabitat during early contamination stages, while the community is still assembling (Olanrewaju et al., 2019). In addition, the rhizosphere offers physicochemical refuges and micro-niches on root surfaces where cells can colonize and persist (Jechalke et al., 2019). However, as the plant and its microbiome mature, increasing competition and community succession, along with induced plant defenses, tend to limit *Salmonella* persistence (Thomas et al., 2024).

As the plant grows and the root microbiota begins to establish, however, plant defenses or competition with beneficial microbes may limit the persistence of *Salmonella* Typhimurium (Olanrewaju et al., 2019; Thomas et al., 2024). Basil (*Ocimum basilicum*) is recognized for its diverse antimicrobial components, primarily found in its essentia (Azizah et al., 2023).The essential oil exhibits a broad spectrum of antimicrobial activity against various microorganisms, including bacteria and fungi. Key components contributing to this activity include eugenol, methyl chavicol, and 1,8-cineole, among others (Kačániová et

al., 2022). This oil shows broad antibacterial activity in foods and on contact surfaces (Chouhan et al., 2017). The eugenol disrupts bacterial membranes altering permeability and causing leakage of intracellular material which helps explain activity against Gram-negative pathogens (Hyldgaard et al., 2012). Consistently, basil essential oil inhibits *Salmonella* Enteritidis in vitro and in foods, supporting a role for basil-derived chemistry in limiting enteric pathogens (Rattanachaikunsopon & Phumkhachorn, 2010).

The reduction in *Salmonella* counts observed by Day 7 may reflect the influence of plant immunity or the action of the root microbiota, which can inhibit pathogen survival (Ilic et al., 2022). These interactions between plant defenses and root microbiota likely play a crucial role in limiting the duration and extent of contamination within the root zone (Garcia-Graells et al., 2020). The root zone is a critical reservoir for pathogens in hydroponic systems, emphasizing the importance of implementing effective sanitation and microbial management measures in this area (Li & Uyttendaele, 2018; Shaw et al., 2016). Since *Salmonella* Typhimurium can attach to both roots and substrates, it is essential to target these regions with appropriate control strategies. Effective interventions could reduce the risk of contamination spreading from the root zone to other parts of the system, thereby mitigating overall pathogen exposure (Ilic et al., 2022). Timing is also a critical factor in pathogen management. The period between Days 1-7 was identified as a window of highest risk for root-associated contamination, with *Salmonella* Typhimurium counts being highest during this time. This suggests that frequent monitoring and early intervention during this critical window could be vital for minimizing pathogen loads in the root zone (Ilic et al., 2022).

The findings of this study are consistent with previous research showing that foodborne pathogens, such as *Salmonella* Typhimurium, can accumulate in plant roots and substrates after contamination through the nutrient solution (Ilic et al., 2022). In closed-loop hydroponic systems like NFT, the continuous circulation of nutrient solutions can facilitate the distribution of pathogens across the

entire system, underscoring the need for constant monitoring and contamination prevention strategies in these environments (Shaw et al., 2016).

Although bacterial levels dropped below quantifiable limits, *Salmonella* was still detectable, indicating its ability to persist at low concentrations, potentially through biofilm formation or attachment to root surfaces. This persistence aligns with previous findings that *Salmonella* can establish on plant roots even when overall populations decline significantly (Gurtler et al., 2013). From a food safety perspective, the continued presence of low-level contamination highlights the risk of pathogen transfer from roots to edible plant tissues or the nutrient solution, emphasizing the need for strict monitoring and preventive strategies in hydroponic production systems.

Swabs.

Table 5 shows the average microbial counts on surface swabs over 28 days. The highest levels were recorded on Day 1 (2.81 ± 0.99 log CFU/mL) and Day 2 (2.52 ± 1.41 log CFU/mL), with no significant difference between them. Counts declined significantly to below quantifiable limits by Day 7 (0.97 ± 0.88 log CFU/mL) and remained very low thereafter till Day 28. These later values did not differ significantly from each other but were significantly lower than those at earlier time points. Overall, the results indicate a continuous decline in surface-associated bacteria, confirming that time had a significant effect on bacterial reduction ($P < 0.05$).

Table 5

Microbial counts of surface swab samples regardless of treatments.

Time	Unit	Log CFU/100 cm^2 ± SD
1	Day	2.81 ± 0.99^{a}
7	Day	0.97 ± 0.88^{b}
14	Day	0.33 ± 0.60^{c}
21	Day	0.15 ± 0.32^{c}
28	Day	0.05 ± 0.19^{c}

Note. Microbial counts (log CFU/mL ± SD) from surface swab samples, averaged across all treatments. Values represent the mean of four replicates per sampling point. $^{a, b, c,}$ Different lowercase letters indicate statistically significant differences between time points ($P < 0.05$). SD**.** standard deviation.

Salmonella Typhimurium was found on swabbed surfaces of NFT channels at the start of production. Counts went down overtime, showing a biphasic pattern: a fast drop between day 1 and day 7, and then a slower reduction from day 14 to day 28. This suggests that many cells could not survive nutrient limitation, osmotic stress, and water pressure in the first phase ((Altendorf et al., 2009; Giaouris et al., 2015). The small number of cells that remained were probably protected by biofilms, which give resistance to stress and cleaning (Habimana et al., 2014). These biofilms can persist in humid, warm, and nutrient-rich conditions of NFT systems (Ilic et al., 2022; Abigail A. Mensah et al., 2024). Greenhouse conditions of 24–29 °C and more than 80% humidity make persistence stronger (Deblais et al., 2019; Orozco R et al., 2008). The extracellular matrix increases tolerance to changes and to antimicrobial products, making cells very hard to remove once attached to surfaces (Garcia-Graells et al., 2020). Even if final counts were very low, the presence of residual cells shows a risk of cross-contamination and the need for strict cleaning (Ilic et al., 2022; Shaw et al., 2016).

This persistence is important for new production cycles. Biofilms left after harvest can be a source of inoculum. Cells inside biofilms may detach and go back into the nutrient solution, increasing the risk of recontamination (Tham et al., 2024). Even small populations can spread in recirculating water and affect the next crops (Ilic et al., 2022). Factors like pH and oxygen also support biofilm stability (Toyofuku et al., 2016). The type of materials also matters: polyethylene and PVC, which are rough and hydrophobic, make adhesion easier (Ivers et al., 2024). Recirculating flows and shear stress also help microbes attach, and rougher surfaces make biofilms more stable (Lee et al., 2020; Thames et al., 2023). These results show that system design and operation can favor colonization and stabilization of *Salmonella*.

Biofilm formation makes *Salmonella* more resistant to sanitation (Yaron & Römling, 2014). They use cellulose and curli fibers to survive longer. Other studies also show survival in hydroponics. Ilic et al. (2022) found that *Salmonella* and Listeria can persist in NFT systems, while Sela Saldinger et al. (2023) reported that pathogens survive in water, infect roots, and form biofilms on wet surfaces. Shaw et al.

(2016) highlight that nutrient rich hydroponic environments can sustain *Salmonella* survival despite overall reductions in concentration.

Leaves.

Results of S. Typhimurium detection on basil leaves showed that no *Salmonella* was detected at any treatment or time point, indicating that leaf tissues did not become contaminated under the experimental conditions. This absence may reflect the plant's natural antimicrobial properties and physical defense mechanisms.

The absence of *Salmonella* Typhimurium on basil leaves across all treatments and time points indicate that foliar tissues did not become contaminated under the experimental conditions. This result was strongly influenced by biosecurity measures implemented in the hydroponic system. Preventive actions such as avoiding nutrient solution splashing, maintaining sanitized greenhouse conditions, and following Good Agricultural Practices (GAP) minimized the risk of cross-contamination from nutrient solution, tools, or human handling (Dong & Feng, 2022; Sela Saldinger et al., 2023). These practices are essential, since in NFT systems pathogens can persist in recirculating solutions and potentially transfer to edible tissues if introduced (Loneragan et al., 2012; Thomas et al., 2024). The additional risk of biofilm persistence on inert surfaces, which makes eradication challenging (Tham et al., 2024), further reinforces the importance of rigorous sanitation protocols.

Beyond management practices, several plant-related and ecological factors also contributed to the absence of contamination. Although *Salmonella* can survive in the root zone, its upward migration into aerial tissues is usually limited or undetectable (Gorbatsevich et al., 2013; Ilic et al., 2022). On foliage, bacterial persistence is constrained by nutrient scarcity and the lack of enzymes required to degrade plant cell walls (Brandl et al., 2013; Karmakar et al., 2018). At the same time, basil possesses strong intrinsic defenses: antimicrobial essential oils such as linalool, estragole, and eugenol with proven antibiofilm activity (Pavone et al., 2025; Rattanachaikunsopon & Phumkhachorn, 2010), and structural traits like a

thick cuticle and glandular trichomes that limit bacterial adhesion (Zdolec et al., 2024). Together, these biochemical, structural, and environmental factors complemented the biosecurity measures, resulting in the consistent absence of *S. Typhimurium* on basil foliage.

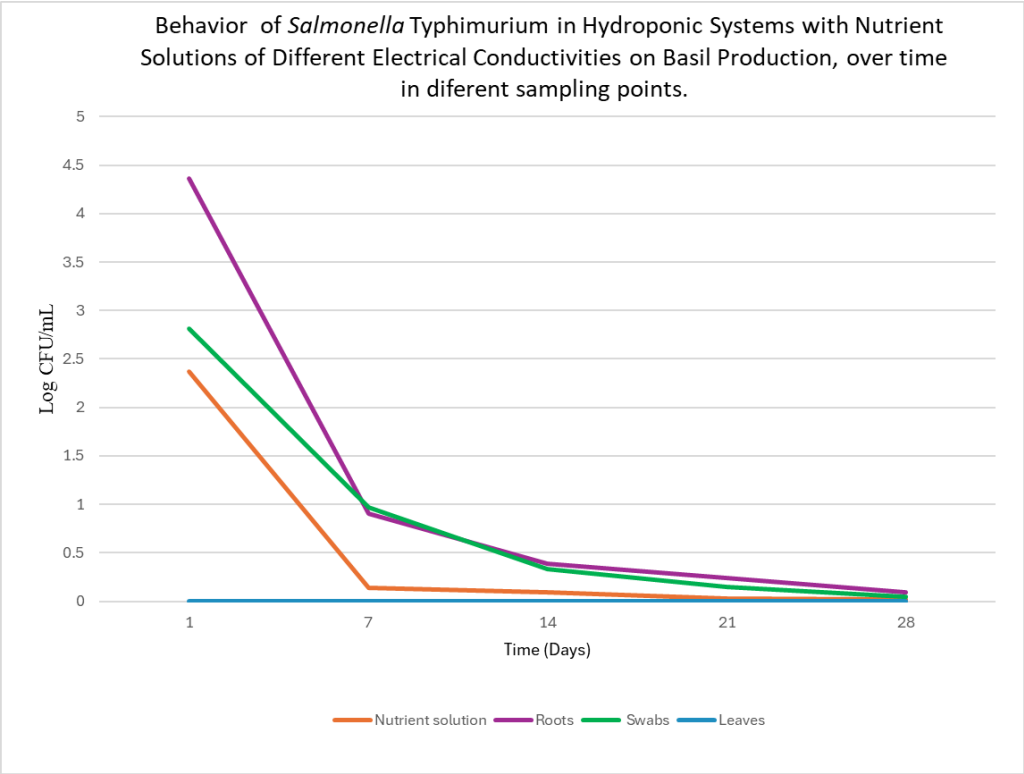
To better evaluate these trends, the following section compares the four sample types in relation to the study hypotheses.

Comparison Across Nutrient Solutions, Roots, Surfaces Swabs, and Leaves.

In this study, during the basil growth cycle, *Salmonella* Typhimurium counts declined steadily over time in the nutrient solution (NS), roots, and swabbed surfaces, following a biphasic pattern with a sharp reduction from days 1 to 7 and slower decreases thereafter (Figure 4). Roots showed the highest initial colonization, swabs reflected partial transfer from the NS, and NS counts dropped progressively until nearly undetectable levels. Leaves consistently tested negative under all conditions, suggesting that neither root translocation nor cross-contamination from the NS occurred and that foliar tissues are unfavorable for pathogen establishment. Overall, these results indicate that survival trends were similar across compartments and that time, rather than sample type or treatment, was the main factor influencing the decline. Moreover, the persistence of *Salmonella* in the NS, roots, and surfaces until day 28 confirms its ability to survive throughout the hydroponic growth cycle.

Figure 4

Graph of Salmonella behavior in four different sample types (nutrient solution, roots, surface swabs, and leaves).



Note. CFU = colony-forming units; mL = milliliter

Based on the results shown in the graph (figure 4), it was clearly determined that the reduction of *Salmonella* is governed by time and follows a biphasic decline. The gradient of microbial counts being higher in roots, then surfaces, and finally, the nutrient solution is consistent with early adhesion and biofilm formation on solid matrices (rhizoplane and inner walls), which protect subpopulations and explain residual persistence despite the overall decrease (Giaouris et al., 2015; Lee et al., 2020). In the NS, the sharper initial drop fits turnover/flush-out dynamics and less opportunity for attachment; once in the circuit, cells can transfer to root surfaces and porous materials (e.g., rock wool) and then colonize channel walls (Guevara et al., 2024; Indira & Sabitha Rani, 2024). As dissolved organic matter is depleted, cells enter starvation, cultivability decreases, and the second slow phase of decline continues (Liu et al., 2018).

Altogether, this explains the time-driven reduction and the matrix gradient observed (Giaouris et al., 2015; Lee et al., 2020).

The consistent non-detection on leaves in all treatments and over time aligns with basil's chemical and structural defenses and with limited translocation. Essential oils (linalool, estragole, eugenol) show broad antimicrobial activity and stability, supporting sustained protection (Rattanachaikunsopon & Phumkhachorn, 2010; Wang et al., 2023). Likewise, the thick cuticle and glandular trichomes reduce bacterial attachment and colonization (Zdolec et al., 2024). Even when *Salmonella* is in the rhizosphere, its arrival to aerial tissues is usually low or undetectable, indicating restricted internal transport; reports agree that root-to-shoot translocation is rare in basil and related species (Xylia et al., 2022). Additional contributors include limited nutrients on the leaf surface and antimicrobial metabolites (Brandl et al., 2013), the lack of enzymes to degrade plant cell walls (Brandl et al., 2013; Karmakar et al., 2018), and the effect of light, which reduces motility and internalization. (Gonzalez & Aranda, 2023). Even strains tolerant to linalool do not improve survival inside plant tissues (Kalily et al., 2017). Plant phenolics inhibit *S. Typhimurium* at low MIC, which helps explain why root counts approach zero toward the end (Ecevit et al., 2022).

The critical microbial risk window is concentrated in the first week, mainly in roots and surfaces, which emerge as priority persistence niches for scheduled sanitation and targeted monitoring (verification of biofilm reduction), in line with literature in hydroponic systems (Lee et al., 2020). As complementary tools, major oil constituents such as linalool and 1,8-cineole show potential to strengthen microbial safety in fresh herbs (Rattanachaikunsopon & Phumkhachorn, 2010).

Conclusions

This study evaluated the effect of different electrical conductivity (EC) levels on the survival of *Salmonella* Typhimurium in nutrient film technique (NFT) hydroponic basil production. The results showed that EC levels (1.0 - 2.0 $mS \cdot cm^{-1}$) did not significantly influence *Salmonella* Typhimurium survival in nutrient solutions, roots, surfaces, or leaves. Instead, time was the primary factor driving the observed decline in microbial counts across all compartments.

Salmonella persisted in the nutrient solution, roots, and surfaces throughout the 28-day growth cycle, although at progressively lower concentrations, confirming its ability to survive in hydroponic systems. Migration to roots and transfer to surfaces was evident, with the two sample types having consistently higher microbial counts than the nutrient solution by day 1. In contrast, no contamination was detected on basil leaves under any treatment, suggesting that physical and biochemical defense mechanisms, combined with strict biosecurity measures, prevented foliar colonization.

Overall, these findings did not support hypothesis 1 (that at least one EC level would affect survival), as no significant differences in pathogen survival were observed among the four EC treatments. However, hypothesis 2 (that survival would differ between roots, leaves, nutrient solution, and surfaces) and hypothesis 3 (that *Salmonella* would survive for the entire growth cycle) were supported. However, the absence of an EC effect suggests that survival dynamics appear to be more strongly driven by temporal and plant-related factors, such as root exudates, biofilm formation, or microbial competition, rather than nutrient concentrations. This paragraph is more discussion than conclusions

The findings of this study add to the growing evidence that foodborne pathogens are capable of surviving in diverse hydroponic conditions, raising concerns about the potential for persistence and transfer along fresh produce supply chains.

Recommendations

Repeat the experiment using nutrient solutions with different nutrient profiles (not only different EC levels), to explore if the specific composition of the solution (for example, higher nitrate, calcium, or potassium) influences the survival of the pathogen beyond the general electrical conductivity.

Evaluate future mitigation strategies directly in the NFT system, such as adding natural antimicrobial compounds (like plant extracts or organic acids) to the nutrient solution, to reduce microbial load from early stages without affecting basil growth.

Evaluate pre-harvest biological control by introducing beneficial microorganisms (e.g., lactic acid bacteria or Bacillus spp.) or *Salmonella* targeting bacteriophages into the nutrient solution or rhizosphere. Monitor colonization, pathogen suppression, and any impacts on the microbiome and plant performance

Future studies should investigate how other abiotic factors (e.g., pH, dissolved oxygen, and temperature), in combination with EC, influence pathogen survival, and whether interactions with beneficial plant-associated microbiota may offer a more effective means of reducing contamination risk.

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Appendices

Appendix A

Microbial counts by sampling time of the nutrient solution separated by treatments.

Time	Unit	Treatments			
		1.0 mS·cm⁻¹	**1.3 mS·cm⁻¹**	**1.6 mS·cm⁻¹**	**2.0 mS·cm⁻¹**
		Log CFU/ml ± SD			
0	Hour	5.28 ± 0.41	5.08 ± 0.44	5.17 ± 0.33	5.26 ± 0.32
4	Hour	5.17 ± 0.29	5.41 ± 0.06	5.34 ± 0.17	5.46 ± 0.25
8	Hour	5.10 ± 0.33	5.13 ± 0.4	5.14 ± 0.43	5.23 ± 0.43
12	Hour	5.04 ± 0.39	5.23 ± 0.25	4.98 ± 0.32	5.09 ± 0.65
1	Day	2.43 ± 0.28	2.41 ± 0.07	2.06 ± 0.55	2.57 ± 1.06
2	Day	1.38 ± 0.35	1.26 ± 0.66	1.03 ± 0.5	1.46 ± 0.38
7	Day	0.18 ± 0.00	0.13 ± 0.08	0.13 ± 0.08	0.13 ± 0.08
14	Day	0.09 ± 0.09	0.09 ± 0.09	0.04 ± 0.08	0.13 ± 0.08
21	Day	0.04 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.09
28	Day	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.09

Microbial counts (log CFU/mL ± SD) from nutrient solution samples, across all treatments. Values represent the mean of four replicates per sampling point.

SD: standard deviation.

Appendix B

Microbial counts by sampling time of the roots separated by treatments

Time	Unit	Treatments			
		1 mS·cm⁻¹	1.3 mS·cm⁻¹	1.6 mS·cm⁻¹	2 mS·cm⁻¹
		Log CFU/ml ± SD			
1	Day	4.82 ± 0.37	4.66 ± 0.22	5.00 ± 0.29	4.96 ± 0.21
7	Day	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	1.30 ± 0.9
14	Day	0.19 ± 0.34	0.58 ± 0.34	0.58 ± 0.34	0.19 ± 0.34
21	Day	0.19 ± 0.34	0.39 ± 0.39	0.39 ± 0.39	0.00 ± 0.00
28	Day	0.19 ± 0.34	0.19 ± 0.34	0.00 ± 0.00	0.00 ± 0.00

Microbial counts (log CFU/mL ± SD) from root samples, averaged across all treatments. Values represent the mean of four replicates per sampling point

SD: standard deviation.

Appendix C

Microbial counts by sampling time of the swabs separated by treatments.

Time	Unit	Treatments			
		1.0 mS·cm⁻¹	**1.3 mS·cm⁻¹**	**1.6 mS·cm⁻¹**	**2.0 mS·cm⁻¹**
		Log CFU/ml ± SD			
1	Day	2.75 ± 1.14	2.52 ± 0.93	2.65 ± 0.44	3.31 ± 0.97
7	Day	1.46 ± 1.19	0.39 ± 0.39	0.78 ± 0.00	1.23 ± 0.79
14	Day	0.19 ± 0.34	0.39 ± 0.39	0.00 ± 0.00	0.74 ± 0.89
21	Day	0.19 ± 0.34	0.39 ± 0.39	0.00± 0.00	0.00± 0.00
28	Day	0.19 ± 0.34	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00

Microbial counts (log CFU/mL ± SD) from surface swabs samples, across all treatments.

SD: standard deviation.