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A meta-analysis of factors influencing the inactivation of Shiga toxin-producing *Escherichia coli* O157:H7 in leafy greens

Joshua Ombaka Owade[1,2] | **Teresa M. Bergholz**[1] | **Jade Mitchell**[2]

[1]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan, USA

[2]Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, Michigan, USA

Correspondence
Jade Mitchell, Department of Biosystems and Agricultural Engineering, Michigan State University, 524 S. Shaw Lane, Farrall Hall 100A, East Lansing, MI 48824, USA.
Email: jade@msu.edu

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Abstract

Recent advancements in modeling suggest that microbial inactivation in leafy greens follows a nonlinear pattern, rather than the simple first-order kinetics. In this study, we evaluated 17 inactivation models commonly used to describe microbial decline and established the conditions that govern microbial survival on leafy greens. Through a systematic review of 65 articles, we extracted 530 datasets to model the fate of Shiga toxin-producing *Escherichia coli* O157:H7 on leafy greens. Various factor analysis methods were employed to evaluate the impact of identified conditions on survival metrics. A two-parameter model (jm2) provided the best fit to most of both natural and antimicrobial-induced persistence datasets, whereas the one-parameter exponential model provided the best fit to less than 20% of the datasets. The jm2 model (adjusted R^2 = .89) also outperformed the exponential model (adjusted R^2 = .58) in fitting the pooled microbial survival data. In the context of survival metrics, the model averaging approach generated higher values than the exponential model for >4 log reduction times (LRTs), suggesting that the exponential model may be overpredicting inactivation at later time points. The random forest technique revealed that temperature and inoculum size were common factors determining inactivation in both natural and antimicrobial-induced die-offs.. The findings show the limitations of relying on the first-order survival metric of 1 LRT and considering nonlinear inactivation in produce safety decision-making.

KEYWORDS
condition, die-off, factor analysis, first order, nonlinear, survival metrics

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1 | INTRODUCTION

With increasing consumption of leafy greens including Romaine lettuce, foodborne pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 have increasingly posed greater threats with serious health impacts on the consumer populations (Astill, 2019). Between 2009 and 2023, approximately 36 outbreaks of STEC O157:H7 in leafy greens were confirmed or suspected in the United States and Canada (CDC, 2024; Chen et al., 2023). Consuming these leafy greens in their raw state poses risks due to the absence of critical pathogen-killing steps, similar to heat treatment found in thermal processing. Antimicrobial treatments such as chlorine washing aim to prevent cross-contamination of produce during processing rather than the removal; thus, the possibility of pathogen survival continues to pose risks to consumers (Abnavi et al., 2021; Nou et al., 2011). Predictive microbiology, including pathogen inactivation modeling, plays a pivotal role in developing effective controls to mitigate microbial risks associated with the consumption of raw leafy greens.

Microbial inactivation in leafy greens has been shown to follow a biphasic pattern, a rapid initial die-off followed by a slower rate of reduction, rather than simple first-order kinetics (Belias et al., 2020; McKellar, Peréz-Rodríguez, et al., 2014; Munther et al., 2020; Seidu et al., 2013). The exponential (log-linear) model assumes a constant die-off rate in the microbial population, with much likelihood of underestimating the survival of the residual pathogens at higher log reductions. However, the die-off of STEC O157:H7 on leafy greens is affected by a number of factors, including the type of produce (Belias et al., 2020), population heterogeneity (Munther et al., 2020), weather and environmental conditions (Belias et al., 2020; Brandl et al., 2023; Moyne et al., 2020), among others, and the effect of these factors needs to be accounted for in the model. Some of the environmental factors that affect inactivation of STEC O157:H7 on leafy greens include substrate and oxygen and carbon dioxide concentrations (Abadias et al., 2012; Elhadidy & Álvarez-Ordóñez, 2016), temperature (Delaquis et al., 2007; Elhadidy & Álvarez-Ordóñez, 2016), pH (Kim et al., 2013), osmotic potential (Elhadidy & Álvarez-Ordóñez, 2016), and competition due to presence of other microorganisms (Van Elsas et al., 2011). Of these, temperature was found to be the most important factor as abuse of the set controls was reported to be responsible for 32% of the outbreaks of foodborne illnesses (Franz et al., 2010). At low temperatures of 8°C, there was a decline in the total culturable STEC O157:H7 on lettuce; however, the viable-but-non-culturable (VBNC) fraction increased (Dinu & Bach, 2011). Thus, the effect of low temperature is not singularly linked to microbial population reduction but leads to increased formation of VBNC cells that survive better under stressful conditions. The biphasic tailing in pathogen survival demonstrated by McKellar, Peréz-Rodríguez, et al. (2014) and Mitchell and Akram (2017) points to this fraction of the microbial population that, if not accounted for, would result in an underestimation of microbial risk.

Multiple microbial inactivation models that have been utilized to describe the behavior of STEC O157:H7 on leafy greens have demonstrated differences in inactivation parameters based on microbial population characteristics (Brandl et al., 2023; Munther et al., 2020), type of vegetables (Belias et al., 2020), and environmental and weather conditions (Belias et al., 2020; Brandl et al., 2023; Munther et al., 2020). The documented studies separately highlight the effect of the various conditions using modeling approaches, pointing out relevant conditions that explain shown inactivation patterns. For instance, the studies by Belias et al. (2020) and Brandl et al. (2023) evaluated weather parameters, highlighting the relative humidity (RH), temperature, and light intensity as some of the core factors affecting the inactivation of STEC O157:H7 on leafy greens. It is noteworthy that in addition to weather conditions, Belias et al. (2020) considered the effect of vegetable type, whereas Brandl et al. (2023) included the effect of microbial population characteristics. Understanding the effect of the various conditions on the inactivation parameters of the pathogens reduces uncertainty in quantitative microbial risk assessment (QMRA) (Dean & Mitchell, 2022). This study hypothesizes that the inactivation of STEC O157:H7 in leafy greens was better described by the biphasic (nonlinear) models than the log-linear model. Furthermore, the study presupposes that the nonlinear models better describe the effect of multiple conditions on the inactivation parameters of time taken to attain a specific log reduction, hereafter referred to as a log reduction time (LRT), of STEC O157:H7 in leafy greens.

The study is built upon a systematic review that identified articles with sufficient data to model the pathogen die-off of STEC O157:H7 in leafy greens using two or three-parameter nonlinear models. The modeling in this study employs two or three-parameter nonlinear models, which are widely recognized in the literature for describing the inactivation kinetics of pathogens in leafy greens (Belias et al., 2020; Dean & Mitchell, 2022; McKellar, Peréz-Rodríguez, et al., 2014; Mitchell & Akram, 2017; Seidu et al., 2013). Previous meta-analyses that modeled the inactivation of STEC O157:H7 on leafy greens (McKellar, Peréz-Rodríguez, et al., 2014) focused on on-field experiments; however, this study documented the farm-to-retail chain, evaluating the significance of experimental setting on microbial survival. Moreover, our study utilizes the more objective response parameter of LRT that factors in nonlinear patterns to elucidate the conditions that influ-

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ence pathogen survival. Remarkably, as far as this study is concerned, it stands out as the sole meta-analysis to utilize the most extensive array of inactivation models for evaluating pathogen survival in leafy greens. Additionally, we elucidate the effect of diverse conditions on the survival of the pathogen to inform food control processes in the supply chain of leafy greens.

2 | MATERIALS AND METHODS

2.1 | Sourcing of the data

Using the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA), we identified 65 articles that investigated the fate of STEC O157:H7 on raw leafy greens such as lettuce and spinach. From these selected articles, we extracted 530 datasets detailing the conditions such as environmental factors (including temperature and RH), storage conditions (including packaging), processing conditions (including sanitization process), and biological characteristics (such as type of produce, bacteriophage, and inoculum size) (Table 1). Microbial concentration data were also extracted for modeling the inactivation of pathogens in leafy greens. The details of the data that were extracted are discussed in the successive sections.

2.1.1 | Antimicrobial treatment

The inactivation of STEC O157:H7 in leafy greens can be attributed to various factors, including environmental conditions and antimicrobial treatments. Natural die-off, as observed in studies by Belias et al. (2020) and Moyne et al. (2020), occurs due to inherent environmental and atmospheric factors. Conversely, antimicrobial treatments, such as washing with chlorine, contribute to microbial reduction (Abnavi et al., 2021; Doering et al., 2009). Leafy green processes that included washing with sanitizers such as chlorine (Davidson et al., 2014; Lee & Baek, 2008) and organic acids (Davidson et al., 2017) and treatment with chlorine dioxide gas (Mahmoud & Linton, 2008), pulsed light (Mukhopadhyay et al., 2021), cold nitrogen plasma (Cui et al., 2018), and electrolyzed water (Ding et al., 2009) were categorized as antimicrobial treatment and analyzed separately from natural die-off. From the analysis of the extracted data, we established that the reduction in microbial population occurred due to natural conditions such as environmental temperature and RH (hereafter shall be referred to as natural die-off) or antimicrobial treatments such as washing with chlorine (hereafter shall be known as antimicrobial-induced die-off). The time and concentration of antimicrobial agents were also extracted, with chlorine concentration preferably reported in free chlorine (FC).

2.1.2 | Bacteriophage

Bacteriophages were evaluated for their effect in reducing the microbial population on leafy greens (Boyacioglu et al., 2013). Though not a conventional intervention in the removal of microorganisms, the data were extracted due to the reported high log reduction in the treated produce (Cui et al., 2018). A binary classification of "yes" and "no" was used in indicating the presence of bacteriophages on produce; without such indication, the phages were assumed to be absent.

2.1.3 | Enumeration technique

This detailed the method of quantification of microbial cells, whereby binary classification of culture-based and molecular was used. Culture-based techniques included the most probable number method (Abnavi et al., 2021) and plate-count techniques (Chang & Fang, 2007; Choi et al., 2011). Quantification techniques that analyzed microbial genetic material such as deoxyribonucleic acid (DNA) including real-time polymerase chain reaction (rtPCR) (Ibekwe et al., 2009) were classified as molecular techniques. These molecular techniques offer higher sensitivity and specificity for pathogen detection. Modifications, including the use of propidium monoazide (PMA), have enabled the quantification of total viable cells, including the nonculturable fraction (Moyne et al., 2013). Herein, these quantification methods represented the culturable cells for lack of additional information on the nonculturable fraction. Although the growth conditions of the cells can impact the microbial kinetics of the cells following inoculation, our study had insufficient data to evaluate the effect of, for instance, growing cells in the exponential phase rather than the stationary phase. Furthermore, there were limited studies that evaluated or used injured cells in survival experiments. Of the 270 datasets, only four clearly stated that the cells were not grown in a new media before inoculation.

2.1.4 | Experimental site

Experimentation was conducted both in outdoor (Moyne et al., 2011, 2013) and indoor experimental plots and in lab-scale settings (Dixit et al., 2021; Ibekwe et al., 2009). Experiments that were conducted in growth chambers

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TABLE 1 Summary of the datasets that that were included in the study.

Condition	Type of variable	Categories	Number of datasets
Experimental site	Binary	Yes	62
		No	208
Irrigation	Binary	Yes	95
		No	175
Modified atmosphere packaging	Binary	Yes	29
		No	241
Plating	Binary	Culture-based	237
		Molecular	33
Predation	Binary	No	261
		Yes	7
Relative humidity	Numeric	NA	173
Temperature	Numeric	NA	264
Sanitization	Categorical	Chlorine washing and chlorine dioxide	58
		Cold nitrogen plasma	9
		Irradiation	1
		Organic acids	6
Shredding	Binary	Yes	162
		No	108
Size of inoculum	Numeric	NA	270
Soil conditions	Binary	Contaminated	29
		Not contaminated	241
Stage in the value chain	Categorical	Primary production	129
		Processing	51
		Storage	86
		Transport	4
Type of vegetable	Categorical	Cabbage	4
		Lettuce	223
		Spinach	43

(Ibekwe et al., 2009) and greenhouses (Dinu & Bach, 2011) were classified as lab-scale experiments. The lab-scale experiments were characterized by high inoculum counts and control of experimental conditions (McKellar, LeBlanc, et al., 2014) compared to the outdoor experiments that had a mix of factors influencing the microbial kinetics (Belias et al., 2020). Experimental conditions that were reported in the experimental settings were extracted in case no results were reported for individual time points.

2.1.5 | Irrigation

Irrigation processes are undertaken during the primary production of the leafy green (Tyagi et al., 2019) and have a binary classification of "yes" and "no." We acknowledge that different irrigation techniques such as overhead and drip may result in differences in microbial concentrations; however, a number of selected studies that had irrigation did not explicitly report these differences. Of the 65 articles reviewed, only seven articles explicitly studied irrigation. Of the extracted datasets that were included in this study, only 13 datasets detailed drip irrigation. The remaining 45 datasets did not specify the technique of irrigation, leading to the broad classification.

2.1.6 | Modified atmosphere

This was indicated by modifying the concentration of gases, such as reducing the oxygen concentration or expelling the gases in the packages of leafy greens (Boyacioglu et al., 2013). Packaging films that had reduced oxygen permeability, and for which specific measures had to be indicated, were classified as modified atmosphere packaging (MAP) (Oliveira et al., 2010). A binary response of "yes" to indicate the use of MAP and "no" to indicate no use was used for this condition.

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2.1.7 | Relative humidity

A continuous variable of RH was provided as part of the experimental conditions evaluating microbial inactivation in leafy greens (Belias et al., 2020; Tyagi et al., 2019). In primary production, atmospheric RH (Belias et al., 2020) was taken as the measure, whereas in storage conditions such as in a modified atmosphere, the RH in the package or in the storage environment was included (Choi et al., 2011). Wherever a range of values or estimates with more than one value was available for an experiment, an average of the given values was calculated.

2.1.8 | Size of the inoculum

The size of the inoculum included the counts of the microbial cells in the culture introduced to the leafy green (Choi et al., 2011). The initial inoculum size was extracted from the indicated microbial population of the culture used, or if absent, the initial count of the cells at time zero was used. In cases where a range of values was reported to estimate the concentration, the lognormal distribution was assumed, and average log values were calculated. The growth conditions of the culture can also impact the inactivation kinetics of the cells.

2.1.9 | Shredding

Experiments on leafy greens were either conducted on whole (Bezanson et al., 2012) or cut produce (Abnavi et al., 2021). In experiments where leafy greens were chopped before inoculation (Davidson et al., 2014), the conditions were indicated as "yes," whereas in cases where the inoculum was added on whole leaves, the conditions were indicated as "no."

2.1.10 | Soil contamination

This included the intentional addition of inoculum to the soil, including the use of contaminated manure and fecal slurry (Chase et al., 2017). A binary response of "yes" and "no" was used to indicate whether the soil was contaminated or not, respectively.

2.1.11 | Stage in the value chain

The supply chain of the produce was classified as primary production (Belias et al., 2020; Chase et al., 2017), processing (Davidson et al., 2017), and transportation and storage (Zeng et al., 2014). Primary production is composed of tillage (Chase et al., 2019), seed treatment (Dixit et al., 2021), weeding and fertilizer application on the growing plants (Chase et al., 2017), irrigation (Markland et al., 2013), and harvesting of the produce (Moyne et al., 2020), all of which contribute to the production of leafy greens. The included studies did not detail the effect of the harvesting equipment on microbial kinetics. Of the 270 datasets, 241 did not specify the harvesting equipment. The 29 datasets that specified the harvesting equipment involved harvesting by cutting with a knife. Activities that solely focused on plant operations such as sanitizing and washing procedures (Davidson et al., 2017; Gleeson & O'Beirne, 2005) were included as processing. Transportation included documenting conditions for produce in transit, and storage included both retail and consumer (Zeng et al., 2014). Whenever a produce was washed with a sanitizer and subjected to storage conditions, such experiments were classified under storage (Lee & Baek, 2008).

2.1.12 | Temperature

The temperature of the experimental studies was captured from the reported empirical values. In primary production, the atmospheric or environmental temperature within the growth chamber was captured (Belias et al., 2020; Tyagi et al., 2019), whereas, in transportation, processing, and storage, the environmental temperature was captured (Likotrafiti et al., 2014; Zeng et al., 2014). If a narrow range or estimate of values (over 1–2°C, such as 4–5°C) is provided for a given period, the mean of those values was calculated.

2.1.13 | Type of leafy green

In this experiment, three leafy greens were studied, including lettuce (Likotrafiti et al., 2014), spinach (Choi et al., 2011), and cabbages (Uzeh & Adepoju, 2013). Romaine lettuce, leaf lettuce (Boyacioglu et al., 2013), and iceberg lettuce (Abnavi et al., 2021) were all classified as lettuce.

2.1.14 | Microbial concentration data

The extracted datasets had to have a minimum of four time points showing empirical values of microbial concentrations to be eligible in the study for ease of fitting two- and three-parameter nonlinear models as described in previous works (Dean & Mitchell, 2022). For datasets that were presented graphically, the open-access Web Plot Digitizer tool (https://automeris.io/WebPlotDigitizer/) was used to extract the values. For experiments that were presented in log reduction values (LRVs), the initial concentration values were used to gen-

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erate the log-transformed ($\log_{10}$ concentration) data and eventually the concentration data. Microbial inactivation data were transformed ($\log_{10}$ (N_0/N_t)) before fitting the models.

2.2 | Selection of datasets for modeling

To distinguish the inactivation from the growth data, a linear regression model with the *y*-intercept fixed at zero was fitted to the 530 datasets. In our study, the dependent variable was LRVs, and time was the predictor variable. The fundamental assumption of this regression modeling was that LRV would be zero at the starting time ($t = 0$). Only datasets exhibiting a statistically significant ($p < .05$) negative trend were considered for inclusion in the subsequent inactivation modeling. Datasets that reached the limit of detection (LOD) at the second data point were excluded from the modeling process. We had 206 (76.3%) of the datasets not reporting the LOD in our study. In instances where the LOD was not explicitly reported in the dataset, a criterion was established: datasets with a >1-log reduction at the second time point, no absolute differences of <0.1 for successive data points, and fewer than 5 data points in the time series were deemed to have reached the LOD. These datasets displayed model diagnostic plots with a 90° angle, forming an L-shape pattern.

2.3 | Inactivation models fitted to survival data

The selected datasets were fitted using a suite of 17 log-linear and nonlinear models, which are broadly classified as shown. Survival models describing pathogen inactivation in leafy greens have been described using four trends, including log-linear, shouldering, tailing, and both shouldering and tailing. The model suite was built to evaluate inactivation patterns based on the four-pathogen behavior. Although some of the models have defined parameters for the shape of the fitted survival model, others may have more than one survival behavior depending on the specific value of the parameters.

2.3.1 | Log-linear survival curve

The exponential, also known as the log-linear (ep) model, follows the first-order kinetics and thus has a constant rate of pathogen die-off (Chick, 1908; Watson, 1908). This model depicts an immediate and steady microbial die-off under stressful conditions until the population is depleted (Crane & Moore, 1986). The simplicity of this model has been the main factor driving its use. Survival metrics such as 1 LRT, the time taken to reduce population by 90%, is the reciprocal of the constant (Peleg et al., 2003).

2.3.2 | Survival curve with a shoulder

There are several reasons for the shouldering behavior in survival curves. Shouldering is indicative of a lag that results from initial resistance to die-off or microbial cell replication that is equal to the death rate (Xiong et al., 1999). Logistic (lg1) (González, 1995), Fermi (lg2) (Peleg, 2006), Weibull (wb) (Aragao et al., 2007), log-normal (ln) (Aragao et al., 2007), and double exponential (dep) (Abraham et al., 1990) models depict delays in inactivation that have been attributed to factors such as the clumping of microbial endospores (Enger et al., 2018), the period required for the resynthesis of cells and vital compounds, the cumulative rather than immediate effect of stress resulting in a lag (Xiong et al., 1999), or the protective effect of the medium or other components of the matrix (Bevilacqua et al., 2015). Microbial populations with different physiological states show varied resistance to stress and are likely to exhibit this lag time (Coroller et al., 2006).

2.3.3 | Survival curve with a tail

The tailing effect in survival patterns indicates that certain fractions of the microbial population exhibit more inherent resistance to die-off than others (Xiong et al., 1999). The two-stage Juneja and Marks (jm1) (Dean & Mitchell, 2022; Juneja et al., 2001) and exponential damped (epd) (Cavalli-Sforza et al., 1983; Dean & Mitchell, 2022) are some of the survival models that show tailing behavior. The tailing effect was assumed to result from population heterogeneity, with some cells showing a degree of resistance to stress (Juneja et al., 2001). The first phase depicts a major population that is sensitive to stress, followed by a second phase of tailing of a resistant fraction, with varying degrees of resistance across strains and microbial species (Coroller et al., 2006). It has also been posited that this residual population can result from a fraction that adapts to or is inaccessible to the lethality of the stress (Bevilacqua et al., 2015). The tailing can be exhibited as zero or small slope tailing, indicating a biphasic trait of different survival kinetics of two distinct fractions of microbial populations (Xiong et al., 1999).

2.3.4 | Survival curve with a shoulder and tailing

These are sigmoidal curves that show both shouldering and tailing behavior (Xiong et al., 1999). The log-logistic

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TABLE 2 Inactivation models used in fitting the selected datasets.

Model	Model equation	Reference
Exponential (ep)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(e^{-k_1 t})$	Crane & Moore, 1986
Logistic (lg1)	$\log_{10}(\frac{N_t}{N_0}) = \frac{2}{1+e(k_1 t)}$	González, 1995
Broken-line (bi)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(e(-k_1 t)),\ t < k_3$	Rogers et al., 2011
Exponential-damped (epd)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(1-(1-e^{k_1 t})^{k_2})$	Mitchell & Akram, 2017
Fermi (lg2)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(\frac{1}{1+e(-k_1(t-k_2))})$	Peleg, 1996, 2021
Gompertz 2 (gz)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(e[\frac{-k_1}{k_2}e((k_2 t)-1)])$	Wu et al., 2004
Gamma (gam)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(e\{(t^{k_1-1})e^{(\frac{-t}{k_2})}\})$	Van Gerwen & Zwietering, 1998
Juneja and Marks 1 (jm1)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(1-(1-e^{-k_1 t})^{k_2})$	Juneja et al., 2001
Juneja and Marks 2 (jm2)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(\frac{1}{1+e^{k_1+k_2 \mathrm{Int}}})$	Juneja et al., 2006
Log-normal (ln)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(1-\{(\ln(t)-\frac{k_1}{k_2})\})$	Aragao et al., 2007
Weibull (wb)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(10^{-((\frac{t}{k_1})^{k_2})})$	Peleg, 2003
Broken-line 2 (bi2)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(e(-k_1 t)),\ t < k_3$	Rogers et al., 2011
Double exponential (dep)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(k_3 e^{-k_1 t} + (1-k_3)e^{-k_2 t})$	Abraham et al., 1990
Gompertz 3 (gz3)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(10^{k_1 e[-e(\frac{-k_2 e(1)(k_3-t)}{k_1}+1)]})$	Gil et al., 2011
Gompertz–Makeham (gzm)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(e(-k_3 t - \frac{k_1(e(k_2 t)-1)}{k_2}))$	Jodrá, 2009
Sigmoid type A (sA)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(10^{\frac{(k_1 t)}{[(1+k_2\times t)(k_3-t)]}})$	Peleg, 2006
Sigmoid type B (sB)	$\log_{10}(\frac{N_t}{N_0}) = \log_{10}(10^{-\frac{(k_1 t^{k_3})}{(k_2+t^{k_3})}})$	Peleg, 2006

Juneja and Marks (jm2) (Dean & Mitchell, 2022; Juneja et al., 2006), sigmoid type A (sA) and B (sB) (Peleg, 2006), double exponential (dep) (Peleg, 2006), and Gompertz 3 (gz3) (Gil et al., 2011) models display a sigmoidal inactivation of the bacterial population under stress. Microbial inactivation displays a shoulder, followed by a linear phase of accelerated die-off, and finally a tail of residual population (Gil et al., 2011).

2.4 | Describing models in the suite

The suite had 17 models including 2 one-parameter, 10 two-parameter, and 5 three-parameter as discussed in this section (Table 2).

2.4.1 | One-parameter models

Exponential (ep)
The model assumes a constant rate of die-off, with a constant k for the death rate (Crane & Moore, 1986). The parameter, k_1 (Equation 1), is the die-off rate, and the reciprocal of this value is the 1 LRT, the time taken to inactivate 90% of the microbial cells, popularly called the *D*-value.

Logistic (lg1)
The logistic model was designed to account for the existence of a lag in the inactivation of the microbial population (González, 1995). The parameter k_1 denotes the inactivation rate of the microorganism, which is nonlinear. The model is symmetric at the inflection point compared to the two-parameter Gompertz model, which is a marked difference between the two.

2.4.2 | Two-parameter

Broken-line (bi)
The model is biphasic, where there is a phase of rapid decline followed by slower decline. The model combines two first-order inactivation equations (Rogers et al., 2011). The k_1 parameter is the die-off rate of the first segment of the inactivation curve, and this equation applies if $t < k_3$, where t is the time for the log reductions and k_3 is the transition time marking the end of stage 1.

Exponential-damped (epd)
This model provides the advantage of capturing the tailing behavior of the residual population (Mitchell & Akram, 2017). While the exponential damped model captures

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the tailing behavior in microbial survival, the U-shaped tendency of the model has limited its application in inactivation modeling in food (Dean & Mitchell, 2022). The model is reduced to the exponential model when the k_2 is zero and attains the initial population counts when both k_1 and k_2 are zero (Dean & Mitchell, 2022).

Fermi (lg2)
The model accounts for short and long lags in the inactivation of microbial cells (Peleg, 1996). The parameter k_1 is the inactivation rate at the linear part of the curve, whereas k_2 is the inflection point. As k_2 increases, the variance explained by k_1 also increases, and the distribution becomes unimodal (Peleg, 2021).

Gompertz 2
The model is a two-parameter model. The k_1 and k_2 are model parameters >0, with k_1 representing the maximum inactivation rate and k_2 representing the lag for the shouldering behavior. When k_2 approaches zero, this model tends to an exponential (Wu et al., 2004).

Gamma (gam)
It describes microbial inactivation under environmental conditions such as temperature and pH. The k_1 and k_2 denote shape parameters of the inactivation model (Van Gerwen & Zwietering, 1998). The parameters of this model are pathogen specific and can be sourced from the literature (Zwietering et al., 1996).

Juneja and Marks 1 (jm1)
The two-stage Juneja and Marks (jm1) model was the first in the food industry to describe microbial thermal inactivation in poultry products (Juneja et al., 2001). The k_1 and k_2 are indicative of the inactivation rates, and when $k_2 = 0$, this model is reduced to a log-linear model (Dean & Mitchell, 2022).

Juneja and Marks 2 (jm2)
The log-logistic Juneja and Marks (jm2) model was generated from different sets of differential equations that described the dynamics of bacterial inactivation during thermal processing (Juneja et al., 2006). It is based on logistic probability distribution, and the k_1 and k_2 parameters are considered dispersal and location parameters, respectively (Dean & Mitchell, 2022).

Log-normal (ln)
The model describes the inactivation pattern with shouldering, resulting from cell damage accumulation if $k_2 > 1$ and tailing when $k_2 < 1$ (Aragao et al., 2007). The distribution is unimodal, and when the standard deviation is greater than the mean and mode, then the shouldering is similar to the Weibull distribution.

Weibull (wb)
The Weibull model was described by Coroller et al. (2006) to account for populations with cells with different physiological states. The k_1 and k_2 parameters are indicative of the inactivation rates of the different fractions of the subpopulations, and these parameters are subject to change with increasing intensity of the stress.

2.4.3 | Three-parameter

Broken-line 2 (bi2)
This model is similar to a broken-line model and is generated when $t \geq k_3$ (Rogers et al., 2011). The k_1 and k_2 are die-off rates for the first and second segments, respectively, with k_3 representing the transition time between the two segments, the time the first segment ends.

Double exponential (dep)
The parameter k_1 is the inactivation rate of the culturable (active) cells, whereas k_2 is the activation rate of the dormant population (Abraham et al., 1990). The k_3 is a parameter for the activation of the dormant fraction. If k_1/k_2 is zero, then the stress has no effect on the microbial population, and at the beginning, it results in shouldering, whereas if the value is negative, the population declines.

Gompertz 3 (gz3)
This model was reparametrized from the earlier Gompertz equation to describe the sigmoidal trend in bacteria inactivation in food (Gil et al., 2011). In the model, k_1, k_2, and k_3 are model parameters describing the behavior of the pathogen in inactivation. The parameter k_1 is derived as the asymptote of the function and denotes the tailing, k_2 is the maximum inactivation rate and is calculated as a derivative at the point of inflection, and k_3 is the interception of an extrapolated tangent line through the inflection point with the time axis.

Gompertz–Makeham (gzm)
The model utilized in this meta-analysis has exponential parametrization. k_1, k_2, and k_3 are parameters denoting pathogen die-off, with k_1 representing the initial die-off rate and k_2 and k_3 representing an exponential increase in the die-off rate (Jodrá, 2009). The k_1 parameter is a die-off rate that depends on time function, with a higher value indicating an increasing die-off rate over time. The k_2 and k_3 parameters represent intrinsic microbial

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die-off rate that is not time dependent and die-off rate due to other factors including environmental conditions, respectively.

Sigmoid type A (sA)

Microbial inactivation follows a rapid die-off of the weaker fraction of the population, followed by a decline in the more resistant fraction (Peleg, 2006). The parameters k_1, k_2, and k_3 are shape parameters that were first derived from temperature profiles with microbial spores (Peleg, 2003). If $k_1 = 1$, $k_2 = 1$, and $k_3 = 1$, then the model is reduced to first order (Peleg & Normand, 2004).

Sigmoid type B (sB)

The curve has a sigmoidal shape depicting accumulated damage of weaker microbial cells, with the more resistant fraction remaining (Peleg, 2006). The inactivation pattern has a semilogarithmic pattern with a shoulder and sometimes a tailing behavior. The parameters k_1, k_2, and k_3 denote the shape of the curve (Peleg, 2003).

2.5 | Evaluating the model fitting

The model parameterization was conducted using maximum likelihood estimation in a three-step optimization process for initializing parameters in each dataset, employing the optim function in the R programming language (R Core Team, 2022). Models that had unstable parameter estimates in the optimization or failed to converge were removed. Model selection was done by calculating the Bayesian information criterion (BIC), and the goodness of fit was evaluated using the normalized root mean square error (nRMSE) and adjusted R-squared (R^2). Lower BIC values (Equation 1) indicated a better fit, with differences >2 showing significantly less fit (Dean & Mitchell, 2022). Since the units of measurement for the pathogens differed from each other, nRMSE (Equation 2) was used instead of the root mean square error (RMSE). Of the 17 models in the suite, only one was log-linear, while the rest were nonlinear; thus, adjusted R^2 (Equations 3–4) was calculated for each successfully fitted model rather than R^2.

$$\mathrm{BIC} = 2\mathrm{nll} + k\log(n), \tag{1}$$

where nll is the negative log likelihood, k is the number of parameters in the model, and n is the number of data points.

$$\mathrm{nRMSE} = \frac{\sqrt{\frac{\sum\left(\mathrm{LR}_{\mathrm{pred}} - \mathrm{LR}_{\mathrm{obs}}\right)^2}{n}}}{\max(\mathrm{LR}_{\mathrm{obs}}) - \min(\mathrm{LR}_{\mathrm{obs}})}, \tag{2}$$

$$R^2 = 1 - \frac{\sum\left(\mathrm{LR}_{\mathrm{pred}} - \mathrm{LR}_{\mathrm{obs}}\right)^2}{\sum\left(\mathrm{LR}_{\mathrm{obs}} - \overline{\mathrm{LR}_{\mathrm{obs}}}\right)^2}, \tag{3}$$

$$R^2 = 1 - \frac{\left(1 - R^2\right)(n-1)}{(n-p-1)}, \tag{4}$$

where $\mathrm{LR}_{\mathrm{pred}}$ are the log-reduction values predicted by the model, $\mathrm{LR}_{\mathrm{obs}}$ are the observed LRVs, $\overline{\mathrm{LR}_{\mathrm{obs}}}$ is the average value of observed log reductions, n is the number of data points, k is the number of parameters in the model, max is the maximum value, and min is the minimum value.

2.6 | Pathogen survival metrics

The survival metric LRT was calculated for datasets that had at least one model selected as the best fit. Linear fitting of the observed value against the predicted values was done for the log-linear and the most selected models to determine LRVs that can be generated. This linear fitting of the values determined that the model could generate LRVs ranging from 1 to 7. In cases where the multiple models were selected based on the BIC, the model averaging approach was used to find the most representative model averaging approach that assigns weighting values (ω_i) to the best fitting models (Dean et al., 2020) (see Equation 5). The monotonic survival metrics were log-transformed to rescale the data and reduce the effect of the outliers, making the outputs more interpretable:

$$\omega_i = \frac{\mathrm{e}\left(-\frac{1}{2}\mathrm{BIC}_i\right)}{\sum_{j-1}^{i}\mathrm{e}\left(-\frac{1}{2}\mathrm{BIC}_j\right)}. \tag{5}$$

2.7 | Exploratory analysis of survival metrics

Various factors were extracted from the literature, including temperature (continuous), RH (continuous), method of quantification (culture-based and molecular), experimental site (field and laboratory), irrigation (yes and no), stage in the value chain (primary production, processing, storage, and transportation), soil conditions (contaminated and not contaminated), packaging (air and modified), type of vegetable (lettuce, spinach, and cabbage), and inoculum size (high and low), as well as the type of produce (whole and shredded), as detailed in Table

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S1. The log-transformed survival metric (LRTs), log10 days, was assessed for its distribution and summarized with descriptive statistics, including mean and median values. In cases of bimodal distributions, the mixtools package (Benaglia et al., 2009) was used to compute the descriptives of the different modes.

2.8 | Factor analysis of the survival metrics

The effect of the identified conditions, mentioned earlier, on the survival metrics was evaluated using cause–effect tests. Linear modeling techniques of linear regression and the Kruskal–Wallis test assessed the effect of continuous and categorical predictor conditions, respectively, on the survival metrics. For binary predictor conditions, the pairwise Wilcoxon test was used to evaluate their effect on the survival metrics. Spearman correlation test was used to evaluate the relationship between binary and continuous conditions and the survival metrics.

Conditions displaying a noticeable cause–effect relationship with the survival metrics were incorporated into the factor analysis. For the factor analyses, the multilevel groups within the conditions were dummy-coded. An important consideration in factor analysis is the risk of overfitting the model when using the training dataset, which may not be representative of the overall trend. By using the dredge function in the Mumin package (Barton, 2023), the individual and combined effects of various factors were evaluated through the sequential addition of conditions to the model for assessment. The factor variables of temperature, RH, stage in the value chain, enumeration technique, inoculum size, sanitization, soil conditions, and type of produce were all included in the generalized liner model (GLM) for performance evaluation. Factors that were included in the GLMs with a difference ≤2 in the corrected Akaike information criteria (ΔAICc) compared to the lowest value were subsequently considered in the successive factor analysis methods (Fabozzi et al., 2014). Diverse factor analysis methods including linear regression, GLM, random forest, quantile regression, regression trees, and principal component regression were used in the evaluation of the conditions. The data were first divided into training and testing datasets using the Caret package (Max et al., 2021). The predict function in R was used to compute the RMSE from the predicted values against the observed and the predictive power of the model tested by evaluating performance on the testing dataset. The random forest consistently gave lower RMSE values of all the techniques. From this analysis, a random forest was selected to evaluate the effect of the conditions on the LRTs. Variable importance in the random forest was established by calculating the increase in the mean square error (MSE) when the variable was removed from the analysis.

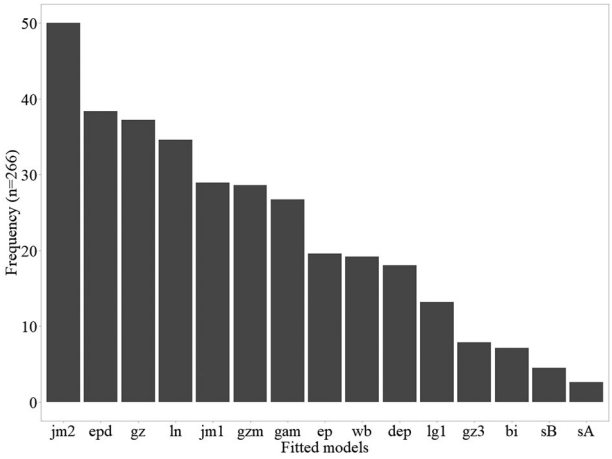

FIGURE 1 Proportion of the datasets best fitted by inactivation models on the survival data of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 in leafy greens. bi, biphasic exponential; bi3, biphasic (3 parameters); dep, double exponential; ep, first order exponential inactivation; epd, exponential damped; gam, gamma; gz, Gompertz 2; gz3, Gompertz-3; gzm, Gompertz–Makeham; jm1, two-stage model Juneja and Marks; jm2, log-logistic model Juneja and Marks; lg1, general logistic model; ln, log-normal; sA, sigmoid type A; sb, sigmoid type A; wb, Weibull.

3 | RESULTS

3.1 | Models describing survival of STEC O157:H7 in leafy greens

Out of the 530 datasets extracted from the literature, 270 exhibited a significant linear reduction ($p < .05$) suitable for inactivation modeling. Among these, 266 datasets (98.2%) had at least one model that successfully fit the data. These datasets were further categorized into 195 representing natural die-offs and 71 representing antimicrobial-induced die-offs. The jm2 model was the most selected best-fit model for most (50%) of the datasets, whereas the ep model was the best fit for 19.5% of the datasets (Figure 1). For both natural and antimicrobial-induced die-offs, the jm2 model proved to be the best fit for the highest number of the datasets (50.8% and 47.8%, respectively) (see Figure S1). The nonlinear models fitted a higher proportion of the datasets in each of the four stages of the value chain (Figure S2). The fitted linear curve of the predicted against the observed values of the datasets showed better fitting in the jm2 (adjusted $R^2 = .89$) model than the exponential (adjusted $R^2 = .58$) (see Figure S3). Only in one dataset was the exponential model selected as the sole model that fit the data, compared to the jm2 model that

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was selected as the sole best-fitting model for 23 datasets. A summary of the parameters of the optimized models is provided in Table S1.

3.2 | Factors influencing inactivation of STEC O157:H7 in leafy greens in natural die-off

3.2.1 | Survival metrics

Upon evaluating the model performance by fitting the observed values against the predicted points generated from both the exponential and model-averaged approaches, it was observed that up to 7 LRTs can be calculated (Figure S3). From the median values of the survival metrics, the exponential model generated higher values for 1–3 LRTs compared to the model averaged; however, for >4 LRTs, the averaged model yielded higher values than the exponential model (Figure S4). Pairwise Wilcoxon test showed that the pairs of 4 and 5, and 6 and 7 LRTs were not significantly different from each other (Figure S6). Although 1 LRT was attained within slightly over a day for natural die-off (median = 1.57 days, mean = 3.83 days), attaining a 5-log reduction took around a month (median = 22.58 days, mean = 81.33 days) (see Table S2).

3.2.2 | Factor analysis

Temperature and RH data were captured in 191 and 146 datasets, respectively. Descriptive statistics for the conditions were summarized in frequencies for the categorical variables and mean for the continuous variables (Table 3). The average temperature in natural die-off in this meta-analysis was 14.57°C, and the RH was 68.95%. Besides soil conditions and type of vegetables, all other conditions significantly ($p < .05$) affected the survival metrics (Table S3).

In natural die-off, it was observed that STEC O157:H7 exhibited higher LRTs at the processing stage of the value chain compared to primary production (Figure 2). Temperature, RH, and the size of the inoculum were all weakly correlated ($r < .3$) with the survival metrics for 1 and 5 LRTs.

A variety of factor analysis techniques were used to establish the effect of the different conditions on the survival metrics, 1 and 7 LRTs, in natural die-off. Only conditions that were statistically significant (Table S4) were included in the factor analysis. The dredge function was used to further select variables that provided a best-fit model to avoid overfitting our model in the factor analysis. In evaluating the effect of conditions on the survival metrics, the random forest method consistently had the lowest RMSE values compared to other factor analysis methods in this meta-analysis (Table S5). The performance power evaluated how best the trained model performed, whereas the predictive power showed how best the trained model predicted the testing dataset.

Despite all the survival metrics being evaluated in factor analyses, the presented results will narrow on 1 and 5 LRTs. The regulatory threshold set by the United States Food and Drug Administration (FDA) requires that the sequential mitigation steps and controls for green leafy vegetables achieve a 5-log reduction (USFDA, 2022); thus, this value must be considered. Random forest explained 40.85% and 31.46% (500 trees, three variables at each split) of variance in 1 and 5 LRTs, respectively. In both 1 and 5 LRTs, temperature and RH were the most important variables explaining the inactivation of STEC O157:H7 in leafy greens (Figure 3). Partial dependence plots and *H*-statistics identified RH, size of inoculum, and temperature as factors with interactive effects (Figures S7–S10). The effect of RH and size of inoculum on 5 LRTs had interactions with other conditions (Figure S9). Specifically, RH interacted with temperature and size of inoculum to affect 5 LRTs (Figure S10). With increasing RH and decreasing temperature, 5 LRTs decreased (Figure S11). At a lower size of the inoculum, a higher LRT (5 LRTs) was generated; however, with increasing RH despite the low size of the inoculum, a lower LRT (5 LRTs) was generated. The effect of the size of inoculum on 5 LRTs was also affected by the storage of the produce (Figure S10). At a lower inoculum size, a higher LRT (5 LRTs) was reported, with consistently higher values reported during the storage of the produce (Figure S11). Stage in the value chain, enumeration technique, and temperature were ranked as the core factors affecting exponentiated survival metrics in natural die-off (Figure S15).

3.3 | Factors influencing inactivation of STEC O157:H7 in leafy greens in antimicrobial-induced die-off

3.3.1 | Survival metrics

The distribution of the survival metrics of STEC O157:H7 in the antimicrobial-induced die-off of leafy greens had a bimodal distribution (Figure S5). The descriptive statistics of the LRT in antimicrobial-induced die-off are summarized in Table S3. The 1–3 LRTs generated from the exponential model had relatively higher values than those from the model averaging approach, whereas at >4 LRTs, the latter had relatively higher values than the former. The median time taken to achieve a 1-log reduction

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TABLE 3 Descriptives statistics of the conditions of influencing inactivation in natural die-off.

Condition	Statistics	Categories	Values
Enumeration technique ($n = 195$)	Frequency	Culture-based	162
		Molecular	33
Experimental site ($n = 195$)	Frequency	Field	54
		Lab-scale	141
Modified atmosphere storage ($n = 195$)	Frequency	No	151
		Yes	44
Irrigation ($n = 195$)	Frequency	Yes	115
		No	80
Relative humidity ($n = 146$)	Mean, %		68.952 (min = 0, max = 100)
Size of inoculum ($n = 195$)	Mean, log CFU/g		6.263 (min = 3, max = 9)
Shredding ($n = 195$)	Frequency	No	115
		Yes	80
Soil conditions	Frequency	Contaminated	28
		Not contaminated	167
Stage in the value chain ($n = 195$)	Frequency	Primary production	128
		Processing	10
		Storage	53
		Transport	4
Temperature ($n = 191$)	Mean, °C		14.645 (min = 1, max = 30.7)
Type of vegetable ($n = 195$)	Frequency	Cabbage	4
		Lettuce	156
		Spinach	32

was 0.397 days, whereas, for a 5-log reduction, it was 12.383 days. Test for differences in the survival metrics established that except for the pairs of 2 and 3 LRTs, 4 and 5 LRTs, and 6 and 7 LRTs, the values were significantly (Wilcoxon test, $p < .05$) different (Figure S6).

3.3.2 | Factor analysis

Washing with chlorine was the most studied antimicrobial treatment in the articles included in the meta-analysis (Table 4). The average temperature and RH for studies in antimicrobial-induced die-off were 10.98°C and 69.65%, respectively. The concentration of chlorine washing solution (FC) and chlorine dioxide gas averaged 7.66 (0.12–50) and 18.5 (0.5–50) ppm, respectively. The average concentration of organic acids used for antimicrobial treatments was 40.1 (0.5–50) ppm. Except for the size of the inoculum and soil conditions, all other conditions significantly ($p < .05$) affected the survival metrics in antimicrobial-induced die-off (Table S4). The random forest technique provided the lowest RMSE values for the performance and predictive powers in natural die-off (Table S5). Although the rank of importance of the factors changed with exponentiated survival metrics, factors that most affected both the 1 and 5 LRTs remained the same (Figure S15).

Nonconventional antimicrobial treatments such as irradiation took comparatively longer time than washing with chlorine to achieve 1- and 5-log reductions (Figure 4). A comparison of processing and storage points of the value chains established that processing had lower 5 LRTs than the former in the antimicrobial-induced die-off. In defining the antimicrobial treatment contact time such as the time taken to wash leafy greens with chlorine, 41 datasets were extracted to detail this step. Washing in chlorine (chlorine water) took a comparatively similar time to achieve 1- and 5-log reductions as chlorine dioxide; however, the nonconventional treatments took a longer time (Figure 5). Using the Spearman rank correlation test, the temperature was shown to be correlated to 1 LRT ($r = -.4$) and weakly correlated to 5 LRTs ($r = -.26$). RH and inoculum size both had weak correlations (<.3) with LRTs.

Among the factor analysis methods, the random forest had consistently the lowest RMSE values for the performance and predictive power across various survival metrics (Table S6). The random forest model explained 64.95% and 53.28% (number of trees 500 and two

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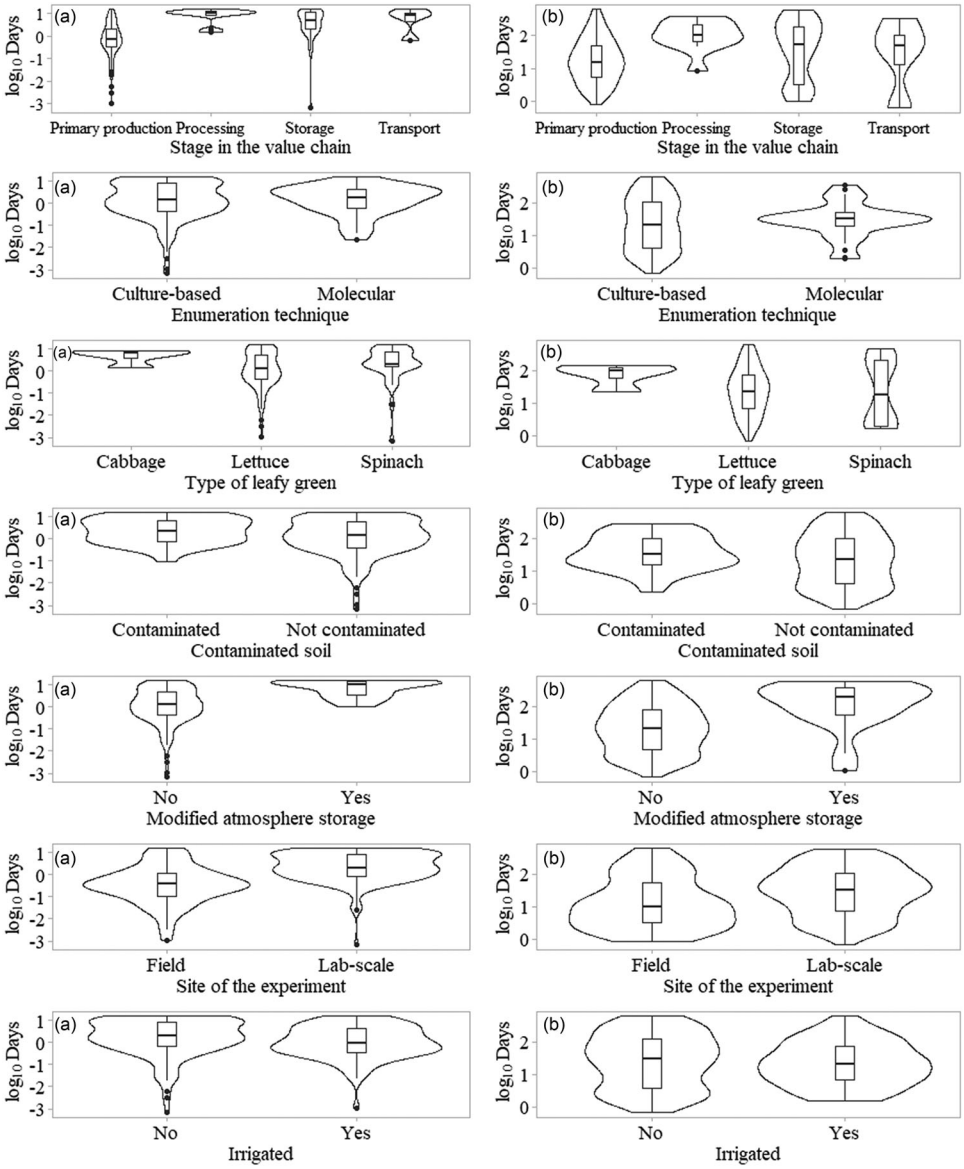

FIGURE 2 Combined violin and boxplots of 1 (a) and 5 (b) log reduction times in natural die-off of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 in leafy greens. The violin plots were used to describe the distribution of the log reduction times. The lines in the boxplot are the 25th percentile, median value, and the 75th percentile of the log reduction times.

variables in each split) of variance in the 1 and 5 LRTs for the inactivation of STEC O157:H7 in leafy greens. Inoculum size, temperature, and the stage in the value chain were found to be the most important variables, as the permutation of any of these three resulted in the highest increase in the percent MSE (Figure 6). The interactive effects among the conditions were not statistically significant (*H*-statistic <.1), as shown in Figures S11–S13. Similar to the model averaging approach, inoculum size, stage in the value chain, and temperature were ranked the most important conditions affecting 1 and 5 LRTs in exponentiated survival metrics in antimicrobial-induced die-off (Figure S15).

4 | DISCUSSION

4.1 | Natural die-off of STEC O157:H7 in leafy greens

Microbial inactivation due to weather conditions and other environmental factors has been studied previously using experimental approaches, with the nonlinear models best fitting the pattern (Belias et al., 2020; Munther et al., 2020). The higher median values for ≥4 LRTs depict the tendency of tailing in the inactivation pattern of STEC O157:H7 in leafy greens, a feature not captured by the first-order exponential models that rely majorly on 1 LRT. In this study,

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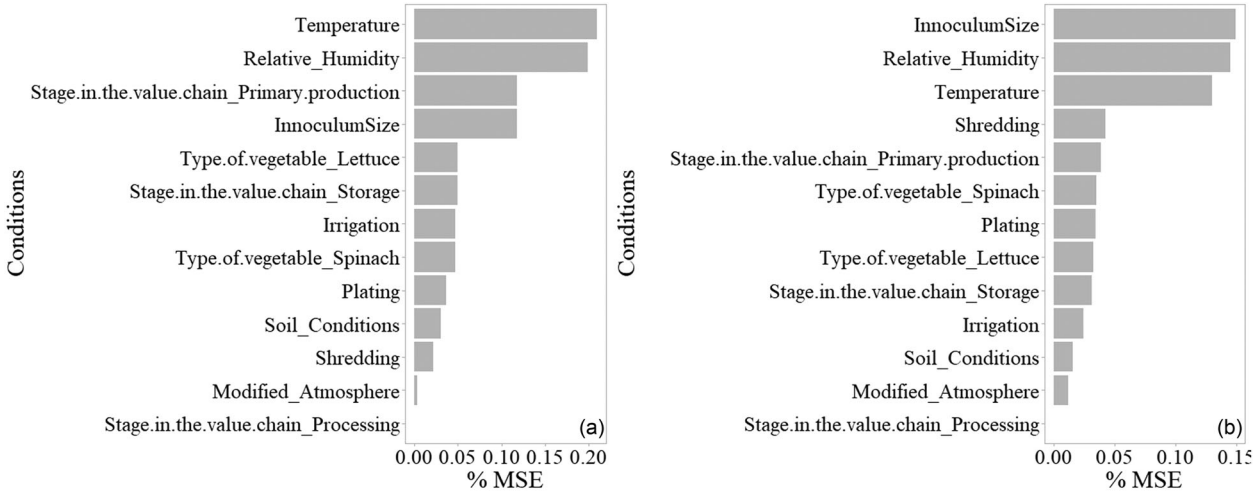

FIGURE 3 Variable importance of conditions influencing 1 (a) and 5 (b) log reduction times of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 in natural die-off. The %MSE (mean square error) shows the percent increase in the error in case the variable is permuted during analysis.

TABLE 4 Descriptive statistics of the conditions of inactivation of Shiga toxin-producing *Escherichia coli* (STEC) in leafy greens in antimicrobial-induced die-off.

Condition	Statistics	Categories	Values
Antimicrobial treatments	Frequency	Chlorine dioxide	13
		Chlorine water	45
		Non-conventional	7
		Organic acids	6
Enumeration technique ($n = 71$)	Frequency	Culture-based	71
Modified atmosphere storage ($n = 71$)	Frequency	No	60
		Yes	11
Irrigation ($n = 71$)	Frequency	No	57
		Yes	14
Relative humidity ($n = 26$)	Mean, %		69.653 (min = 45, max = 97)
Size of inoculum ($n = 71$)	Mean, CFU/g		6.963 (min = 4, max = 9)
Shredding ($n = 71$)	Frequency	No	7
		Yes	64
Soil conditions ($n = 71$)	Frequency	Contaminated	1
		Not contaminated	70
Stage in the value chain ($n = 195$)	Frequency	Processing	41
		Storage	30
Temperature ($n = 69$)	Mean, °C		11.106 (min = 4, max = 25)
Type of vegetable ($n = 195$)	Frequency	Lettuce	60
		Spinach	11

the nonlinear model of jm2 best fitted more individual datasets than the first-order kinetics. Since the model fitting the highest number of datasets differed by stage of the value chain, the jm2 model performed relatively better in describing pathogen survival during inactivation. While previous studies have attempted to create model forms to account for the effect of individual conditions on the inactivation of STEC O157:H7 in leafy greens (Belias et al., 2020; Ottoson et al., 2011), this research highlights the limitations of such models due to the multitude of factors and their diverse effects. Inactivation rates had the greatest effect in the sensitivity analysis of QMRA of STEC O157:H7 in

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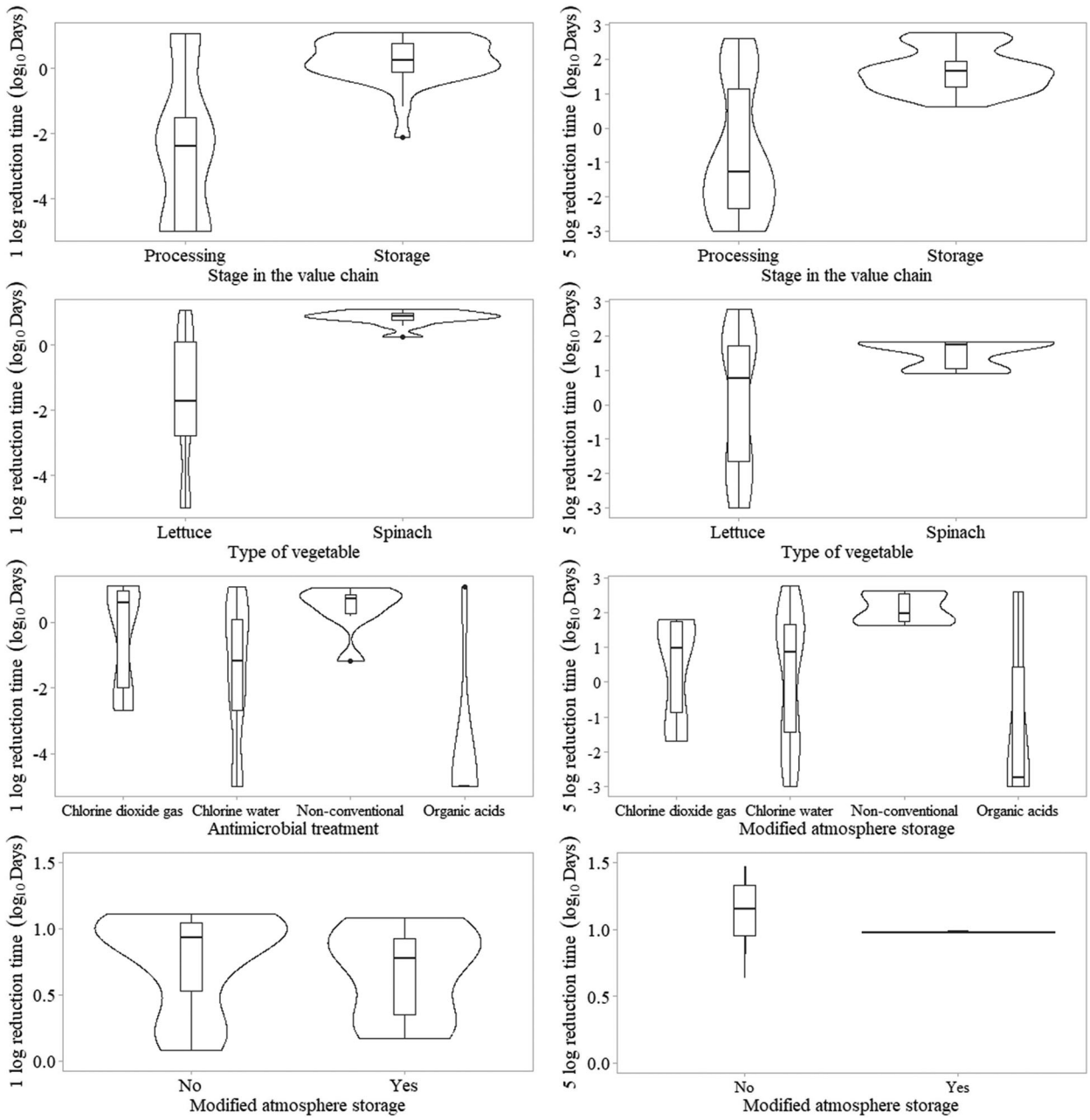

FIGURE 4 Combined violin and boxplots of 1 and 5 log reduction times in antimicrobial-induced die-off. Nonconventional antimicrobial treatments in this case entailed neutralized alkaline water, cold plasma, and irradiation, which are not widely used in the industry in the sanitizing process of the green leafy vegetables.

lettuce (Ottoson et al., 2011). To address this gap, it is essential to employ model forms that offer more accurate parameter estimates. Beyond its superior performance in fitting datasets related to both natural and antimicrobial-induced die-offs, the jm2 model exhibited better overall fitting in the pooled survival data.

Temperature and RH were ranked as the most important factors influencing microbial survival in leafy greens. The systematic review established that the interaction of RH and temperature influenced the inactivation of STEC O157:H7. For instance, light intensity and low temperature were found to induce the pathogen's inactivation (Moyne et al., 2020). However, this study did not explore this interaction, as only a single study evaluated inactivation under these conditions while assigning and associating specific inactivation data to the presence or absence of light. The interaction of temperature and RH established in this study was also reported in the study by Choi et al. (2011). Although there was no significant reduction (<1.0 log) of STEC O157:H7 on spinach stored at 100% RH at 12°C, at the same temperature and 43% RH, a 1.7-log reduction was reported over 5 days in natural die-off. Our study, how-

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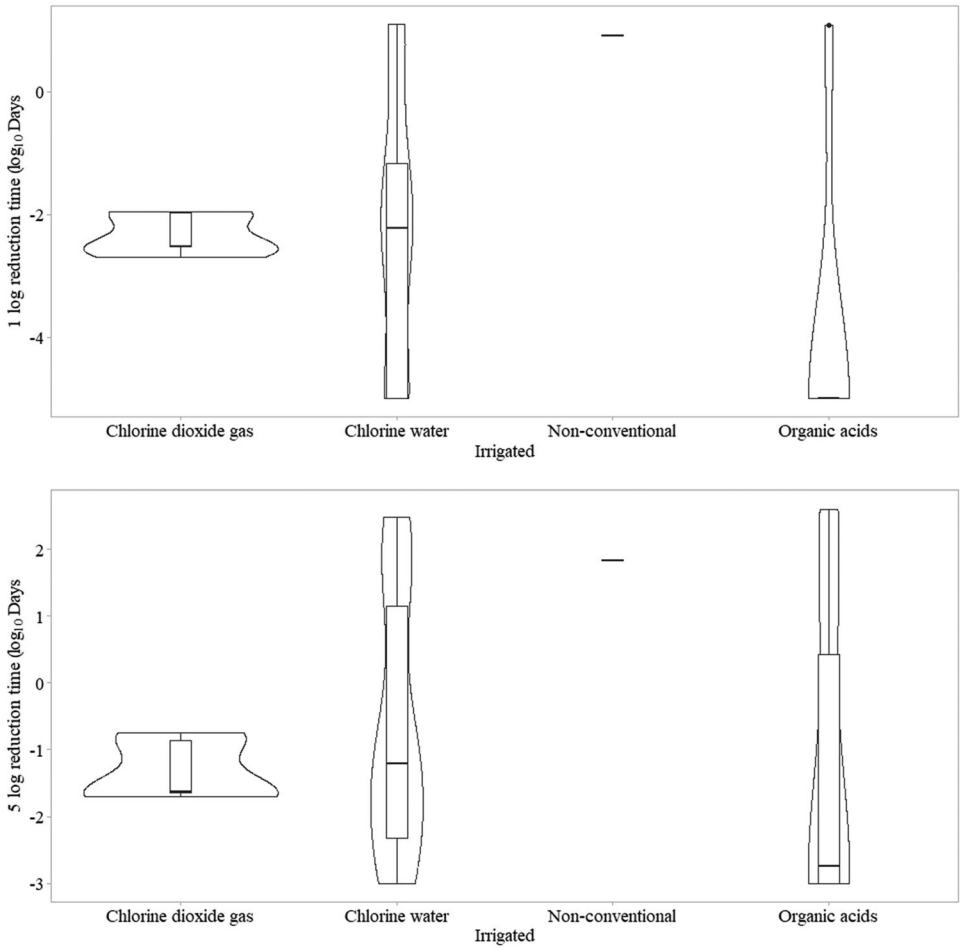

FIGURE 5 Survival metrics during antimicrobial treatments contact time of leafy greens. Contact time in this case refers to the duration for which leafy greens were dipped in chlorine water or treated with other antimicrobials to achieve a 1- or 5-log reduction in the antimicrobial-induced die-off.

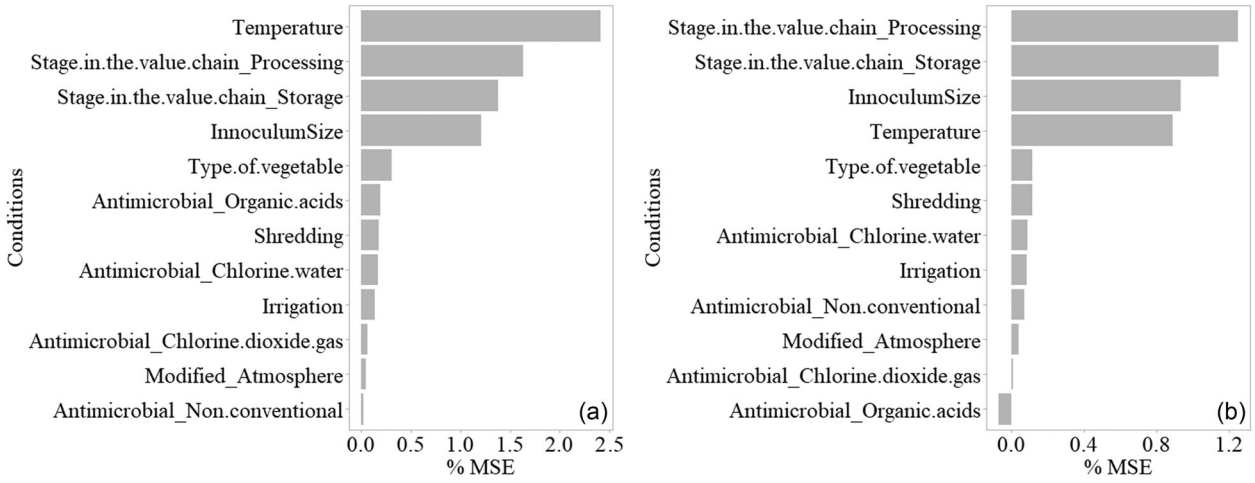

FIGURE 6 Variable importance of conditions influencing 1 (a) and 5 (5) log reduction times of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 in antimicrobial-induced die-off. The % MSE (mean square error) shows the percent increase in the error in case the variable is permuted during analysis.

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ever, acknowledges that the concentrations reported by the studies were largely (83.1%) culturable counts and may overlook the nonculturable fraction due to stressful conditions such as low temperature, which have increased the resistance to natural die-off (Dinu & Bach, 2011; Moyne et al., 2013).

This study also established an interaction between the size of the inoculum and the RH. Another interaction established in this study is increasing survival of the pathogen at a lower size of inoculum, while consistently lower values will be reported if not stored. Initial inoculum size positively correlates with the die-off rate (McKellar, Peréz-Rodríguez, et al., 2014), and increasing survival of the pathogen has been reported during temperature abuse in storage such as cold (8–15°C) and room temperature (25°C) (Choi et al., 2011). Our study established that the mean temperature of studies in this meta-analysis for the storage step was 9°C, supporting evidence of the reported high survival. This suggests that models focusing on one condition while neglecting the other may yield less accurate die-off parameters when such interactions are at play.

4.2 | Inactivation of STEC O157:H7 in antimicrobial-induced die-off in leafy greens

In the case of antimicrobial-induced die-off, the decline in microbial population is primarily attributed to antimicrobial treatments such as washing with sanitizers, which makes it different from natural die-off. As expected, the generated LRTs were shorter in antimicrobial-induced die-off than natural die-off. While no single model best fitted the majority of the datasets in antimicrobial-induced die-off, the jm2 model fitted the highest number of datasets compared to other models. The shift from the first-order kinetics to the nonlinear models in antimicrobial-induced die-off has been supported by findings showing that, under stress, STEC O157:H7 transitions into a nonculturable state with increased resistance to inactivation (Chhetri et al., 2020; Dinu & Bach, 2011). Notably, while the first-order kinetics generated higher values for lower LRTs (1–3), suggesting higher microbial survival, the model likely underestimated survival at the higher LRT (5 LRTs), which is a crucial threshold for microbial safety of leafy greens (USFDA, 2022). It is noteworthy that the random forest technique explained a higher variance in survival metrics generated from antimicrobial-induced die-off than natural die-off.

Considering the diverse antimicrobial treatments used in the selected literature, we focused on sanitizers that have been approved for industrial use and are subject to established standards (Chang & Fang, 2007; Davidson et al., 2014). The recommended concentration of total chlorine in the wash water for fresh produce processing is 50–200 ppm (Sun et al., 2012). Since microbial inactivation is influenced by the concentration of FC, studies have established that 10 ppm is the lowest effective concentration of FC for the treatment of produce during washing (Fu et al., 2018; Luo et al., 2018). Notably, our study found an arithmetic mean of 7.66 ppm of FC, which is lower than this threshold. It is worth noting that within the reported ranges of chlorine water and chlorine dioxide, similar survival metrics were observed. However, nonconventional methods like cold plasma, irradiation, and electrolyzed water did not offer any advantage in improving pathogen inactivation in produce. While our review identified an interaction between antimicrobial treatments and temperature, the combined effect of these factors had little impact on explaining the variation in the reported survival metrics in this study. Due to the limited number of studies exploring different concentrations of the sanitizers, our study did not explore important chemical conditions such as the concentration of the antimicrobial treatments.

4.3 | Recommendations for future research

The findings of this study challenge the overdependence of first-order kinetics, including *D*-values, in describing microbial inactivation in leafy greens. To account for the effects of conditions on the survival of STEC O157:H7 in leafy greens, future QMRA studies must move beyond first-order kinetics and account for nonlinear trends. The inactivation parameters generated from this study, while useful for future studies, should be extrapolated beyond the studied leafy greens with caution. Different types of fresh produce have distinct characteristics that may affect microbial survival. Funding future projects to fill this critical gap is essential and should include an exploration of produce–pathogen characteristics that influence microbial kinetics. Furthermore, gaps including properties of microbial culture including growing conditions of the culture that may induce injury in the cells, affecting the inactivation pattern that if studied can be included in the modeling. The study presented herein can help establish a framework for designing a project to create a searchable database of specific inactivation rates for produce–pathogen pairings, which would provide a better description of pathogen die-off for risk assessment and microbial safety of leafy greens within the food industry.

The study herein also evaluated a mix of individual and interactive effects of conditions both in natural and laboratory environments. Experiments in the

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natural environment had sufficient observations to evaluate the interactive effects across multiple conditions. However, the majority of laboratory studies did not adequately measure or report data on the interactive effects, such that the study herein could not provide conclusive findings on how these effects influenced inactivation models. Factors including inoculum growth conditions and inactivation kinetics beyond the LOD were also not comprehensively studied due to limitations in the data. We view these as potential points of data that can further contribute to minimizing model uncertainty.

5 | CONCLUSION

Based on our study, we concluded that the jm2 model would best fit the inactivation data of STEC O157:H7 in leafy greens. When using the model averaging approach and exponential model to generate survival metrics, the latter may likely underestimate pathogen survival on the produce, particularly for higher LRVs (>4). Although temperature and RH were the core factors influencing inactivation under natural die-off conditions, inoculum size, temperature, and stage of the value chain influenced inactivation most in the antimicrobial-induced die-off. While the use of conventional antimicrobials such as organic acids, chlorine water, and chlorine dioxide achieved no advantage over each other, they provided better efficiency in the removal of the microbial cells on leafy greens than nonconventional methods. Our findings demonstrate the need for sequential mitigation steps that consider different conditions influencing the survival of the pathogen. This finding aligns with recommendations, including the use of Hazard Analysis Critical Control Point (HACCP) to attain a 5-log reduction of the target pathogen in fresh produce. Furthermore, the study provides an alternative modeling approach for generating inactivation parameters for inputs in risk assessment. The study further shows that the continued reliance on first-order kinetics due to its simplicity, for instance in establishing 1 LRT, may be insufficient especially when the interest is to achieve produce safety. The findings of this study have far-reaching applications, including addressing gaps in pathogen modeling and enhancing the precision of inactivation rates utilized in QMRAs, a process that informs risk management approaches such as temperature controls in leafy greens. Additionally, we found that future experimental studies should detail conditions under which pathogen inactivation data were collected.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used in this study can be availed upon reasonable request to the corresponding author.

ORCID

Jade Mitchell https://orcid.org/0000-0003-2199-4190

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