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Effects of Fumigation on the Reduction of *Salmonella enterica* in Soil

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Abstract

Due to the phaseout of methyl bromide (MeBr), there is a need for broad-spectrum soil fumigation alternatives for pest management. Little is known about the impact of fumigation alternatives on foodborne pathogens, such as *Salmonella*, in agricultural soils. This study investigated the effect of MeBr alternative fumigants on *Salmonella* reduction in soil. Sandy loam soil was collected from a conventional farmed vegetable field and inoculated with either *Salmonella* Newport J1892 or Typhimurium ATCC 14028 (5.9 ± 0.3 $\log_{10}$ colony-forming unit [CFU]/g). Each of the four fumigants labeled for pest management (1,3-dichloropropene, chloropicrin, dimethyl disulfide, and metam sodium) was applied at labeled maximum application field levels to soil in pots and stored for a 2-week period. Sterile water was used as a control. Following the 2-week period, *Salmonella* concentrations in soil samples were enumerated at 1, 7, 14, and 21 days postfumigation. The mean concentration of *Salmonella* Newport was significantly higher than that of *Salmonella* Typhimurium 1 day after fumigation ($p = 0.015$). Fumigation using 1,3-dichloropropene or dimethyl disulfide significantly reduced *Salmonella* Newport and *Salmonella* Typhimurium concentrations, compared with the sterile water control. The rate of *Salmonella* reduction in soil treated with dimethyl disulfide was higher (0.17 ± 0.02 $\log_{10}$ CFU/g/day), compared with soil treated with the other fumigants (0.10–0.12 $\log_{10}$ CFU/g/day). Due to the reduction of *Salmonella*, alternative fumigation treatments may mitigate potential *Salmonella* contamination in soil within farm environments.

Keywords: *Salmonella*, fumigation, soil, methyl bromide, preharvest, food safety

Introduction

Salmonella is a bacterial pathogen of public health concern as it is one of the leading causes of foodborne illnesses, hospitalizations, and deaths in the United States annually (CDC, 2018; Dhaliwal et al., 2021; Scallan et al., 2011). Over the last decade, there has been an increasing number of *Salmonella* outbreaks associated with fresh produce (Bell et al., 2015; Carstens et al., 2019; Lynch et al., 2009). Since fruits and vegetables are often consumed raw, mitigating contamination early in the supply chain is a priority for the industry. Previous research has shown that *Salmonella* can be introduced in the preharvest environment by a variety of routes, including from soil, water (e.g., irrigation, application of pesticide sprays), and/or wild animal intrusion in production fields (FDA 2015; Franz and Van Bruggen, 2008; Honjoh et al., 2014; Stine et al., 2011; Suslow, 2010).

When contaminated water or soil amendments are applied to fields, it may directly or indirectly contaminate fresh produce (FDA, 2015; Natvig et al., 2002; Sallach et al., 2015; You et al., 2006). *Salmonella* has frequently been isolated from waterways and agricultural soil in the Mid-Atlantic region, with *Salmonella* Newport and *Salmonella* Typhimurium among the predominant serovars isolated (Bell et al., 2015; Gu et al., 2019; Marine et al., 2015; Micallef et al., 2012; Murphy et al., 2023; Sharma et al., 2020). In addition, soil composition (e.g., silt, sand, clay), management

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practices, temperature, and moisture can affect the survival of *Salmonella* in soil (Bardsley et al., 2021; Chandler and Craven, 1980; Gu et al., 2013b; Holley et al., 2006; Natvig et al., 2002).

Chemical fumigation of agricultural soil has been shown to be effective against plant pests of concern (plant pathogens, insects and weeds), and has been proposed as a possible antimicrobial strategy against foodborne pathogens to optimize existing preharvest practices (Miller et al., 2022). Methyl bromide (MeBr) was a commonly used broad-spectrum fumigant and has been suggested as an effective mitigation method to reduce *Escherichia coli* O157:H7 concentration in soil, and transfer from soil to lettuce leaf (Ibekwe et al., 2007). However, MeBr was classified as a class I ozone-depleting substance due to its capability to deplete the ozone layer (United States Environmental Protection Agency [US EPA], 2021). As such, the use of MeBr as a soil fumigant has been slowly phased out by the EPA since 2005, in accordance with the Montreal Protocol (US EPA, 2022; US EPA, 2011).

Researchers and growers have spent the last two decades searching for fumigation alternatives to MeBr that are effective on production of pests faced in produce fields. Therefore, there is a need to evaluate the synergistic and antagonistic effects on food safety hazards when using MeBr alternatives, specifically the impact of these fumigants on *Salmonella* concentrations in preharvest agricultural soils.

In the Mid-Atlantic region, MeBr alternative fumigants have successfully provided weed, plant disease, and nematode control (Kuhar et al., 2020). Registered and commonly suggested fumigants include chloropicrin, 1,3-dichloropropene, metam sodium, and dimethyl disulfide (McAvoy and Freeman, 2013; Shi et al., 2022). 1,3-Dichloropropene is a colorless liquid organochlorine compound, which is widely used in the United States as a pesticide, applied specifically as a preplant fumigant (US EPA, 2000). Currently, registered in many states, as well as globally, dimethyl disulfide is an organic chemical applied preplant to fields, and is commonly used during the production of berries, cucurbit vegetable, fruiting vegetable (e.g., tomato, pepper), field-grown ornamental, and forest tree nursery crops (US EPA, 2012). Chloropicrin is a liquid chemical compound used as a preplant soil fumigant to manage a broad spectrum of fungi, bacteria, insects, and other harmful pests. It is commonly used in combination/coformulation with 1,3-dichloropropene (US EPA, 2014). Metam sodium is the sodium salt of methyl-dithiocarbamate, which is a nonselective soil fumigant with fungicidal, herbicidal, insecticidal, and nematicidal properties. As one of the most widely used agricultural soil fumigants in the United States, metam sodium is currently labeled for use on most food, feed, and fiber crops. It is used preplant on turfgrass to control invading plant roots and in drains and sewers (US EPA, 2013). Thus, the objective of this study was to evaluate the effect of four MeBr fumigant alternatives on the concentration of *Salmonella* serovars Typhimurium and Newport in inoculated agricultural soil during and postfumigation.

Materials and Methods

Salmonella *inoculum preparation*

Similar to previous research in the Mid-Atlantic region (Han and Micallef, 2014), *Salmonella* serovars Typhimurium and Newport were chosen with the purpose of addressing the gap in knowledge surrounding these two pertinent serovars. *Salmonella enterica* serovar Newport strain J1892 (*Salmonella* Newport; associated with a previous tomato-borne outbreak) was obtained from the U.S. Centers for Disease Control and Prevention (CDC, Atlanta, GA). *Salmonella enterica* serovar Typhimurium strain ATCC 14028 (*Salmonella* Typhimurium) was obtained from the American Type Culture Collection (Manassas, VA).

Both bacterial cultures were stored in Luria-Bertani (LB) broth containing 20% glycerol at −80°C until used in this study. Before each experiment, cultures were reinoculated into LB broth and incubated at 37°C for 20 h. Each overnight culture was centrifuged at 4000 rpm for 15 min at 22°C, and the pellets were suspended in 100 mL of sterile water to an optical density (600 nm) of 0.3 ($\sim$8 $\log_{10}$ colony-forming unit [CFU]/mL) for use as inoculum in this study.

Soil collection

Sandy loam soil was obtained from vegetable production fields at the Virginia Tech's Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA. Plastic containers with 3.07 L capacity (Glad, Amherst, VA) were used to collect 4 kg soil samples. A total of 36 soil samples were collected and transported to a biological safety level 2 greenhouse at the ESAREC. Soil collected for this study had average nitrogen (N), phosphorus (P), and potassium (K) amounts of 320, 136, and 103 mg/kg, respectively, and an average pH of 6.2. Soil was tested and negative for *Salmonella*.

Salmonella *inoculation and soil fumigation*

This study consisted of two independent trials organized in a randomized complete block design in the study. Of the 36 soil samples, 15 were inoculated with *Salmonella* Newport and 15 with *Salmonella* Typhimurium in plastic containers (3.07 L) by mixing each soil sample with 40 mL of the corresponding *Salmonella* suspension to reach a target initial concentration of $\sim$6 $\log_{10}$ CFU/g. To ensure homogeneity, each container was subjected to 5 min of shaking. Six soil samples (without inoculation and fumigation) were used as negative controls.

Fumigants used in this study included 1,3-dichloropropene (Telone II; Teleos Ag Solutions, Pinehurst, NC), chloropicrin (Chloropicrin 100; Cardinal Professional Product, Gilroy, CA), dimethyl disulfide (Paladin; Arkema, King of Prussia, PA), and metam sodium (Vapam HL; AMVAC Chemical Corporation, Newport Beach, CA). Immediately following inoculation, each of the four fumigants were applied to three *Salmonella* Newport and three *Salmonella* Typhimurium inoculated soil samples, respectively, at equivalent maximum application levels in fields (Table 1). Sterile tap water was applied to control samples. Each tested fumigant was mixed with sterile water to a final volume of 1 mL and evenly drop-applied (50 μL per drop) onto the surface of each soil sample. After applying the fumigant to the soil sample, each container with the soil sample was then covered with a 0.03-mm-thick Blockade impermeable plastic mulch (Berry Plastics Corp., Evansville, IN) and sealed with transparent Scotch tape (3M, Saint Paul, MN).

Table 1. List of Fumigants and Application Doses

Fumigants	*L/ha*[a]	*mL/4 kg*[b]
1,3-Dichloropropene	168,000	0.156
Chloropicrin	132,400	0.123
Dimethyl disulfide	442,400	0.411
Metam sodium	700,000	0.65
Control	N/A[c]	1

[a]Application amount in fields.
[b]Equivalent application amount in study.
[c]Sterile tap water: none applied in field.

Sealed containers with treated soils were placed in a biological safety level 2 greenhouse equipped with ridge vents, a cooling air conditioning unit, and a gas heater at ESAREC for a 2-week fumigation period at 24°C ± 3°C. Soil pH data were collected for each sample after fumigation using an Orion 5-Star Benchtop Multiparameter Meter (Thermo Fisher Scientific). To maintain sandy loam soils within the typical moisture range (10–12%), the soil moisture was adjusted and maintained at 10% ± 2% during the study by manually adding sterile tap water (Bardsley et al., 2021; Rawls and Brakensiek, 1982).

Soil sampling and Salmonella *detection*

Three soil samples (4 g each) were removed immediately after inoculation of soil. *Salmonella* was enumerated in soil samples as described below and was recorded as the starting concentration. Following the 2-week fumigation period, soil samples (4 g each) were also collected at 1, 7, 14, and 21 days ($n = 3$/time point) and *Salmonella* concentrations were enumerated. Specifically, each 4 g soil sample was diluted in 40 mL of sterile water. Serial dilutions in sterile water were performed and 0.1 mL of each dilution plated directly onto xylose-lysine-tergitol 4 (XLT-4; Thermo Scientific, Waltham, MA) agar plates using an Eddy Jet 2 spiral plater (IUL Instruments, Barcelona, Spain), and incubated at 37°C for 24 h.

After enumeration, presumptive *Salmonella* colonies with characteristic colony formation (black or black-centered colonies with a yellow or pink periphery) were quantified using a Neutec Flash & Go automated colony counter (Neutec Group, Inc., Farmingdale, NY). From each plate, up to three colonies were restreaked onto XLT-4 plates and confirmed as *Salmonella* by polymerase chain reaction for the *invA* gene, as previously described (Luo et al., 2014).

Statistical analyses

All the statistical analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC). *Salmonella* concentrations were converted to $\log_{10}$ CFU/g, and reductions in *Salmonella* concentrations for each fumigation treatment were determined for each time point. The effects of fumigation application on soil samples (concentration reduction) were examined by analysis of variance (ANOVA; $p \le 0.05$). The rates of decline and intercept of *Salmonella* concentration density in soil samples were calculated by fitting $\log_{10}$-transformed data to the linear model, as previously described (Gu et al., 2013a). Estimated values of the parameters were analyzed by multivariate analysis of variance (MANOVA; $p \le 0.05$). Soil pH values of each sample before and after fumigation, and among treatments, were compared by *t*-test and ANOVA, respectively ($p \le 0.05$).

Results

Reduction of Salmonella *in soil due to fumigation*

The average initial concentration of *Salmonella* in inoculated soil samples before fumigation was 5.9 ± 0.3 $\log_{10}$ CFU/g. Regardless of the fumigant used, $\log_{10}$ reductions were significantly higher in *Salmonella* Typhimurium (1.4 ± 0.2 $\log_{10}$ CFU/g) compared with *Salmonella* Newport (1.1 ± 0.1 $\log_{10}$ CFU/g) immediately postfumigation ($p = 0.015$; Fig. 1A). For individual fumigates, irrespective of serovar, 1,3-dichloropropene showed the greatest $\log_{10}$ reduction of *Salmonella* immediately postfumigation (2.4 ± 0.1 $\log_{10}$ CFU/g), compared with all other treatments ($p < 0.05$, Fig. 1B). Furthermore, *Salmonella* reductions in dimethyl disulfide (1.6 ± 0.3 $\log_{10}$ CFU/g) treatments were significantly greater, compared with the control (0.6 ± 0.1 $\log_{10}$ CFU/g), chloropicrin (0.7 ± 0.1 $\log_{10}$ CFU/g), and metam sodium (1.0 ± 0.1 $\log_{10}$ CFU/g) treatments ($p < 0.05$; Fig. 1B).

Soil pH was not significantly different before and after fumigation, or among different fumigant treatments ($p > 0.05$; data not shown). No *Salmonella* was detected from negative control samples.

Salmonella *die-off in soil after fumigation*

Postfumigation (day 1), the concentration of *Salmonella* was significantly higher in the control soils (5.29 ± 0.02 $\log_{10}$ CFU/g) and soils treated with chloropicrin (5.17 ± 0.18 $\log_{10}$ CFU/g) compared with metam sodium (4.90 ± 0.05 $\log_{10}$ CFU/g)-, 1,3-dichloropropene (3.48 ± 0.16 $\log_{10}$ CFU/g)-, and dimethyl disulfide (4.23 ± 0.13 $\log_{10}$ CFU/g)-treated soils (Table 2). *Salmonella* concentrations in soil samples with 1,3-dichloropropene and dimethyl disulfide treatment fell below the limit of detection (<1.0 $\log_{10}$ CFU/g) by day 21 postfumigation (Table 2). *Salmonella* concentrations significantly decreased between 1 and 21 days postfumigation, regardless of the fumigation treatment applied ($p < 0.05$; Table 2 and Fig. 2). Due to the fact that *Salmonella* concentrations were significantly different by treatment postfumigation (day 1; Table 2), a $\log_{10}$ linear model was used to describe the reduction rate ($\log_{10}$ CFU/g/day; Table 3).

The linear model used to describe *Salmonella* concentrations captured the majority of variance with a coefficient of variation (R^2) of 0.94 (Table 3). In addition, since the die-off rates by *Salmonella* serovars were not statistically significant ($p = 0.18$), data in Table 3 and Figure 2 reflect data for the serovars combined. The observed rates ($\log_{10}$ CFU/g/day) of *Salmonella* concentration reduction in inoculated soil samples were significantly different between the fumigation treatments (Wilk's Lambda significance = 0.0001). The rates of *Salmonella* reduction in inoculated soil samples treated with dimethyl disulfide (0.17 ± 0.02 $\log_{10}$ CFU/g/day) treatment was significantly faster, compared with all other treatments as well as the control ($p < 0.05$; Table 3). No significant differences were observed when comparing the rate of *Salmonella* reduction in soil samples treated

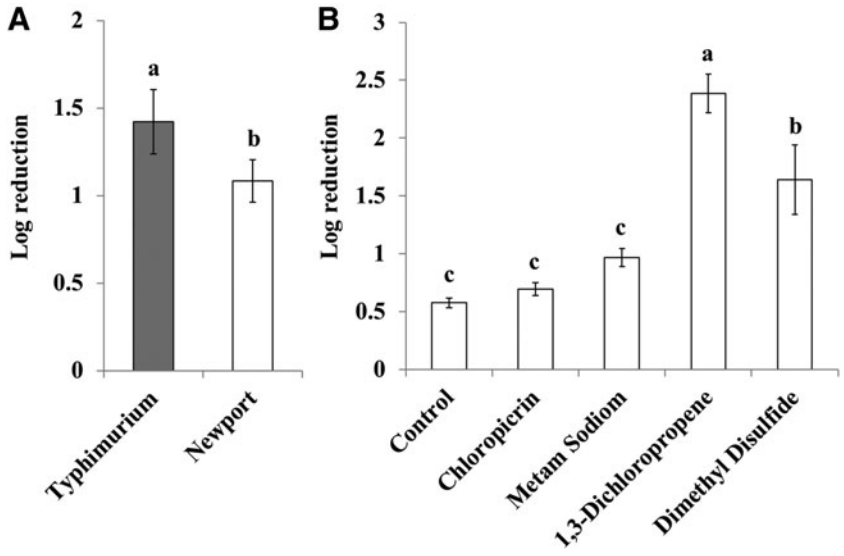

FIG. 1. Log_{10} reduction of *Salmonella* in inoculated soils 1 day after the 2-week fumigation. **(A)** Log_{10} reduction of *Salmonella* by serovar. **(B)** Log_{10} reduction of *Salmonella* by fumigant treatment. Different letters (a–c) denote significant differences between variables ($p < 0.05$). Bars represent standard errors of the reduction levels.

with 1,3-dichloropropene, chloropicrin, metam sodium, and control with die-off rates of 0.10–0.12 log_{10} CFU/g/day ($p > 0.05$).

Discussion

Several salmonellosis foodborne outbreaks associated with fresh produce have been attributed to serovars Newport or Typhimurium (Bell et al., 2015; Callejón et al., 2015; Jackson et al., 2013). Furthermore, soil, including soils with biological amendments, has been reported to be one of the primary sources of *Salmonella* contamination in produce (FDA, 2015; Honjoh et al., 2014; Natvig et al., 2002; You et al., 2006). Previous studies evaluating the prevalence of *Salmonella* serovars in biological soil amendments and survival in soil after the addition of an amendment have reported Newport and Typhimurium to be among the most commonly identified serovars (Gu et al., 2019; Gu et al., 2018; Murphy et al., 2022). In this study, immediately following the 2-week fumigation treatments, recovered concentrations of *Salmonella* Newport were significantly higher than those of *Salmonella* Typhimurium, suggesting that *Salmonella* Newport may exhibit a greater tolerance to fumigation (at least initially).

Salmonella survival was not observed to be significantly different between serovars postfumigation treatment (up to 21 days). Further research on the bactericidal effect on other serovars of *Salmonella* commonly implicated in fresh produce-related outbreaks could provide additional insight into the synergistic effects or dynamics of integrated pest management strategies with fumigation on *Salmonella* concentrations in soil.

This study demonstrated that there was a significant die-off in *Salmonella* concentration after 21 days in the soil. The total reduction of *Salmonella* due to chloropicrin (2.29 log_{10} CFU/g) and metam sodium (1.76 log_{10} CFU/g) treatments was not significantly different from the control (1.96 log_{10} CFU/g) treatment. This suggested that these two fumigants had a minimal additional antimicrobial effect on reducing *Salmonella* in soil. Chloropicrin works as a broad-spectrum fumigant by penetrating the bacterial cell wall and membrane, disrupting functions such as bacterial replication and cell division (Ajwa et al., 2010; Gray et al., 2013). Metam sodium affects bacteria by producing reactive substances that damage cells, inhibiting their metabolism and energy production (Li et al., 2022). It is important to note that these two fumigants, which have varying modes of action, did not facilitate enhanced *Salmonella* survival, and thus did not increase the food safety risk.

On the contrary, the total reduction of *Salmonella* concentrations after 21 days in the soil treated with 1,3-dichloropropene (>2.48 log_{10} CFU/g) or dimethyl disulfide

Table 2. Mean and Standard Deviation of *Salmonella* Concentrations (log_{10} CFU/g) in Soils After Treatment with Fumigation

Time (days)	*1,3-Dichloropropene*	*Chloropicrin*	*Dimethyl disulfide*	*Metam sodium*	*Control*
1	3.48 ± 0.16	5.17 ± 0.18	4.23 ± 0.13	4.90 ± 0.05	5.29 ± 0.02
7	2.85 ± 0.10	4.63 ± 0.10	3.45 ± 0.22	4.48 ± 0.16	4.29 ± 0.16
14	2.13 ± 0.03	3.33 ± 0.19	1.60 ± 0.26	3.09 ± 0.21	3.34 ± 0.26
21	<1.00[a]	2.88 ± 0.13	<1.00[a]	3.14 ± 0.27	3.33 ± 0.24

Data for both *Salmonella* serovars combined.
[a]Concentrations fell below the limit of detection (<1.0 log_{10} CFU/g).
CFU, colony-forming unit.

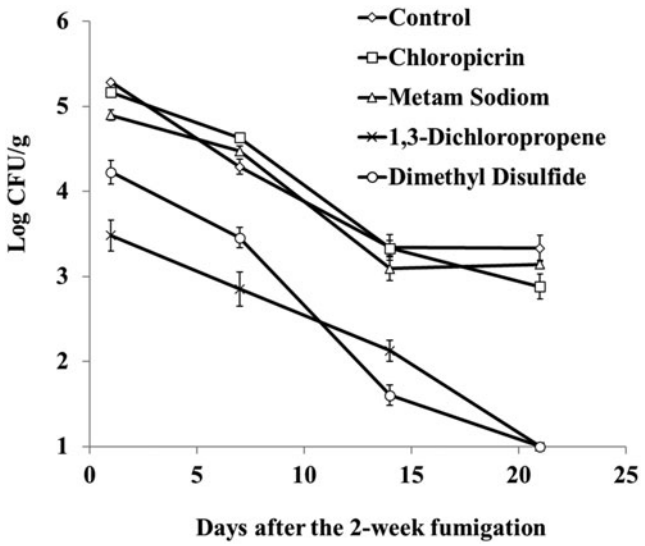

FIG. 2. Survival of *Salmonella* in soils postfumigation period (1–21 days after the 2-week fumigation). Bars represent standard error of *Salmonella* concentration densities at each sampling point. CFU, colony-forming unit.

(>3.23 $\log_{10}$ CFU/g) was significantly different from that of the control (1.96 $\log_{10}$ CFU/g). *Salmonella* concentrations in those soil samples were reduced below the limit of detection. The effect was comparable with the total reduction of the foodborne pathogen *E. coli 0157:H7* in soil by MeBr, as previously reported (Ibekwe et al., 2007). Both 1,3-dichloropropene and dimethyl disulfide have the same mode of action that works by inhibiting bacterial virulence and related gene expression by interfering with bacterial enzyme systems that are involved in energy production (e.g., respiration and electron transport) (Ajwa et al., 2010; Antunes et al., 2010).

In addition, the *Salmonella* reduction rate after dimethyl disulfide treatment was significantly higher during the 3 weeks postfumigation when compared with control and other fumigant treatments. Overall, the significant reduction in *Salmonella* concentrations both immediately after fumigation and up to 21 days post-treatment suggested that the use of these four fumigants does not enhance *Salmonella* survival, compared with control samples.

Table 3. Parameters of the $\log_{10}$ Linear Model[a] Describing the Reduction Rate (Mean ± Standard Deviation) and Intercept (Mean ± Standard Deviation) *Salmonella* Concentrations in Soils After Treatment with Fumigation

Fumigant	*Reduction rate (log_{10} CFU/g/day)*	*Intercept (log_{10} CFU/g)*
1,3-Dichloropropene	0.12 ± 0.05A[b]	3.48 ± 1.11B
Chloropicrin	0.12 ± 0.03A	5.31 ± 0.13A
Dimethyl disulfide	0.17 ± 0.02B	4.41 ± 0.39B
Metam sodium	0.10 ± 0.06A	5.00 ± 0.19A
Control	0.10 ± 0.02A	5.14 ± 0.22A

[a]Coefficient of variation (R^2) of 0.94.
[b]Different letters denote significant differences within a column ($p \leq 0.05$).
CFU, colony-forming unit.

It is well-established that fumigants impact the agricultural production environment beyond plant pathogen and pest targets (Castellano-Hinojosa et al., 2021; Dangi et al., 2017; De Neve et al., 2004; Ibekwe et al., 2001; Pietri and Brookes, 2008; Zhang et al., 2019). Chloropicrin and 1,3-dichloropropene have been reported to lower soil pH (Cheng et al., 2020), which may negatively impact nitrification rates in the amended soil and result in decreased soil fertility. However, this study showed that none of the fumigants used significantly impacted soil pH, suggesting that the use of these products with the intent to minimize foodborne pathogens in biologically amended soil does not directly negatively impact the primary purpose of the initial amendment. This study indicated that fumigation using 1,3-dichloropropene or dimethyl disulfide, which are labeled for broad-spectrum plant pest management, may also minimize the potential risks associated with *Salmonella* contamination in sandy loam soils.

However, a limitation of this study is that only one soil type was investigated with four sampling time points, thus understanding the reduction of *Salmonella* in fumigate-treated soils with a variety of compositions and moisture contents with more intensive sampling is of interest. Furthermore, similar to previous studies (Gu et al., 2019; Lee et al., 2019; Shah et al., 2019), XLT4 was used to enumerate *Salmonella* populations in soil samples; however, further research with the application of solid agar overlay may benefit the recovery of injured cells (Kang and Siragusa, 1999). Future research is also needed to evaluate the efficacy of other MeBr alternative chemicals or the combination of fumigants, such as both 1,3-dichloropropene and dimethyl disulfide, on the reduction of *Salmonella* in agricultural soil to reduce potential contamination risks.

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Authors' Contributions

G.G.: Data collection, data analysis, article writing, and article revisions. C.M.M.: Article writing. J.Z.: Experimental design and article revisions. X.N.: Article revisions. S.L.R.: Funding, experimental design, data analysis, and article revisions. L.K.S.: Funding, data analysis, article writing, and article revisions.

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No competing financial interests exist.

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