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Effect of pesticide application on *Salmonella* survival on inoculated tomato leaves

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Abstract

Outbreaks of Salmonellosis have been traced to contaminated tomato. The produce production environment poses a risk for *Salmonella* contamination; however, little is known about the effects of pest management practices on *Salmonella* during production. The study objective was to evaluate pesticide application on the inactivation of *Salmonella* on tomato leaves. Thirty greenhouse-grown tomato plants were inoculated with *S. enterica* serovars Newport or Typhimurium. Inoculation was performed by dipping tomato leaves in an 8-log CFU/mL *Salmonella* suspension with 0.025% (vol/vol) Silwet L-77 surfactant for 30 s, for a starting concentration of 6–7 log CFU/mL. Plants were treated with one of four pesticides, each with a different mode of action [acibenzolar-*S*-methyl, copper-hydroxide, peroxyacetic acid (PAA), and streptomycin]. Pesticides were applied at manufacturers' labeled rate for plant disease management with water as a control treatment. *Salmonella* was enumerated at 0.125 (3 h), 2, 6, and 9 days post-inoculation (dpi), and counts log-transformed. Growth of *Salmonella* was not observed. At 2 dpi, PAA and streptomycin significantly reduced surface *Salmonella* concentrations of inoculated tomato leaves (0.7 and 0.6-log CFU/g, respectively; $p \leq 0.05$), while significant *Salmonella* log reduction occurred in the ground tomato leaves after copper hydroxide treatment (0.8-log CFU/g; $p \leq 0.05$), compared to the control. No significant differences in *Salmonella* populations on tomato leaf surface and in ground leaves were observed from 2 to 9 dpi, regardless of pesticide application. These findings suggest single in-field pesticide applications may not be an effective mitigation strategy in limiting potential *Salmonella* contamination. Future research, including multiple in-field pesticide applications, or pesticide use in combination with other mitigation strategies, may offer intriguing management practices to limit possible preharvest contamination.

1 | INTRODUCTION

Tomato bacterial diseases, such as those caused by *Xanthomonas vesicatoria* (bacterial spot), *Clavibacter michiganensis* (bacterial canker), and *Pseudomonas syringae* pv. *tomato* (bacterial speck), poses a major threat to tomato production in the United States and worldwide (Jones, Zitter, Momol, & Miller, 2014). Multiple pesticides, possessing various modes of action, are commercially available and routinely applied to manage bacterial diseases of fresh market tomatoes (Kuhar et al., 2020). Copper hydroxide is a widely used pesticide labeled to suppress levels of several bacterial and fungal diseases in tomatoes. Copper-containing materials are broad spectrum, multi-site, and active on bacterial pathogens through disruption of cellular proteins and enzymes, such as aggregation of ROS-independent protein and inhibition of peptidoglycan LD-transpeptidases (Baena, Marquez, Matres, Botella, & Ventosa, 2006; Mahovic, Gu, & Rideout, 2013; Ritchie, 2004). Acibenzolar-*S*-methyl stimulates the plant's natural defenses through mimicking the natural systemic activated resistance (SAR) response, which allows the host plant to ward off infection from certain bacterial and fungal pathogens (Graves & Alexander, 2002). Peroxyacetic acid (PAA) is advertised to offer broad spectrum bacterial and fungal control of diseases in tomato when field applied to crops (Huang, de Vries, & Chen, 2018). PAA is also used as a surface sanitizer and postharvest water treatment additive to reduce potential cross-contamination in dump tanks/flumes (Mari, Bertolini, & Pratella, 2003; Sargent, Ritenour, Brecht, & Bartz, 2000; Sisquella, Casals, Viñas, Teixidó, & Usall, 2013). Streptomycin is an aminoglycoside antibiotic with antibacterial activity, and is labeled for control of bacterial diseases, especially during greenhouse transplant production (Mahovic et al., 2013).

The use of multiple pesticides in the production environment is a comprehensive approach to quality and integrated pest management (IPM) programs, allowing different modes of action to suppress or eliminate a variety of phytopathogens; thus, also reducing the likelihood of bactericidal resistant plant pathogen strains. This approach attempts to optimize preharvest pesticide use within the context of the microbial diversity of the phyllosphere by promoting the use of pesticides with multiple purposes; leading this study to investigate impacts against foodborne pathogens (Miller, Ferreira, & LeJeune, 2022). This may be of particular importance as some studies have observed plant pathogens to enhance the survival of foodborne pathogens, for example, *Xanthomonas perforans* and *X. campestris*, in production environments (Barak & Liang, 2008; Potnis et al., 2014). However, despite investigation on the efficacy of these commonly applied pesticides for tomato disease prevention, few studies have examined the activity of these pesticides on foodborne pathogen prevention for tomatoes during pre-harvest production.

Between 1990 and 2017, fresh tomatoes were linked to 38 outbreaks in the United States, resulting in 4,028 illnesses and four deaths (Bennett, Littrell, Hill, Mahovic, & Behravesh, 2015; Jackson, Griffin, Cole, Walsh, & Chai, 2013; Krug, Valadez, Chapin, Schneider, & Danyluk, 2020; Lynch, Tauxe, & Hedberg, 2009). Of these 38 fresh tomato outbreaks, *Salmonella* was confirmed as the causative agent in 30, with serovar Newport accounting for 11 of the *Salmonella* tomato-borne outbreaks (Bennett et al., 2015, Jackson et al., 2013, Krug et al., 2020, Lynch et al., 2009). *Salmonella* causes ~1.2 million illnesses, and the most foodborne bacterial hospitalizations and deaths, annually in the United States (Scallan et al., 2011). While traceback investigations may not ultimately identify the initial point source of contamination, it is hypothesized that most of the *Salmonella* Newport outbreaks associated with tomatoes resulted from pre-harvest contamination (Bell et al., 2015; Greene et al., 2008; Gu et al., 2018a, Gu et al., 2018b; Gu, Strawn, Zheng, Reed, & Rideout, 2019; Truitt et al., 2018). For example, in two multistate outbreaks of *Salmonella* Newport (2002 and 2005) from Virginia tomato fruits, the outbreak strain was isolated and genotypically identified using pulse-field gel electrophoresis (PFGE) from pond water that was used to irrigate the tomato fields (Greene et al., 2008). Multiple studies have investigated produce contamination pathways in the pre-harvest environment, including through biological soil amendments of animal origin (BSAAO), irrigation water, domestic and wild animals, and pesticide applications (Bell et al., 2015; Danyluk et al., 2008; Gorski et al., 2011; Gruszynski et al., 2014; Gu et al., 2018a, Gu et al., 2018b; Gu et al., 2019; Lopez-Velasco, Tomas-Callejas, Diribsa, Wei, & Suslow, 2013; Micallef et al., 2012; Stine, Song, Choi, & Gerba, 2011; Zheng et al., 2013). For instance, one study (Gu et al., 2018a, Gu et al., 2018b) observed the likelihood of *Salmonella* contamination on tomato leaves was significantly higher than on the tomato fruit in sampled fields. Another set of studies (Bolten et al., 2020; Soto, Chavez, Baez, Martinez, & Chaidez, 2007) found the adjacent leaves and debris could be the main cross-contamination source during harvesting and post-harvest handling; thus, inactivating or reducing *Salmonella* on tomato leaves may be a management practice to reduce contamination downstream. Studies have also observed *Salmonella* can survive and even grow in water containing commercial pesticides and fungicides labeled for tomato production (Danyluk et al., 2008; Gorski et al., 2011; Gu et al., 2019; Jones et al., 2014). While previous studies have evaluated the efficacy of copper, chlorine, and peracetic acid on *Salmonella* mitigation on fresh produce during post-harvest handling and processing (Bolten et al., 2020; Rahn et al., 1992; Silveira et al., 2018; Soto et al., 2007; Zaengle-Barone et al., 2018), little research has examined the effects of pesticides on pre-harvest applications. In addition, washing with commonly used sanitizers (i.e., antimicrobial pesticides) could not eliminate *Salmonella* on inoculated tomatoes, and the primary function of sanitizer in wash water was to minimize cross-contamination during the washing stage (Bolten et al., 2020; Soto et al., 2007). Given that the available post-harvest processing intervention strategies cannot sufficiently be relied on to mitigate *Salmonella* contamination risks, the prioritization of strategies that minimize contamination during pre-harvest (i.e., production) are imperative. However, knowledge of the evaluation of different pre-harvest pesticide applications on *Salmonella*-contaminated tomato plants is still limited. Thus, the objective of this study was to investigate the impact of four commercial pesticides (each with a different mode of action) on *Salmonella* serovar Newport and Typhimurium concentrations on tomato leaves.

2 | MATERIALS AND METHODS

2.1 | *Salmonella* preparation

Salmonella serovar Newport strain J1892, isolated from a previous tomato-borne *Salmonella* outbreak, was originally obtained from the US Centers for Disease Control and Prevention (CDC; Atlanta, GA). *Salmonella* serovar Typhimurium strain ATCC 14028 was obtained from the American Type Culture Collection (ATCC; Manassas, VA).

Both bacterial cultures were stored in Luria-Bertani broth (LB; Thermo Fisher Scientific, Waltham, MA) containing 20% glycerol at −80°C. Prior to each experiment, bacterial cultures were re-inoculated into LB broth and incubated at 37°C. After overnight growth, the cultures were harvested by centrifugation at 1,750 × *g* for 15 min at 22°C. To reach the desired initial bacterial concentration of 8 log CFU/mL, bacterial pellets were re-suspended in 100 mL of phosphate buffered saline (PBS; Thermo Fisher Scientific) to an optical density (600 nm) of 0.3.

2.2 | Tomato plant growth

Red round tomato seeds of the cultivar "BHN602" (BHN Seed, Immokalee, FL) were sowed into 128-cell Styrofoam plug trays (Speedling Inc., Sun City, FL) containing Premier Pro-mix HP (Premier Tech Horticulture, Quakertown, PA). Approximately one-month post-seeding, seedlings were transplanted into 30-cm diameter pots containing sandy loam soil collected from agricultural fields at Virginia Tech's Eastern Shore Agricultural Research and Extension Center (ESAREC; Painter, VA). Transplanted tomato plants were maintained in a BSL-2 greenhouse at the ESAREC. Air temperature in the greenhouse during experiments ranged from 23 to 33°C, with an average temperature of 28°C. Water was applied manually to the pots at 2 days intervals and fertilization was applied every 2 weeks using Miracle-Gro Water Soluble Plant Food (The Scotts Company LLC, Marysville, OH). No additional lighting was provided in the greenhouse.

2.3 | *Salmonella* inoculation and pesticide applications

The experimental setup is schematically summarized in Figure 1. Inoculation was accomplished 7 weeks after tomato transplanting by dipping three leaflets from each of four branches per plant into an 8 log CFU/mL *Salmonella* suspension with 0.025% (vol/vol) Silwet L-77 surfactant (Momentive Performance Materials, Inc., Waterford, NY) for 30 s, as described in a prior study (Gu, Cevallos-Cevallos, Vallad, & van Bruggen, 2013). Three of the six plants (about 50–80 cm tall) were inoculated with *S. enterica* serovar Newport and the other three with *S. enterica* serovar Typhimurium. Sterile tap water with 0.025% (vol/vol) Silwet L-77 was used as a control. After dip inoculation, leaves were left to air dry until pesticide application (24 h). Two independent experiments were performed in triplicate in a completely randomized design in a BSL-2 greenhouse ($N = 6$).

One day after inoculation, pesticides were applied to tomato plants. Pesticides evaluated for this study included acibenzolar-*S*-methyl (the active ingredient in Actigard 50WG; Syngenta Crop Protection, LLC, Greensboro, NC), PAA (OxiDate 2.0 L; BioSafe Systems, LLC, East Hartford, CT), copper hydroxide (Kocide 3000 46WG: Certis USA, LLC; Columbia, MD), and streptomycin sulfate (Firewall 17WP: AgroSource, Inc., Tequesta, FL). Pesticides were applied to tomato plants at their maximum allowed application rate according to the labels. Formulated pesticides were obtained commercially and mixed into sterile DI water. For each treatment, six plants were sprayed using a 710 mL spray bottle (Gempler's Farm & Home Supply Co., Janesville, WI) containing 470 mL and calculated amount of the pesticide to simulate a grower spray output of 935 L/ha (Actigard 50WG @ 27 mg/L, Kocide 3000 46WG @ 985 mg/L, OxiDate 2.0 L @ 2.5% (vol/vol), and Firewall 17WP @ 200 mg/L). Water without pesticides was applied as a control.

2.4 | Tomato leaf sampling and *Salmonella* detection

Treated tomato leaflets were sampled at 0.125 (0 day after pesticide application), 2 (1 day after pesticide application), 6 (5 days after pesticide application), and 9 days (8 days after pesticide application) after leaflet inoculation. At each sampling time, three inoculated leaflets were removed from each of the three plants of each treatment. Four 12-mm leaf discs were taken with a sterile cork-borer from each inoculated leaflet, and weighed (g). Two of the four leaf discs were dipped in 1 mL sterile water with 0.025% (vol/vol) Silwet L-77 and sonicated in FS20 Ultrasonic Cleaner (Thermo Fisher Scientific) for 15 min to collect *Salmonella* cells on the surface of inoculated leaves. The other two leaf discs were surface disinfected by dipping the discs in 70% alcohol for 20 s, rinsing three times with sterile distilled water, and ground in 1 mL PBS using sterile micro pestles, as previously described (Gu et al., 2013). The rinsate from sonicated leaf discs and extract of surface disinfected leaf discs were diluted in a 10-fold series in PBS. Aliquots (100 μL) of the appropriate dilutions were spread onto Xylose Lysine Tergitol-4 agar (XLT-4; BD Biosciences, Franklin Lakes, NJ) and incubated at 37°C for 24 h. *Salmonella* colonies were enumerated using the Neutec Flash & Go automated colony counter (Neutec Group Inc., Farmingdale, NY). *Salmonella* concentrations were determined and expressed in log CFU/mL or log CFU/g, where appropriate. Up to three colonies from each plate were re-streaked on XLT-4 for PCR confirmation targeting the *invA* gene, as previously described (Rahn et al., 1992).

2.5 | Statistical analysis

In each of the two trials, a total of 36 tomato plants were tested with six replicates per pesticide treatment (Figure 1). *Salmonella* concentration in the rinsate was used to estimate *Salmonella* on the surface of inoculated leaves and the concentration enumerated from the surface disinfected leaf discs was used to estimate *Salmonella* inside inoculated leaves. Pesticide treatment impact on *Salmonella* concentration was calculated as the log reduction using the equation: log reduction $= \log_{10} (CFU_{control}/CFU_{treatment})$. Effects of pesticide application among treatments were analyzed by analysis of variance (ANOVA) of the log reduction. The decline rate (slope) and intercept of *Salmonella* concentration densities on and in inoculated tomato

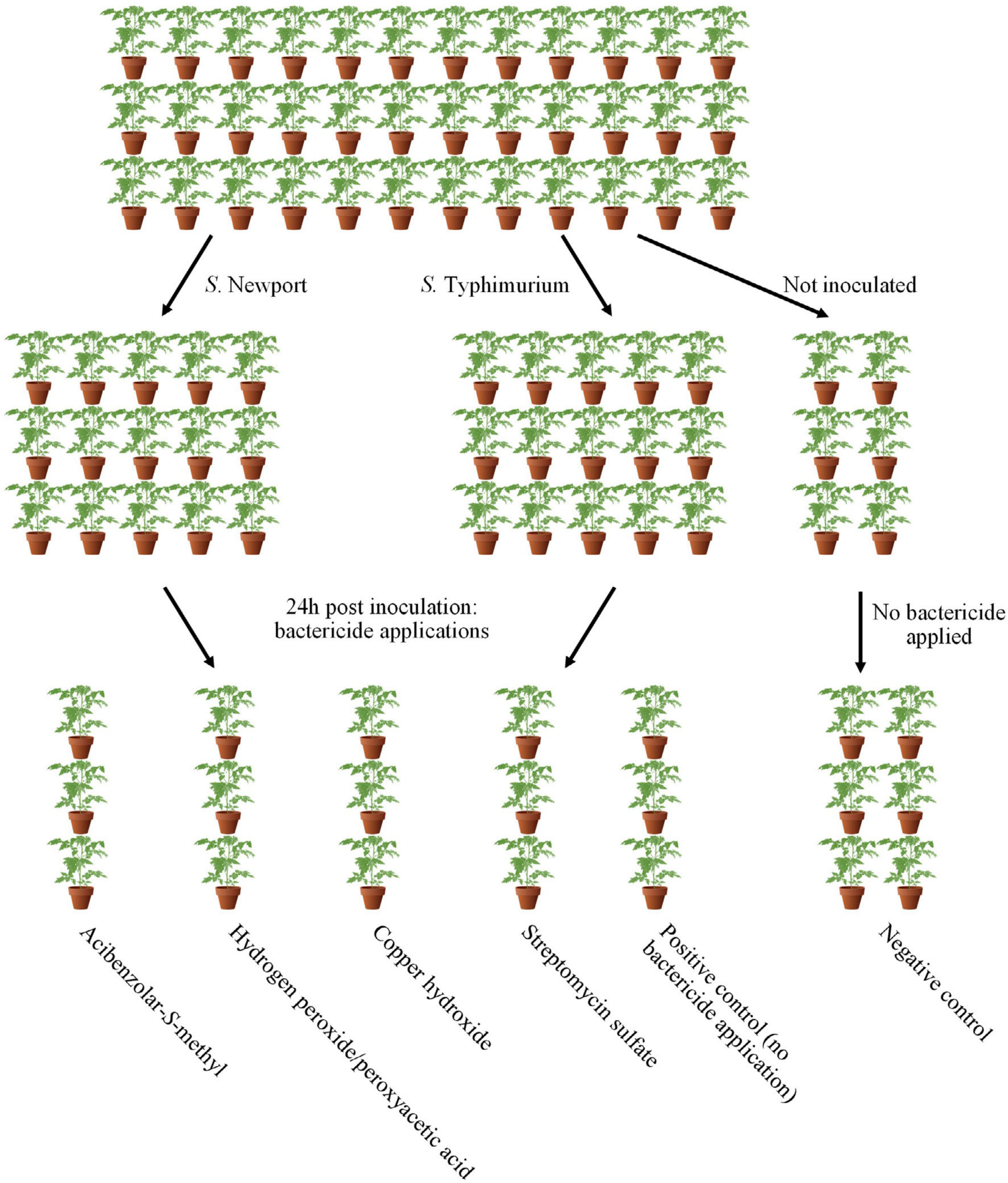

FIGURE 1 Schematic presentation of experimental design on *Salmonella enterica* inoculation and pesticide application on tomato leaves. Two independent experiments were performed in triplicate ($n = 6$). For each treatment, 470 mL pesticide was applied with the maximum label concentration of Actigard 50WG at 27 mg/L, Kocide 3000 46WG at 985 mg/L, OxiDate 2.0 L at 2.5% (vol/vol), and Firewall 17WP at 200 mg/L. Water without pesticides was applied as control.

leaves with pesticide treatment were each compared to the control by fitting log-transformed data (separately for each replication) to the linear model as described previously (Kuhar et al., 2020). Estimated values of parameters were subjected to multivariate analysis of variance (MANOVA). Statistical analyses (ANOVA, MANOVA, and linear regression) were performed using SAS (SAS release 9.2, SAS Institute Inc., Cary, NC), and differences were considered significant at $p \leq 0.05$.

3 | RESULTS AND DISCUSSION

Previous studies indicated that *Salmonella* persistence or growth on and in tomato plants may vary by *Salmonella* serovar (Shi, Namvar, Kostrzynska, Hora, & Warriner, 2007; Zheng et al., 2013). However, for the two serovars (Newport and Typhimurium) evaluated in the study reported here, *Salmonella* concentration in leaf rinsate and ground tomato leaves (after surface disinfection) were not

TABLE 1 Log reduction (mean ± SE) of *Salmonella enterica* 24 h (1 day) after pesticide application in rinse water (i.e., rinsate) of inoculated leaves and after surface disinfection (with 70% ethanol) and grounding of inoculated leaves.

Treatment	Reduction in rinse water (log CFU/mL)	Reduction after surface disinfection (log CFU/g)
Control	0.00 ± 0.07c*	0.00 ± 0.20bc
Acibenzolar-*S*-methyl	0.15 ± 0.11bc	−0.2 ± 0.10c
Copper hydroxide	0.41 ± 0.12abc	0.79 ± 0.16a
Peroxyacetic acid	0.70 ± 0.24a	0.24 ± 0.16b
Streptomycin sulfate	0.60 ± 0.06ab	0.14 ± 0.09bc

*Letters in each column denote the significance levels among pesticide treatments (p < 0.05).

significantly different ($p > 0.05$) at each sampling point; thus, data were grouped for analyses.

As expected, 1 day after PAA and streptomycin application, the log reduction of *Salmonella* concentrations in leaf rinsate was significantly greater than the inoculated control samples without pesticide application ($p \leq 0.05$) (Table 1). The antibacterial effects of PAA on *Salmonella* have been well-documented for produce postharvest washing (Singh, Kim, Shepherd, Luo, & Jiang, 2011; Yuk, Bartz, & Schneider, 2006); however, the success of streptomycin against *Salmonella* has primarily been limited to a medical treatment strategy as a therapeutic antibiotic for Salmonellosis (Bohnhoff, Drake, & Miller, 1954; Kaiser, Diard, Stecher, & Hardt, 2012; Seligmann, Barash, & Cohlan, 1947). In comparison to *Salmonella* concentrations on tomato leaves, PAA and streptomycin treatments had no effect on *Salmonella* concentrations in ground leaves (Table 1). While PAA does not penetrate plant tissue, it has been shown to exhibit some phytotoxicity in hydroponic tomato operations at 0.5–5 mg/L (Vines, Jenkins, Foyer, French, & Scott, 2003). These effects were not observed in this study, suggesting that its use as a foliar application is less damaging to the plant; however, the lack of reduction in *Salmonella* concentrations in the ground leaves support PAA may be more optimally utilized in surface washing or multi-hurdle approaches (Huang et al., 2018; Lippman, Yao, Huang, & Chen, 2020). In contrast to PAA, streptomycin is considered a partially systemic pesticide (McManus & Stockwell, 2000). The isolation of streptomycin-resistant *Salmonella* from fresh produce and meat products at retail has been increasingly reported (Abatcha, Effarizah, & Rusul, 2018; Peng et al., 2016; Whichard et al., 2010); although, the lack of streptomycin-resistant *Salmonella* has also been reported in pre-harvest environments (Peng et al., 2016). While this study's observation of significant log reductions in *Salmonella* concentrations on tomato leaves was promising, additional research is needed to evaluate multiple pesticide applications at different time-points during tomato pre-harvest production.

Acibenzolar-*S*-methyl treatment did not significantly reduce *Salmonella* concentrations in leaf rinsate and ground tomato leaves 1 day after application, compared with the control (Table 1; $p > 0.05$). Acibenzolar-*S*-methyl has been reported to be effective in managing plant bacterial diseases by stimulating plant defense responses (Takeshita et al., 2013); although, the findings reported here suggest acibenzolar-*S*-methyl alone did not significantly reduce *Salmonella* concentrations on or in leaf tissue. This is supported by a previous study of acibenzolar-*S*-methyl's impact on tomato phyllosphere microflora in Virginia (Ottesen et al., 2015); as well as, additional research that showed the use of systemic acquired resistance (SAR) stimulating chemicals were ineffective at preventing *Salmonella* colonization of tomato leaf tissue (Phannareth, 2015). However, a recent study observed the use of acibenzolar-*S*-methyl as a priming agent could prevent internalized colonization of fresh produce by *Salmonella* (Chalupowicz et al., 2021). Future studies may investigate acibenzolar-*S*-methyl as an intervention to minimize active internalization using a syringe or vacuum inoculation methods, versus passive internalization methods (as the study here).

A previous study indicated that copper has antibacterial effects against *Salmonella* (Zhu, Elguindi, Rensing, & Ravishankar, 2012); however, in the study reported here, the application of copper hydroxide did not result in a significant reduction of *Salmonella* concentrations on leaf surfaces (Table 1). This finding is supported by existing literature evaluating the use of copper-based compounds against epiphytic *Salmonella* populations (Mahovic et al., 2013; Ottesen et al., 2015). In contrast, *Salmonella* concentration had a significant log reduction in the ground leaves after copper hydroxide treatment, compared to the control (0.79 ± 0.16, $p \leq 0.05$). Copper ions (Cu^{2+}) may be able to enter tomato leaves and suppress *Salmonella* inside leaves (Bain, 1902), but the actual mechanism of suppression needs to be investigated further. Additionally, further research should determine copper hydroxide's impact on development of resistance in agricultural systems (Wightwick, Reichman, Menzies, & Allinson, 2013; Yu, Wang, Shen, Fang, & Yu, 2022).

Salmonella populations decreased on the surface and in ground inoculated tomato leaves up to 8 days after treatment applications (Figure 2 and Table S1). The linear model used to describe survival of *Salmonella* in leaf rinsate and ground leaf samples explained 90.5% and 89.5% of the observed variation, respectively ($R^2 = 0.905 \pm 0.470$ and 0.895 ± 0.350). Among all treatments, *Salmonella* rates of decline in leaf rinsate and grounded tomato leaves were less than 0.2 and 0.4 log/day, respectively, which were not significantly different, compared to the control ($p > 0.05$). The initial *Salmonella* concentrations after inoculation (0.125 days/3 h) in leaf rinsate and ground tomato leaves were 6.87 ± 0.14 log CFU/mL and 6.35 ± 0.13 log CFU/g, respectively (Figure 2). *Salmonella* concentrations were reduced by up to 2.81 log CFU/mL on the surface, and up to 1.54 log CFU/g in tomato leaves throughout the experiment (Figure 2 and Table S1). This finding was similar to a prior study (Zhao, Silva, Van der Linden, Franco, & Uyttendaele, 2021) that also observed reductions in *Salmonella* on spinach plant and leaf tissues after the use of a biological control agent (*Bacillus thuringiensis*). The results reported here, suggest that a single pesticide application does not eliminate or significantly reduce *Salmonella* concentrations on the surface of or in ground tomato leaves. However, *Salmonella* concentrations did not increase on the surface of or in ground tomato leaves after pesticide

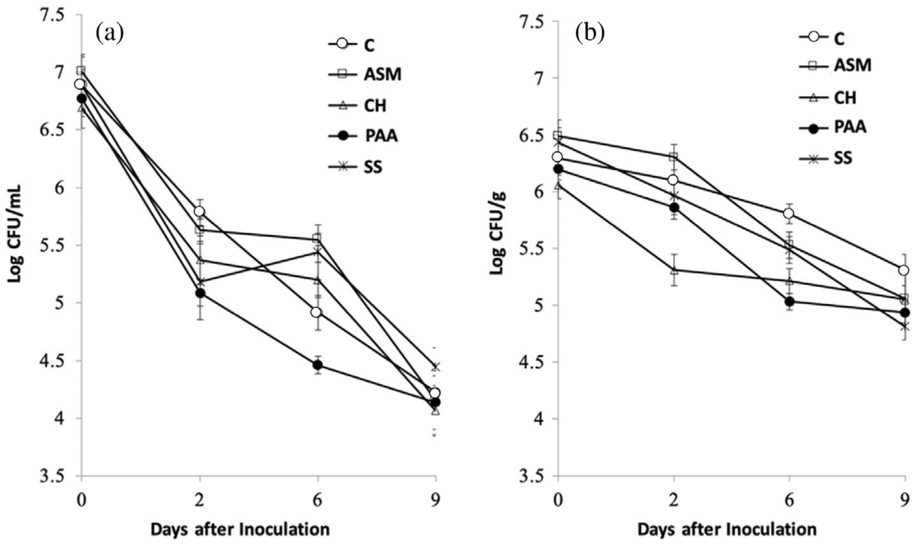

FIGURE 2 Survival (mean ± SE) of *Salmonella enterica* after pesticide application (control [C], acibenzolar-*S*-methyl [ASM], copper hydroxide [CH], peroxyacetic acid [PAA], streptomycin sulfate [SS]) in (a) leaf rinsate and (b) ground leaves.

application, for up to 8 days. These results suggest that the use of pre-harvest foliar pesticide applications does not increase food safety risks associated with *Salmonella* contamination of tomato plants when foliar pesticides are made using water that is not considered high risk (e.g., surface water). Future studies are needed to examine the effect of multiple applications of a single pesticide, or in combination as a multi-hurdle approach, for reducing potential *Salmonella* contamination, as it is common practice in IPM programs to apply multiple applications of a pesticide, or multiple pesticides with different modes of action during production.

4 | CONCLUSIONS

This study investigated the effects of commercial pesticides labeled for tomato production on the reduction of *Salmonella* concentrations on the surface of, and in ground inoculated tomato leaves. This study addressed a central question that agricultural industry personnel have queried regarding the efficacy of using existing pesticide applications, as part of IPM programs, for pre-harvest food safety. PAA and streptomycin significantly reduced *Salmonella* concentrations on the surface of inoculated tomato leaves 1 day after treatment, while copper hydroxide significantly reduced *Salmonella* concentrations in the ground tomato leaves, compared to the control. The use of a single application of acibenzolar-*S*-methyl did not reduce *Salmonella* concentrations in leaf rinsate and ground tomato leaves within 24 h post-treatment. These findings suggest that while some applications of pesticides (e.g., PAA, streptomycin) resulted in reductions of *Salmonella* on tomato leaves, a single in-field pesticide application early in tomato plant production was not an effective intervention for mitigating *Salmonella* contamination in the study reported here. *Salmonella* did not grow on the surface of, or in ground tomato leaves after pesticide application during the 8-days study duration. However, there are some limitations of the study presented here. However, the study presented here inoculated samples at one level (6–7 log CFU/mL) of *Salmonella*, further research investigating various levels is needed to investigate other contamination scenarios. Additionally, this research was a laboratory study, and may not precisely replicate field conditions; as well as, only a single application of pesticides was applied over the study. Therefore, future research, including multiple in-field pesticide applications or pesticides used in combination with other technologies or management strategies (e.g., cropping schemes), might offer intriguing pre-harvest contamination preventions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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