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ORIGINAL ARTICLE

Effect of surrounding vegetation on microbial survival or die-off on watermelon surface in an agriculture setting

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Abstract

Preharvest contamination of produce with food borne pathogens has been a major food safety issue. In this study, we investigated the effect of surrounding vegetation on the survival of natural and inoculated generic *Escherichia coli* on watermelon rinds in an agricultural field setting. There was no significant difference ($p > .05$) on the populations of natural generic *E. coli* (1–1.46 log Most Probable Number (MPN)/sample) and coliforms (<3.99 log CFU/cm^2) on watermelons harvested from low, medium, and high levels of vegetation. However, the survival rate of generic *E. coli* inoculated on watermelon rind discs was variable with the level of vegetation. A significant reduction in generic *E. coli* count was observed within 12 hr at all vegetation levels. After 108 hr, discs placed at low vegetation level had a highest die-off reduction (3 log Colony Forming Units (CFU)/cm^2) compared to medium and high vegetation levels.

Practical applications

To ensure preharvest produce safety, the Food Safety Modernization Act (FSMA) produce safety rule has suggested a time interval between last irrigation and harvest for potentially contaminating microorganisms to die-off. However, a knowledge gap exists regarding the influence of surrounding vegetation on microbial die-off rates on produce in the agricultural field. The findings of this study emphasize the importance of considering the surrounding vegetation while making decisions for developing preharvest risk management strategies based on microbial die-off rate calculations.

1 | INTRODUCTION

Fruit and vegetable crops have the potential to be contaminated with pathogenic microorganisms in the field. As fresh produce is often consumed raw, a higher risk of foodborne illness is posed with this food group. The number of foodborne illness outbreaks associated with the consumption of fresh fruits and vegetables has noticeably increased in the last decade (Huang & Chen, 2011; Olaimat & Holley, 2012). During 1973–2014, fruit and vegetable crops were the most commonly implicated commodities for several foodborne illness outbreaks (Crowe, Mahon, Vieira, & Gould, 2015; Herman, Hall, & Gould, 2015; Nguyen et al., 2015). Preharvest contamination of produce is commonly originated from the soil, inadequately composted manure,

contaminated irrigation water, and improper human handling of produce (Annous, Solomon, Cooke, & Burke, 2005; Tomas-Callejas et al., 2011). The intrusion of crops by wild animals, birds, reptiles, and rodents, as well as insects and nematodes, act as a vector for transferring various pathogens (Brandl, 2006). The survival and growth of microorganisms is influenced by several environmental factors and agricultural practices such as, exposure to solar UV radiation, temperature changes, humidity, and poor fertilizer regimes (Bezanson et al., 2012; Brandl, 2006; Nyeleti, Cogan, & Humphrey, 2004; Tomas-Callejas et al., 2011; Weller et al., 2017).

Several studies reported that the sunlight of tropical latitudes (Davies & Evison, 1991; Nyeleti, Cogan, & Humphrey, 2004; Obiri-Danso, Paul, & Jones, 2001) and a concomitant increase in the surface

temperature of produce (Tomas-Callejas et al., 2011) have an inhibitory effect against various microbial pathogens. Moreover, sunlight is found to reduce *Salmonella* levels in fresh water sources (Davies & Evison, 1991) and on food contact surfaces such as stainless steel (Nyeleti et al., 2004). Furthermore, microbial populations decline with decreasing nutrient availability because of failing to lower their metabolic rate to adopt the starvation condition (Fontaine, Mariotti, & Abbadie, 2003). All these factors contribute to the natural decline of microbial populations on produce surfaces in the field and should be considered for microbial die-off rate calculations (Davies & Evison, 1991; Reddy, Khaleel, & Overcash, 1981).

To minimize preharvest microbial food safety risk originated from contaminated irrigation water, the FDA Food Safety Modernization Act (FSMA) Produce Safety Rule requires agricultural water used for covered produce, must be of safe and adequate sanitary quality (USFDA, 2015). It should also meet the generic *Escherichia coli* requirements as proposed in the U.S. Environmental Protection Agency's 2012 Recreational Water Quality Criteria. However, the rule provides flexibility to the growers that are not able to meet the microbial water quality criteria initially by extending the time interval between the last irrigation and first harvest to allow for microbes to naturally die (USFDA, 2015). Research on the surface of produce highlights the effect of environmental factors on the die-off rate of microorganisms (Wood, Bezanson, Gordon, & Jamieson, 2010). However, the level of exposure to environmental stressors, in particular, solar UV radiation on the surface of produce may be influenced by growing practices. Presence of weed and/or the surrounding vegetation from the plant may cover fruit surfaces, thus preventing the exposure of the contaminated surface to the natural sunlight. As per our knowledge, the effects of surrounding vegetation have not been considered while calculating microbial die-off rates on produce such as melons. This is especially imperative as melons grow in direct contact with the soil with surrounding vegetation making them at a higher risk of microbial contamination. Several recent outbreaks have been attributed to microbial contamination of melon crops (McCollum et al., 2013). During 1973–2011, watermelon, cantaloupe, and honeydew melons were responsible for 34 food borne disease outbreaks (Walsh, Bennett, Mahovic, & Gould, 2014). Investigating the effect of surrounding vegetation on the microbial die-off rate would help generate data for on-farm food safety risk assessments. Thus, the objective of this study was to determine the effect of surrounding vegetation (weeds and vines) on the survival or die-off rate of generic *E. coli* on watermelon rind surfaces in an agricultural setting.

2 | MATERIALS AND METHODS

2.1 | Experimental overview

The present study was conducted on July and August at the Louisiana State University Agriculture Center (LSU AgCenter) Botanic Gardens in Baton Rouge, LA. The test field was divided into three blocks, and each block contained six plots (12×30 ft^2). Eighteen total plots were tested. Plots were initially treated with one of the following five preemergent herbicides or untreated control. The herbicide treatments were applied 24 hr prior to transplanting. Preemergent herbicide

treatments included Strategy 5 pts/acre (Ethalfluralin & Clomazone), Command 3ME 0.67 pts/acre (Clomazone), Strategy 5 pts/acre plus Sinbar 4 oz/acre, Valor 1 oz/acre (Flumioxazin), Sinbar 4 oz/acre (Terbacil), and an untreated control. Preemergent herbicides were applied using a CO_2 backpack sprayer delivering 15 gal/acre. Twenty-four hours after herbicide application, "Legacy' watermelon seedlings were transplanted. Additional herbicides, Sandea (0.67 oz/acre) and Prowl (1 qt/acre), were applied to the row middles as a lay-by application 14 days after planting. Overhead irrigation was applied to establish plants, until flowering period. Eighty days after initial preemergent herbicide application, multiple types of vegetation (weeds) were observed in each plot. Dominant vegetation (weeds) included Yellow nutsedge (*Cyperus esculentus*), Goosegrass (*Eleusine indica*), Large Crabgrass (*Digitari sanguinalis*), and Barn yard grass (*Echinochloa crusgalli*). The surrounding vegetation (weeds plus vine) in each plot was evaluated using a 0–10 scale, where "0" represented "no vegetation," "5" represented 50% plot coverage in vegetation, and "10" represented completely or 100% coverage of the plot with vegetation (Figure 4). Using this scale, we developed three categories of surrounding vegetation: (a) low level (0–3), (b) medium level (4–6), and (c) high level (7–10). Some plots had fewer numbers of fruits, so the sample collection was based on the surrounding vegetation rather than the plot, it was grown. Our preliminary studies revealed that there was no significant effect of herbicides on the level of naturally coliform and generic *E. coli* on watermelon surface.

A total of 80 watermelons, 30 each from low and medium surrounding vegetation and 20 from high surrounding vegetation (Strategy, Valor, Strategy plus Sinbar, Sinbar & Command: 14 each and Untreated: 10), were harvested from the test plots. We were unable to gather 30 melons from the high vegetation (weed) test areas because the overwhelming presence of weeds made it difficult for watermelon vines to flower and fruit. Each melon was carefully removed from the field using disposable gloves. Gloves were changed with each harvested melon. Melons were labeled with the plot number and the level of surrounding vegetation. The dimensions of each watermelon (i.e., length and width) and fresh weight were recorded to calculate the bacterial count per centimeter square. The harvested melons were then aseptically transferred into a sterile polythene bags (48.3×58.4 cm^2) and immediately transported to the food safety laboratory at 4 °C. After receiving the samples at the lab, a 200 ml of 0.1% peptone water was added to each watermelon bag and was hand massaged intensively for 5 min to dislodge the microorganisms from the melon surface. In our preliminary study, we observed a low number of generic *E. coli* on watermelon surface. Thus, to reduce the dilution level, we used only 200 ml 0.1% peptone water but initiated a longer massaging time. The eluent was then used for the microbial analysis.

2.2 | Testing natural coliform, generic *E. coli* levels and the bacterial pathogens on the watermelon surface

Quanti-Tray 2000-Colilert (IDEXX Laboratories, Portland, ME) and Petrifilm EC plates (3 M Microbiology Products Co, St. Paul, MN) were used to enumerate generic *E. coli* and coliform levels on the watermelon rind surface, respectively. The Quanti-Tray method was used

to enumerate the generic *E. coli* levels because of its lower detection limit (0.30 log MPN/sample) than petrifilm and VRBA plating methods. Briefly, each eluent sample (100 ml) obtained from hand massaging was poured into a sterile plastic container containing Quanti-Tray reagent powder. Contents were thoroughly mixed by gentle agitation and then poured into Quanti-Trays. The trays were sealed using a heat sealer (Quanti-Tray-2X, IDEXX Laboratories) and incubated at 35 ± 0.2 °C for 24 hr. The colors of the wells were compared with a comparator provided by IDEXX laboratories, and the number of wells showing fluorescence under a UV lamp (WL200, Hanovia LTD, Aquionics, United Kingdom) was recorded as generic *E. coli* positive samples. The results were expressed in terms of MPN using a chart provided by IDEXX Laboratories. The enumeration of coliforms on the watermelon rind was done by using 3 M Petrifilm, and the results were expressed in CFU. This is because coliforms were present at higher numbers and the dilution used to detect generic *E. coli* resulted in all wells positive for coliforms. Each sample was also analyzed for bacterial pathogens (*E. coli* O157:H7 and *Salmonella* spp.) using immunomagnetic separation (BeadRetriever, Thermo Fisher Scientific, Waltham, MA) technique followed by spread plating on selective media. However, we did not detect the presence of *E. coli* O157:H7 or *Salmonella* spp. on the watermelon surface.

2.3 | Watermelon disc preparation and inoculation with generic *E. coli*

To bring uniform light exposure on the surface of watermelons and better understand the effect of surrounding vegetation on the die-off rate of bacteria, studies were also conducted by artificially inoculating watermelon discs with generic *E. coli*. Subsequently, these inoculated discs were exposed to natural sunlight in the field under different levels of surrounding vegetation. Briefly, fresh watermelons grown in our test plots were first washed with sterile deionized water and air-dried inside a biological safety cabinet for 1 hr at room temperature. A total of 63 watermelon discs (surface area of 20 cm² and thickness of 0.5 cm) were prepared using sterile stainless-steel knives by coring off the edible portion. The watermelon discs were placed on sterile petri-dishes with the outer epidermal surface facing up.

A cocktail of three generic *E. coli* strains (ATCC 23716, 25922, & 11775) were used in this study. These strains are among the few well-characterized surrogates for use in field trials (Harris et al., 2012). Frozen cultures were activated in three successive passes by following the procedure described by Adhikari, Syamaladevi, Killinger, and Sablani (2015). The final inoculum size was 10^9 CFU/ml. The bacterial cocktail was agitated 25 times in a 30 cm arc to ensure thorough mixing. The surface of the discs placed in sterile petri-dishes was spot inoculated with 50 µl inoculum distributed into 15 small droplets. The inoculated discs were dried inside the biological safety cabinet for 12 hr.

2.4 | Enumeration of inoculated generic *E. coli* on watermelon rind disc

After harvesting all the watermelons, three plots were selected representing one for each level of surrounding vegetation (low, medium,

and high). Inoculated watermelon discs kept on sterile petri plates (n = 21) were placed randomly around in each level of surrounding vegetation plot (Figure 4). Samples of three watermelon discs from each plot were collected at 0, 12, 36, 60, 84, and 108 hr (at 6 p.m.). The discs were stored in an ice chest and transported to the laboratory maintaining 4 °C. Each disc was placed into a sterile stomacher bag containing 90 ml of 0.1% peptone water and hand-massaged for 1 min followed by blending in a stomacher (BagMixer 400S, Interscience, Woburn, MA) for 5 min. The eluent was used for the enumeration of the generic *E. coli* by plating on Violet Red Bile Agar (Criterion, Bio-Rad Laboratories, Inc, Hercules, CA) (with detection limit: 1.65 log CFU/cm²) and incubated at 37 °C for 24 hr.

2.5 | Statistical analysis

Data were analyzed using PROC MIXED feature of SAS 9.4 (SAS Institute, Cary, NC). A significant difference ($p \le .05$) among the mean values of the bacterial count from different vegetation levels was determined using Fisher's Least Significant Difference (LSD) test.

3 | RESULTS AND DISCUSSION

3.1 | Level of natural coliforms and generic *E. coli* on watermelons surrounded by different levels of vegetation

The generic *E. coli* count on the watermelon collected from three different vegetation levels, that is, low (1 log MPN/sample), medium (1.46 log MPN/sample), and high (1.23 log MPN/sample) is shown in Figure 1. The results did not show a significant difference ($p > .05$) in generic *E. coli* count among three different levels of vegetation. Similar results were observed for total coliforms. The coliform counts were 3.7, 3.66, and 3.91 log CFU/cm² on low, medium, and high vegetation levels, respectively (Figure 2). Under the tested conditions, the results of the current study indicate that generic *E. coli* and total coliforms were naturally prevalent (although at low levels) on the surface of watermelons. However, the level of vegetation surrounding the watermelons did not show a significant effect ($p > .05$) on the bacterial count.

Several studies reported a higher level (>5 log CFU/g) of microbial count on fresh produce such as cantaloupe, lettuce, and broccoli (Johnston et al., 2005; Liu, Tan, Yang, & Wang, 2017; Zhang & Yang, 2017). The aerobic mesophilic count was more than 3 log CFU/g in the fresh cut-honeydew melons (Chong, Lai, & Yang, 2015), while the total coliform level on the cantaloupe rinds was 2.4 log CFU/g (Gagliardi, Millner, Lester, & Ingram, 2003). The level of *E. coli* varied with the types of the fresh produce, with higher count (geometric mean of 1.5 log CFU/g) in cantaloupe (Johnston et al., 2005). Preharvest farming practices significantly impact the microbial contamination on fresh produce. Studies reported that biological soil amendments of animal origin and untreated surface water are important sources of generic *E. coli* contamination in the farm (Annous et al., 2005; Tomas-Callejas et al., 2011). In our study, the low prevalence of generic *E. coli* on the watermelons at the time of harvest can be attributed to the
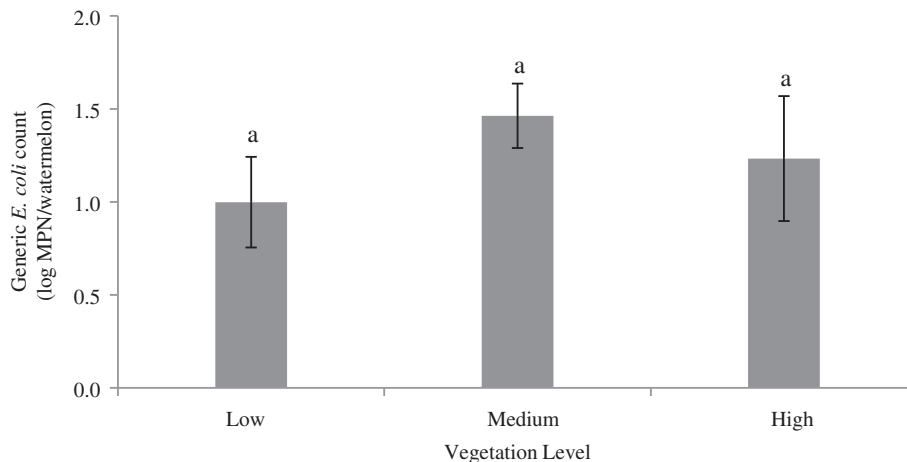
FIGURE 1 Generic *E. coli* levels on watermelon surface harvested from low, medium, and high level of surrounding vegetation plots. The level of surrounding vegetation was converted to numeric value (0–10); 0 meant for no vegetation and 10 meant for highest amount of vegetation. Categorically, those plots with 0–3 were considered as low vegetation plots, 4–6 as medium level of vegetation plots, and 7–10 as high level of plots. The detection limit was 0.30 log MPN/sample. Data are presented as means ± standard deviation. Means with same small case letters are not significantly different

factors such as following effective weed management strategies, avoiding the use of untreated soil amendments as fertilizers, and untreated surface water for irrigation. The municipal water of sound microbiological quality used in the current study for the irrigation of watermelon plots helped to minimize irrigation water-related contamination. Furthermore, the watermelons on the test plots were harvested 80 days after initial transplant. The prolonged exposure to natural sunlight and other environmental stress conditions may be associated with the lower levels of naturally present coliforms and generic *E. coli* on watermelon surface. These results emphasize the importance of following good agricultural practices (GAPs) to mitigate the risk associated with the microbial contamination of produce in a farm environment. However, due to the low prevalence of naturally present generic *E. coli* on the surface of watermelons under the tested conditions, the effect of surrounding vegetation on the microbial die-off rate is not fully understood. This information is critical when the

grower does not meet the water quality requirements of FSMA produce safety rule and solely relies upon on the farm microbial die-off to ensure the safety of harvestable produce. To understand the effect of surrounding vegetation on the microbial die-off rate at high microbial loads, a simulated on-farm study was conducted by artificially inoculating watermelon rind.

3.2 | Effect of surrounding vegetation on the die-off rate of generic *E. coli* on watermelon rind disc

The die-off rate of generic *E. coli* on the watermelon discs under three different levels of vegetation is shown in Figure 3. At all three levels of surrounding vegetation, a significant reduction in the generic *E. coli* was observed from 0 to 12 hr. The highest reduction was observed in the low vegetation level (2.58 log) followed by medium (1.14 log) or high (0.95 log) vegetation level. However, the difference in the
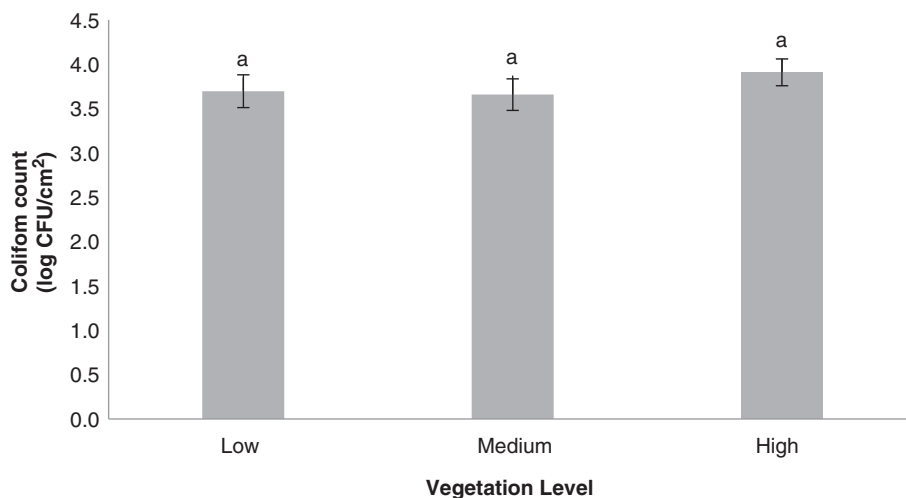

FIGURE 2 Coliform levels on watermelon surface harvested from low, medium, and high level of surrounding vegetation plots. The level of surrounding vegetation was converted to numeric value (0–10); 0 meant for no vegetation and 10 meant for highest amount of vegetation. Categorically, those plots with 0–3 were considered as low level of vegetation plots, 4–6 as medium level of vegetation plots, and 7–10 as high level of vegetation plots. The detectable limit was 0.30 log MPN/sample
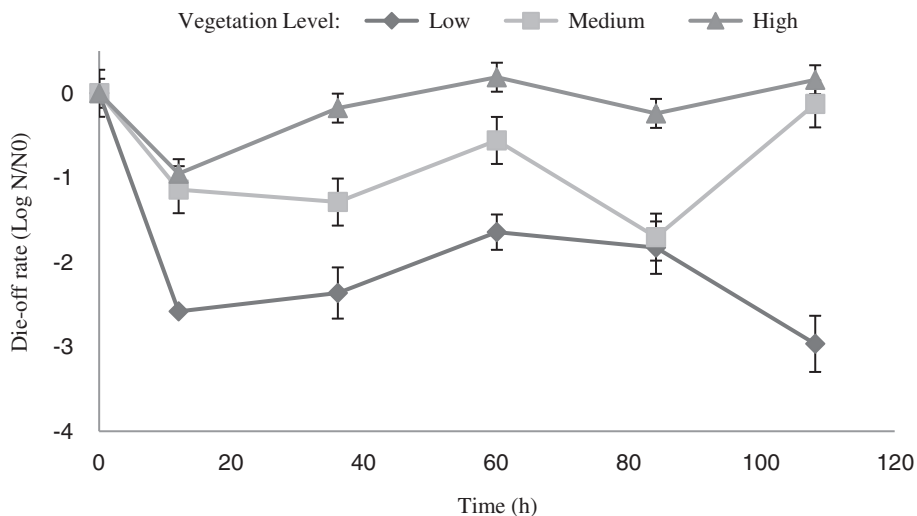
FIGURE 3 Die-off of generic *E. coli* on watermelon discs under three level of surrounding vegetation plots. The level of surrounding vegetation was converted to numeric value (0–10); 0 meant for no vegetation and 10 meant for highest amount of vegetation. Categorically, those plots with 0–3 were considered as low level of vegetation plots, 4–6 as medium level of vegetation plots, and 7–10 as high level of vegetation plots. The detection limit was 1.65 log CFU/cm^2

reduction was statistically not significant ($p > .05$) between medium and high vegetation levels. A relatively high reduction of generic *E. coli* on the surface of watermelon was expected in the first few hours of exposure to on-farm daylight conditions. Brandl (2006) reported that the direct exposure of produce surface to the sunlight has a detrimental effect on the survival of enteric bacteria. In our study, the watermelon discs at low surrounding vegetation were found to be fully exposed to sunlight. While the surface of watermelon was not fully exposed to sunlight at medium and high surrounding vegetation. This explains the possible cause of the high rate of initial reduction of generic *E. coli* at low vegetation level compared to others.

After 12 hr, a gradual increase in the levels of generic *E. coli* was observed in all vegetation levels (Figure 3) indicating that bacterial cells might have adapted to the surrounding environment. In adverse environmental conditions, bacterial cells could maintain structural and genetic integrity by altering their cellular physiology (Brooks, Turkarslan, Beer, Lo, & Baliga, 2011). Berney et al. (2006) reported that *E. coli* could express adaptive response to Ultraviolet A (UVA) radiation, and resume their growth when UVA light is very scarce or when the UVA irradiation is stopped. In our study, the night time (i.e., between 12 hr (6 p.m.) and 24 hr (6 a.m.) may have allowed

adequate time for the bacterial cells to recover from damage and resume their growth. Studies reported that relative humidity and temperature could affect microbial survival or die-off rate on produce surface (Del Rosario & Beuchat, 1995; Stine, Song, Choi, & Gerba, 2005; Weller et al., 2017). During the current study, the relative humidity ranged from 62% to 74%, and the average temperature ranged from 25 °C to 28.56 °C (Table 1). We calculated correlation coefficient to see if the relative humidity and environmental temperature were associated with the bacterial survival. There was a positive correlation ($r = .76$) between the relative humidity and the bacterial survival in the medium vegetation level; however, the correlation was not significant in low and high vegetation levels (data not shown). We observed negative correlation (−.93) between environmental temperature and the bacterial survival in high vegetation level. However, there was no significant correlation in other vegetation levels. The results indicated that the surrounding vegetation could alter environmental factors such as relative humidity and temperature which affect microbial survival.

Several studies reported that the sunlight and its ultraviolet component is a significant factor in inhibiting pathogens (Davies & Evison, 1991; Heaton & Jones, 2008; Nyeleti et al., 2004). Nyeleti et al. (2004) found that simulated sunlight decontaminated *Salmonella* on the stainless-steel surface under field conditions. In addition, longer

Low vegetation level   Medium vegetation level   High vegetation level

FIGURE 4 Inoculated watermelon discs kept on sterile petri plates placed randomly around in each level of surrounding vegetation plot

TABLE 1 Data of on-farm environmental conditions over a period of 108 hr for die-off rate measurement

Time (hr)	Day light length (hr)	Average temperature (°C)	Average relative humidity (%)
0	NA	25	71
12	11:52:23	28.56	64
36	12:15:56	25.55	69
60	12:14:06	25.55	65
84	12:12:17	25.55	62
108	12:10:27	25	74

sunlight exposure may result in an increased surface temperature of fruit and desiccation rate, which ultimately may cause additional stress to the bacterial cells on produce surface (Tomas-Callejas et al., 2011). In another study, Stine et al. (2005) reported that the cantaloupe surface under shading favors longer bacterial survival than those fully exposed to simulated sunlight. In contrast, Erickson et al. (2010) reported that physical shading condition did not significantly contribute to the *E. coli* survival on lettuce leaves than the sunny condition. The effect of direct sunlight on microbial survival on produce surface cannot be underestimated. During the initial stage of bacterial contamination in the agricultural field, the surrounding vegetation could be a critical factor determining the bacterial growth on produce surface.

After 108 hr, the highest reduction was observed in low vegetation level (3 log CFU/cm^2; Figure 3) with an average die-off rate of 0.66 log/day. This result was consistent with the results reported by other studies, especially on lettuce surface matrix. In field conditions, Weller et al. (2017) observed that the average daily die-off rate of *E. coli* on lettuce head was 0.52 log from their 10 days of die-off study. In a similar study, Bezanson et al. (2012) observed an average daily die-off rate of *E. coli* O157:H7 on lettuce by 0.56 log. It was interesting to notice that, in low vegetation level, the generic *E. coli* counts at 108 hr were similar to those of 12 hr. However, in medium and high vegetation level, the counts recovered to the initial level (~6 log CFU/cm^2). This indicates that the on-farm microbial die-off rate estimates based on environmental conditions such as exposure to sunlight, UV fraction of sunlight light, temperature and relative humidity variations, and bacterial recovery mechanisms seem plausible in low vegetation conditions. Whereas, generic *E. coli* exhibited high survival especially when the melon surface was surrounded with medium and high vegetation.

Overall, this study investigated the effect of surrounding vegetation on the survival of generic *E. coli* on the surface of watermelon. Controlling the common contamination sources such as soil and irrigation water, the levels of naturally present generic *E. coli* were very low (<1.5 log MPN/watermelon), and the effect of surrounding vegetation was found to be insignificant. However, there was a significant effect of surrounding vegetation on microbial survival when watermelon rind discs were artificially contaminated with high levels of laboratory strains of generic *E. coli*. The findings of this study emphasize the importance of considering the effect of surrounding vegetation while making decisions based on microbial persistence on produce surface. Future studies should focus on combining contaminated irrigation water or composted and manure-based soil amendments in conjunction with weed control to estimate generic *E. coli* die-off rates on watermelon rind surfaces.

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