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A study on the contamination routes of tomato and bell pepper plants by *Escherichia coli* and *Listeria innocua*

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ABSTRACT

Tomatoes and bell peppers have been the source of recent outbreaks of foodborne illnesses due to contamination by human pathogens such as *E. coli* O157:H7 and *Listeria monocytogenes* in the field. The objectives of the present study were to investigate (i) the potential uptake of bacterial surrogates of *E. coli* O157:H7 and *Listeria monocytogenes*, *E. coli* (EC) and *L. innocua* (LI), from soil into the edible parts of tomato and pepper plants, and (ii) the survivability of EC and LI in the plant environment (soil, rhizosphere and phyllosphere). Mature tomato and bell pepper plants were soil-inoculated with a bacterial suspension (population density of ca. 10^8 cfu/ml) of Nalidixic-acid resistant EC or LI. Tomatoes and peppers were also artificially contaminated on the surface with 1 ml of an overnight culture of Nalidixic-acid resistant EC and LI (population density of ca. 10^9 cfu/ml). Samples of vegetables as well as non-edible parts (soil, roots, stem, foliage) were subjected to microbiological analyses by plating on Eosin Methylene Blue Agar and Listeria Identification Agar supplemented with Nalidixic acid to a final concentration of 50 μg/ml to recover EC and LI respectively. We observed no evidence of internalization of EC or LI into the edible fruits. However, these bacteria were recovered from different non-edible parts of the tomato plant at varying population densities of 3.0-3.6, 1.8-2.2 and < 0.7 log cfu/g in the bulk soil, roots and foliage respectively. They were also found to persist in the soil for up to 4 days post-inoculation. Tomatoes and peppers surface-inoculated with EC or LI were shown to harbor the bacteria for > 48 h. Taken together, findings of this study point to the microbiological and health risks associated with consumption of raw tomatoes and bell peppers due to the possibilities of pre-harvest microbial contamination by human pathogens.

Indexing terms/Keywords

Contamination; *Escherichia coli*; *Listeria innocua*; tomato, pepper; *Ralstonia solanacearum*; *Pseudomonas fluorescens.*

Academic Discipline And Sub-Disciplines

Food Science; Food Safety; Crop Science

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INTRODUCTION

Fresh vegetables contain rich sources of many nutrients and provide numerous health benefits, so nutritionists and health professionals highly recommend increasing consumption of these important foods [1]. Tomatoes and peppers represent some of the vegetables that are most commonly consumed in the raw state. However, these vegetables have also been the source of recent outbreaks of foodborne illnesses in developed countries, which have caused sickness, hospitalizations, and deaths of consumers, as well as serious adverse economic impact on growers and processors [2]. Since 1990, up to 15 outbreaks of salmonellosis have been linked to the consumption of fresh tomato fruits in developed countries such as the United States [3]. Trace-back investigations of outbreaks linked to tomatoes have concluded that the fruits were generally contaminated in the field [4]. Suggested sources ranged from animals in nearby pastures or wetlands to water used for irrigation or pesticide applications [4]. Orozco et al. [5] detected *Salmonella* in 1.8% of tomatoes grown hydroponically in a greenhouse prior to an extreme weather event during which time floodwaters entered several of the houses. Immediately after the floodwaters had disappeared, the contamination rate increased to 9.4% [5]. Bell peppers also represent a major world commodity by virtue of their high content in vitamin A and C as well as the presence of the compound responsible for the irritation ("hotness") called capsaicin [6]. The production of hot and sweet peppers for vegetable uses has increased by more than 21% since 1994 [6]. Peppers are commonly used fresh in condiments, sauces, salads, meats and vegetable dishes [6]. Unfortunately, peppers form increasingly recognized vehicles for transmission of foodborne pathogens [7]. A study conducted on the prevalence of *Salmonella* in peppers showed that 10 out of a total of 27 samples from a pepper production system tested positive for *Salmonella* and were identified as either *Salmonella enterica* serovar Typhimurium (91% of 54 cases) or *Salmonella enterica* serovar Enteritidis (9% of cases) [6].

Given the high frequency of microbial contamination of raw tomatoes and peppers, there has been a concern regarding the potential for human pathogens to become internalized within plant tissue [8]. The phenomenon of human pathogen internalization into food crops is an area of research that has already been well studied in developed countries having a temperate climate [9-12]. However, to our knowledge, no studies have been published on the ability of human pathogens to infiltrate, translocate or internalize in the edible parts of vegetables grown in a tropical climate such as that of Mauritius. In the current study, tomato and pepper plants, belonging to the family of *Solanaceae*, were used as model host systems to study their susceptibility to uptake and persistence of bacterial human pathogens. *E. coli* O157:H7 is one of the most common zoonotic enteric pathogens associated with vegetables given its widespread presence in animal manure used in produce cultivation [13]. *Listeria monocytogenes* on the other hand, is a common geophilic (soil-borne) bacterium and is ubiquitous in vegetation [14]. In addition, the role of plant commensal bacteria such as plant pathogen *Ralstonia solanacearum* and plant beneficial bacteria *Pseudomonas fluorescens* in enhancing or hindering internalization of human pathogens in vegetables is of equal interest. Indeed, previous research has suggested that bacterial plant pathogens can enhance infiltration or internalization of human pathogens in the roots, leaves and fruits of food crops [14]. Moreover, *P. fluorescens* represents one of the most abundant soil resident species that usually confer several benefits to the plants [14]. It is thus hypothesized that the presence of phyto-pathogenic species such as *R. solanacearum* might enhance the uptake of human pathogens in plants due to ability of *R. solanacearum* to produce plant lesions and wounds which may act as sites of co-infection by human pathogens. Plant pathogens may also have the ability to depress the defense mechanisms of plants, thus enhancing colonization and persistence of human pathogens. On the other hand, it is hypothesized that non-pathogenic *P. fluorescens* will discourage uptake or internalization of human pathogens since literature has shown that it acts as an excellent plant competitor against non-resident human pathogenic bacteria.

The objectives of the present study were therefore to: (i) investigate the potential uptake, infiltration or internalization of bacterial human pathogens from soil into the edible parts of tomato and bell pepper plants, (ii) investigate the influence of plant pathogen and plant beneficial bacteria on the uptake or internalization of human pathogens and (iii) investigate the survivability of human pathogens in the soil, rhizosphere and phyllosphere of tomato plants.

MATERIALS AND METHODS

2.1 Assessing the potential for systemic uptake of *E. coli* and *L. innocua* in tomato and pepper plants

2.1.1. Soil sterilization

The oven was preheated to 82-88°C (180°-190°F). Ten kg of soil was spread evenly in a large pan to a maximum depth of 10 cm. The pan was sprayed with water to moisten slightly and then covered tightly with aluminum foil. At the center of the covered baking pan, a thermometer probe was inserted into the soil and the pan placed into the oven. Once the soil temperature reached 82-88°C, the temperature was maintained for 60 minutes following which the pan was removed from the oven and allowed to completely cool. Once cooled, soil was transferred to clean gunny bags. Given the limited capacity of the oven, multiple cycles were run to sterilize several batches of soil.

2.1.2. Plant preparation

Tomato (*Solanum lycopersicum* var. St Pierre) and bell pepper (*Capsicum annum* var. Nikita) seeds were used. Briefly, seeds were disinfected with 70% ethyl alcohol (EtOH) for 3 min, rinsed in sterile water, and soaked in Javel commercial bleach (0.525% sodium hypochlorite) for 15 min. Seeds were then rinsed in sterile water three times (5 min each rinse). Subsequently, they were sowed in steam-sterilized soil contained in Styrofoam plug trays and grown in a Biosafety Level 1 (BSL-1) greenhouse located at the Mauritius Sugar Industry and Research Institute, Reduit. Plants were watered on a daily basis with sterile water. Seedlings were transplanted at 2 weeks of age to potting bags containing steam-sterilized

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soil (~1 kg) placed in plastic saucers to serve as a water reservoir for indirect irrigation. The pH and water activity of the soil were regularly monitored with a pH meter (Mettler Toledo) and a water activity meter (Novasina) respectively. Over the

period of October 2013 to December 2014 chamber temperatures ranged from 21 to 32°C (daytime) and 12 to 23°C (nighttime) and the relative humidity varied between 65 to 81%. The saucer was refilled with ca. 50 ml sterile water daily. Additionally, the soil was supplemented with 'Terreau' or peat (Stender) as per the manufacturer's instructions to maintain plant growth, to speed up harvest time and increase yields.

2.1.3. Experiment Design

Two plant types (tomato and pepper) were investigated in this part of the study. The plants were given one of 7 treatments (Sterile water, EC, EC + R, EC + P, LI, LI +R, LI + P) where EC, LI, P and R stand for *Escherichia coli, Listeria innocua*, *Pseudomonas fluorescens* and *Ralstonia solanacearum* respectively. Each treatment was given in duplicates. The experiment was carried out in two independent replicates. A total of 56 plants (7 treatments x 2 plant types x 2 plants per treatment x 2 replicates) were considered. The different treatments given to the plants are summarized in the Table 1 below.

2.1.4. Soil Inoculation

2.1.4.1. Bacterial cultures

E. coli ATCC 25922 strain was provided by the Food Technology Laboratory of the Ministry of Agro-Industry and Food Security of Mauritius. The strain was plated onto Eosin Methylene Blue medium (HiMedia) and incubated for 24 h at 37°C for confirmatory identification of *E. coli*. *Pseudomonas fluorescens* ATCC 13525 (Microbiologics Ltd) and was revived on Pseudomonas CFC medium. Colonies that were straw coloured with a greenish tinge were presumed to be

P. fluorescens and confirmed by oxidase and catalase tests. *Listeria innocua* ATCC 33090 (Microbiologics Ltd) and was revived on Polymyxin Acriflavin Lithium-Chloride Ceftazidime Aesculin Mannitol (PALCAM) medium (HiMedia). Olive green colonies with dark sunken centers and black haloes were confirmed to be *L. innocua. L. innocua* hydrolyzes aesculin to form aesculetin and dextrose. Aesculetin reacts with ammonium ferric citrate and forms a brown-black complex seen as a black halo around colonies. An environmental isolate of *Ralstonia solanacearum* was generously provided by Dr S. Ganeshan, from the Mauritius Sugar Industry Research Institute. The isolate was obtained from the ooze of a tomato plant suffering from bacterial wilt disease. The isolate was plated onto triphenyl tetrazolium chloride (TTC) medium (Sigma) and incubated overnight at 27°C. Strains were stored at -80°C in glycerol stocks.

2.1.4.2. Inoculum preparation

The cells of the four cultures were adapted to grow on Plate Count Agar supplemented with 100 µg/ml of nalidixic acid (Sigma) (PCA-N) to select for Nalidixic-acid (NA) resistant strains of *E. coli*, *L. innocua*, *P. fluorescens* and *R. solanacearum.* NA-resistant mutant strains were subsequently transferred on fresh Plate Count Agar supplemented with 100 µg/ml of NA and plates incubated overnight at 35°C to yield solid cultures. Stock cultures of NA resistant strains of *E. coli*, *L. innocua*, *R. solanacearum* and *P. fluorescens* were also stored in TSB-N broth containing 25% glycerol (Sigma) at −18°C. To prepare liquid cultures, a single colony of each NA-resistant strain was transferred to 200 ml of tryptic soy broth (TSB-N) and placed on an orbital shaker at 35°C for 18 h.

2.1.4.3. Soil inoculation of plants

On the day of inoculation of the plants, 100 ml of each culture was mixed with 900 ml of sterile water (10-fold dilution of an overnight culture) to serve as the inoculum for the plants. The concentration of each culture was determined by serial dilution and plating on PCA-N. In addition, the population density of *E. coli* and *L. innocua* recovered from the soil immediately after inoculation was also determined. Various treatments were given to the tomato and pepper plants upon fruit set (Table 1). Plants serving as negative controls were treated with sterile water. Tomato plants were staked and strung to bamboo sticks to ensure upright growth. All plants were watered once or twice daily as needed.

2.1.5. Microbiological analysis of vegetables at harvest

Vegetables reaching commercial maturity were harvested by plucking tomato and pepper fruits. Tomatoes and peppers were blended with 0.1% Buffered Peptone Water at a 1:4 ratio. Vegetable samples were blended with 0.1% Buffered Peptone Water at a 1:4 ratio. Vegetables were macerated for 10 minutes into a slurry. The slurry and its serial dilutions were then plated onto Eosin Methylene Blue agar or PALCAM agar supplemented with 100 µg/ml of Nalidixic acid and plates incubated at 44 or 35°C respectively for 48 h. In addition, primary samples suspected to be contaminated with *E. coli* or *L. innocua* were subjected to primary enrichment in Lauryl Tryptose broth (LTB) and Half-Fraser broth respectively and incubated at 44 and 35°C for 24 h. Broths were supplemented with NA to a final concentration of 100 ug/ml. Aliquots of LTB and Half-Fraser Broth were then transferred for secondary enrichment into EC and Fraser broths supplemented with NA, and incubated at 44°C and 35°C for 24 h respectively. A loopful of secondary enrichment broth was then streaked onto EMB-N or PALCAM-N and plates incubated at 44 or 35°C respectively for 24 h. Colonies with characteristic green metallic sheen on EMB-N or olive green colonies with a surrounding black halo on PALCAM-N were presumed to be Nalidixic-acid resistant *E. coli* or *L. innocua* respectively.

2.2. Assessing the translocation potential of E. coli and L. innocua into different sections of the tomato plant

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This experiment was conducted to investigate the translocation potential of soil-inoculated *E. coli* and *L. innocua* into different parts of the tomato plants (*S. lycopersicum* cv. St Pierre). Mature tomato plants (past fruit set) were soil-

inoculated with 200 ml of a 10-fold dilution of a late-log phase culture of NA-resistant *E. coli* or *L. innocua*. The population density of the suspension was ca. 8 log cfu/ml. After 24 h, the plants were cut into 3 sections: the roots, stems and foliage.

2.3. Assessing the persistence of E. coli and L. innocua in rhizosphere soil

Soil microcosms were set up consisting of a polypropylene tray containing 2 kg (dry wt) of soil mixed with live roots of an un-inoculated tomato plant. Initial water activity of the soil-roots mix was ca. 0.3. The microcosm was inoculated with 200 ml of a suspension of NA-resistant *E. coli* or *L. innocua* having a cell density of ca. 10^8 cfu/ml resulting in a theoretical final population density of ca. 10^7 cfu/g of soil. The inoculum was homogeneously stirred into the soil-roots mix and the microcosm covered with aluminum foil. Microcosms were incubated in the dark at 25°C for 7 days with daily addition of 100 ml of sterile water. Soil was collected daily and subjected to microbiological, water activity and pH analyses. In order to determine the population density of bacteria present in the microcosms at daily intervals, about 25 g of soil was taken and mixed with 225 ml of 0.1% buffered peptone water in a sterile stomacher bag. This soil suspension was ten-fold serially diluted in 0.1% buffered peptone water and plated on EMB-N and PALCAM-N agar. Plates were subsequently incubated for up to 48 h at 44°C and 35°C respectively. Soil water activity and pH were determined using a dew point water-activity meter (Novasina) and a pH meter (Mettler-Toledo) respectively.

2.4. Assessing the survivability of E. coli and L. innocua on the surface of tomato and pepper fruits

Tomato and pepper plants were cultivated as described previously. At fruit set, a spot inoculation method was used to artificially contaminate the tomatoes and peppers since it allows the deposition of a known amount of cells onto the surfaces, regardless of weight/size. A total of 54 tomatoes and 30 peppers were used for the spot-inoculation study. Mature red ripe tomato and pepper fruits were spot-inoculated with 1000 ul of late-log phase cultures of Nalidixic-acid resistant *L. innocua* or *E. coli* on the pericarp and calyx using an appropriate micropipettor. In addition, tomatoes and peppers were also spotted with sterile water as a negative control. Tomatoes and peppers were aseptically harvested after 24h and 48h by plucking the fruits together with the stem or peduncle. After aseptically removing the peduncle and calyx, each fruit was then placed in an individual sterile Whirl-Pak filter bag containing 40 ml of 0.1% BPW. To recover bacteria from the surface of fruits, each tomato or pepper fruit was gently hand-massaged for 2 min, and then the rinsate was diluted 10-fold in 0.1% Buffered Peptone Water, and 0.1-ml aliquots of the appropriate dilutions were spread-plated onto EMB-N or PALCAM-N. Plates were incubated and enumerated after 24h as described previously.

RESULTS AND DISCUSSION

3.1. Translocation of E. coli and L. innocua in tomato and pepper plants

In this part of the study, *E. coli* ATCC 25922 and *L. innocua* ATCC 33090, non-pathogenic surrogate microorganisms were used in lieu of the enteric pathogens *Salmonella* or *E. coli* O157:H7 and the ubiquitous soil-borne pathogen *L. monocytogenes* respectively, to avoid introduction of pathogenic agents in the BSL-1 greenhouse. Other authors including Ingham et al. [15] and Wood et al. [16] have also resorted to non-pathogenic surrogates to circumvent this limitation. Examples of surrogates that have been used *in planta* studies include *E. coli* Shiga toxin-negative *E. coli* O157:H7 [9; 17], *Listeria innocua* [18], and avirulent *Salmonella* [9]. In using these surrogates, the assumption has been made that they would respond similarly as the pathogenic agent.

Table 2 summarizes the results obtained for the soil-inoculation experiment of tomato plants. The population density of *E. coli* and *L. innocua* recovered from all tomato fruits was below the limit of detection of the plating methodology (< 1.7 log cfu/g) and the bacteria were not detected after enrichment and streaking in most of the tomato samples tested except for three samples highlighted in bold. These suspect *E. coli* isolates originating from three tomato samples yielded negative results upon biochemical identification, thus confirming their absence.

Table 3 indicates that similar to tomato fruits, *E. coli* and *L. innocua* were also undetectable (< 2.2 log cfu/g) by plating in pepper fruits following artificial contamination of the soil. In other words, our study failed to demonstrate the translocation of these bacteria from soil to fruits despite optimizing the cultivation conditions to promote uptake of the inoculated bacteria in the plant. This was achieved by sterilizing the soil used as a growing medium to get rid of indigenous microorganisms present. Indeed, the high complexity of interactions between the inocula of interest and background microflora have often prompted plant physiologists and microbiologists to use simple 'gnotobiotic' type models, namely sterile or sub-sterile growing media as well as pure cultures of microorganisms [17]. However, it is worth mentioning that these simple models also have some limitations since they tend to mask the role of interactions among the different groups of microorganisms present in the soil and in the rhizosphere. Indeed, plant roots and soil are never sterile; rather they are surrounded or invaded by large numbers of microorganisms with potentially intense biochemical activity.

In our study, *E. coli* ATCC 25922 and *L. innocua* ATCC 33090 were used as non-pathogenic surrogates to mimic *Salmonella* spp. or *E. coli* O157:H7 and *L. monocytogenes* respectively. Similar to our findings, other authors have also reported the inability to detect *Salmonella* in tomatoes that have been artificially contaminated with the microorganisms via soil [19, 20]. Contrary to our findings however, Zheng et al. [21] has shown that *Salmonella* is capable of internalizing in tomato plants through the roots provided there are favorable conditions for this to occur. Zheng et al. [21] also indicated

that uptake of *Salmonella* through the roots of *S. lycopersicum* Micro-Tom grown in sandy loam soil led to the contamination of developing tomato fruits. The authors further noted that fruit contamination rate was much higher with

Salmonella introduction through flowers (70.4%) than through the rhizosphere (5.5%). Hence, the phenomenon of Salmonella enterica internalizing tomato plants through the root system remains a largely controversial issue.

Erickson [17] mentioned that two key factors that influence the chance of *Salmonella* internalization in tomato plants through the root system are the introduction time post-transplant and the bacterial strain used. In other words, the timing of inoculation critically influences *Salmonella* internalization via the root system. Specifically, inoculation within 3 days of transplanting yielded a significantly higher recovery of endophytically colonized *Salmonella* (average of 20%) than did inoculations 1 week after transplantation (0%). Plant wounding or stress induced by abiotic factors during transplantation probably underscores the bias for *Salmonella* entrance [22]. Soon after the transplantation stage, a *Solanaceae* (tomato or pepper) plant is more susceptible to internalization, thereby increasing the chance of *Salmonella* internalization in the plant, and, subsequently, causing an increased risk of *Salmonella* contamination of fruits. In our study, the lack of uptake of *Salmonella* could partly be explained by the fact that inoculation was scheduled after ca. 6 weeks after transplanting. Not surprisingly, our study failed to recover *Salmonella* from tomato and pepper fruits via inoculated soil given the long time lapse post-transplantation.

In addition to the timing of inoculation, the strain used can also have a critical bearing on the probability of translocation of human pathogens from soil to fruits. Despite the existence of a wide variety of *Salmonella* serovars in the environment [23], only a few serovars of *Salmonella* have been repeatedly linked to outbreaks associated with tomatoes and peppers, thus leading to the hypothesis that certain serovars (such. as *S.* Newport and *S.* Montevideo) have a greater propensity for survival in inimical environments such as the acidic interior of tomatoes or the endocarp of peppers known to harbour antimicrobial capsaicin. Guo et al. [19] and Shi et al. [24] both reported that *S.* Montevideo was the most persistent serovar recovered within tomato fruits when introduced. Zheng et al. [21] also found *S.* Montevideo, *S.* Newport and *S.* Javiana, to be well adapted for survival in tomatoes probably due to the presence of genes responsible for acid tolerance. Similarly, *E. coli* O157:H7 has also been shown to be acid-tolerant [25]. However, the nonpathogenic surrogates used in this study, *E. coli* ATCC 25922 and *L. innocua* ATCC 33090, are not known to have these genes. The absence of acid-tolerance traits could partly account for the inability to detect these microorganisms inside the acidic milieu of tomato fruits or inside the hostile environment of the pepper endocarp.

Table 2 and 3 also compared the translocation potential of *E. coli* and *L. innocua* in the presence of plant pathogen *Ralstonia solanacearum* and plant beneficial bacterium *Pseudomonas fluorescens. R. solanacearum* is a soil-borne pathogen that infects the roots of plants including tomatoes and peppers leading to bacterial wilt disease. Bacterial wilt is one of the leading disease problems of tomato production, causing devastating yield and economic losses each year. In some instances, growers have had to forfeit harvesting a field because the bacterial wilt infestation was so severe that it was not possible to make a profit from the harvestable fruit. Moreover, Good Agricultural Practices (GAP) guidelines urge growers not to harvest fruits from diseased plants infected by plant pathogens in fear that the plant's compromised immune system would make them more susceptible to human pathogens such as *S. enterica*, *E. coli* O157:H7 or even *L. monocytogenes* [26]. Indeed, *R. solanacearum* when added to soil has the ability to infect the plant through natural openings or through wounds in the roots [26], thus potentially increasing the chances for ingress of human pathogens. In this study, the influence of *R. solanacearum*, a plant pathogen, on the uptake of pathogen surrogates in food crop plants was thus of academic interest. Table 3 and 4 indicate that systemic uptake of *E. coli* and *L. innocua* from roots to fruits did not occur in the presence of either plant pathogen *R. solanacearum* or plant beneficial bacterium *P. fluorescens*. Contrary to our findings, Pollard et al. [27] demonstrated that *R. solanacearum* could influence *S. enterica* survival and its transportation throughout the internal tissues of tomato plants, causing an increase in *S. enterica* populations on plants [27]. Van der Schoot [28] explained that xylem fluid in tomato stems must pass through pit membranes before moving from a stem into a petiole. Pit membranes have pores of about 0.3 μm in diameter [29], which allow water to move freely but filter out large particles such as bacteria. Phytopathogenic bacteria, such as the wilt pathogen *R. solanacearum*, have the ability to digest these membranes and could thus breach this barrier [30]. Indeed, certain laboratory models have demonstrated internalization of wilt pathogen *Ralstonia solanacearum* by tomato roots and then movement up the xylem of the plant [4].

Similar studies investigating the effects of plant pathogens on *S. enterica* populations on produce have also been conducted. One such study was carried out by Wells and Butterfield [31] who investigated interactions between the causative agent of bacterial soft rot, *Erwinia* spp. and *S. enterica* in fresh fruits and vegetables. They discovered that *S. enterica* populations increased up to 10-fold when co-inoculated with *Erwinia* spp. on the tomato fruit compared with the populations enumerated when the fruits and vegetables were inoculated with *S. enterica* alone. A study conducted by Barak et al. [32] found that *S. enterica* populations on tomato plants increased in the presence of *Xanthomonas campestris*, the causal agent of bacterial spot. Vegetables co-inoculated with *Pseudomonas viridiflava* and *S.* Typhimurium harboured *Salmonella* populations that were approximately three times higher than vegetables inoculated with *Salmonella* alone. Fungi were also shown to benefit human pathogens through habitat modification. Co-inoculation of tomato tissue with *Salmonella* and *Rhizopus* (plant pathogenic fungus) caused a significant increase in *Salmonella*

population sizes compared with its inoculation alone [33]. Similarly the post-harvest fungal pathogens *Alternaria alternata* and *Cladosporium* spp. enhanced the growth of *Salmonella* in ripe tomato fruit, likely via alkalinization of the plant tissue resulting from their proteolytic activity [34]. Hence it appears that fungi provide pathogens with not only enhanced access to growth substrates by degrading the plant tissue, but also reduce environmental stress that may inhibit *Salmonella*.

Overall, findings of the current work indicate that the presence of a prototypic plant pathogen exemplified by *R. solanacearum* and a typical beneficial plant bacterium such as *P. fluorescens* did not have any effect on the susceptibility

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of tomato and pepper plants to uptake of bacterial human pathogens. According to Van der Schoot [28], certain cultivars of *Solanaceae* possess a type of resistance against wilt pathogens and their pit membranes are resistant to digestion.

Resistance to infection by the plant pathogen or resistance to colonization by the plant beneficial bacteria could have explained the absence of any noticeable differences in the presence of these plant commensal bacteria.

3.2. Translocation of E. coli and L. innocua to different sections of the tomato plant

The localization and population density of *E. coli* and *L. innocua* in different parts of the tomato plant is depicted in Figure 1. Our study indicated that *E. coli* and *L. innocua* were recovered from bulk soil and roots at population densities of 3.0-3.6 log cfu/g and 1.8-2.2 log cfu/g respectively 24 hours post-inoculation. However they were undetectable (< 0.7 log cfu/g) in the main stem and foliage (fruits, flowers, stemlets, petiole and leaves) of the tomato plant. Jablasone et al. [35] similarly applied water contaminated with *Salmonella* directly onto the soil of pots containing tomato plants (*S. lycopersicum* cv. Cherry Gold) and also could not recover *Salmonella* from the stems or fruits of the tomato plant although populations in the soil ranged from 2.3 to 3.7 log cfu/g. In addition, another study found no evidence of *Salmonella enterica* serovar Montevideo on the stems, leaves, or fruit of tomato plants (*S. lycopersicum* L. cv. Trust) when soil-inoculated with contaminated water [3]. This is very similar to our data where plants artificially soil-contaminated with *E. coli* and *L. innocua* did not show evidence of translocation of the bacteria to the aerial parts of the plant.

However, presence of *E. coli* and *L. innocua* in the bulk soil as well as in the roots was observed as indicated in Figure 1. Contrary to our findings where we observed a relatively lower population of these bacteria on roots (1.8 – 2.2. log cfu/g) than in the bulk soil (3.0 – 3.6 log cfu/g), Semenov et al. [36] found the densities of *S.* Typhimurium and *E. coli* O157:H7 in bulk soil and rhizosphere (roots) to be similar following addition of manure to soil. The same author further noted that the densities were even higher in the rhizosphere than in bulk soil after slurry application. Similarly, Habteselassie et al. [37] found similar numbers of *E. coli* in bulk and rhizosphere soil when manure was added to pots in which lettuce was being grown. Rhizosphere soil is the thin film of soil adhering to roots. Cooley et al. [13] postulated that motility of the inoculated bacteria played an important role on successful colonization of the rhizoplane (root surface). Cooley et al. [13] showed that inoculation of plants with *S. enterica* and *E. coli* O157:H7 led to invasion of roots at the lateral root junctions but invasion was decreased when non-motile mutants of *S. enterica* were used. The lower populations of *E. coli* and *L. innocua* on roots noted in our study, compared to bulk soil, could be due to reduced chemotaxis or motility of the microorganisms towards the roots or root exudates. Indeed, some researchers have disputed that active motility is important in soil, and as soil dries up, the water films become too thin to support flagella-mediated movement [3]. Overall, prevalence of human pathogens in the bulk soil and on the rhizoplane is not likely to be translocated to aerial parts of the plant.

Contrary to our observation, Hintz et al. [20] reported that repeated application of *Salmonella enterica* serovar Newport to the root zone via irrigation water has the potential to contaminate various tomato plant tissues of the tomato plant *Solanum lycopersicum* cv. Solar Fire. Likewise, Zheng et al. [19] demonstrated that of 22 tomato plants grown with *Salmonella*-infested soil, 22% (4 out of 18) contained endophytically colonized *Salmonella* based on direct plating or enrichment procedures, including two stem samples (11.1%), one leaf sample (5.5%), and one fruit sample (5.5%). *S. enterica* serovar Saintpaul was also isolated from a single positive leaf sample and *S.* Newport was found on the surface and within the single positive tomato sample (5.5%). Evidence for vascular movement of *Salmonella* up the plant was also presented when tomato plants were grown hydroponically in a Hoagland's nutrient solution contaminated with *Salmonella* at a population density of 4.6 log cfu/ml [19]. *Salmonella* was subsequently successfully isolated from hypocotyls, cotyledons, stems, and leaves of plants growing in the contaminated nutrient solution.

3.3. Survival of E. coli and L. innocua in soil mixed with live roots

Plant roots are known to modify their immediate habitat by changing the soil porosity and clustering properties [38] and such physical alterations are likely to impact the microbial community near those roots (i.e. the rhizosphere community). Live roots release root exudates that have the potential to significantly affect the microbial population including the fate of pathogens in the rhizosphere of food crops [39]. These exudates serve as nutrient sources for the bacteria in the vicinity of the roots and could therefore promote the extended survival of pathogens in soil. Taking this into consideration, we thus designed a microcosm consisting of a mix of autoclaved soil and live roots, since previous research has shown that *E. coli* O157:H7 survived longer in rhizosphere soil compared to free soil [40].

The survival curves of *E. coli* and *L. innocua* in the soil-root mix is shown in Figure 2. Both bacterial species exhibited a slow decline from an initial population of 5.2-5.3 to < 0.7 log cfu/g but persisted for up to 96 hours in the soil-roots mix. It is to be noted that conditions of the microcosm were particularly optimized to promote survival of the inocula in the soil-roots mix by protecting against dessication though daily watering with sterile water (soil a_w ~ 0.97-0.99) and protecting against UV radiation by covering with foil. Indeed, a critical factor influencing bacterial persistence in the soil is the moisture availability. In general, survival of microorganisms is greater in moist environments than dry environments [41]. When *E. coli* and *S.* Typhimurium cell suspensions were added to experimental plots, the organisms persisted at higher

concentrations in moist soils compared to dry soils (< 10% moisture) [42]. In fact, the effect of different soil types on *Listeria monocytogenes* survival was attributed to the ability of clay soils to hold more moisture than sandy soils [43]. In addition to dessication stress, pathogens in surface soils are subjected to radiation stress due to ultraviolet light from the sun. Radiation has been cited as the principal cause for *Salmonella* mortality in a study exposing soil to wastewater [44]. Hence, it is probable that bacterial persistence would be considerably shorter in the open field where water availability and UV exposure are not artificially controlled. Erickson [3] also indicated that *Salmonella* survived for weeks in soil provided that conditions were favorable i.e. high moisture retention in soil, high relative humidity in the air and limited sunlight.

Islam et al. [9] indicated that survival curves generally exhibit a concave curvature with initial decreases that are log-linear. The death curve of *E. coli* also had the characteristic concave curvature with slightly higher death rate in the first 12 hours. Literature has shown the variable persistence of different microorganisms in different agricultural niches [45]. Ecological

surveillance data on tomato farms by Bell et al. [23] and Micallef et al. [46] provided evidence that *Salmonella* persists in the tomato-growing environment including the soil. Bernstein et al. [47] reported that *S*. Newport is capable of persisting in potting medium for 4.7 to 10 weeks. Even among *Samonella* serovars, there were considerable differences in their persistence; *S*. Newport and *S.* Javiana appeared to persist in sandy loam soil more efficiently than other serovars, including *S.* Montevideo, *S.* Saintpaul, and *S.* Typhimurium. In addition to *Salmonella*, enteric bacteria such as *E. coli* O157:H7 as well as other fecal microorganisms have been demonstrated to survive for extended periods in soils. Reported survival times of *E. coli* O157:H7, *E. coli* O26, *Salmonella*, *Listeria, Campylobacter* and *Cryptosporidium* in soil are up to 6 months, 3 years, 2 years, 20 days and 3 months respectively [3, 34]. Indeed, there is considerable evidence to support the fact that pathogens can survive for widely varying periods of time in the soil and even on produce [33]. The relatively short survival times of *E. coli* and *L. innocua* noted in our study (≤ 4 days) could be due the high air temperatures (27-31°C) of the greenhouse during the experiment. Indeed lower survival rates were noted by Fremaux et al. [41] with increasing temperatures. Semenov et al. [36] also reported that the survival of *S.* Typhimurium and *E. coli* O157:H7 declined with increasing mean soil temperature. Other factors that may have contributed to the shorter survival are the relatively low levels of easily available nutrients in the soil [37, 48] and relatively low clay content [49].

3.4. Survival of E. coli and L. innocua on the surface of tomato and pepper fruits

Tables 4a and 4b show the population density of *E. coli* and *L. innocua* recovered after 24 and 48h from tomatoes that have been spot-inoculated with the bacteria. Our results show that tomatoes surface-contaminated with *E. coli* still harbored the bacteria after 24 h at varying density of 3.0-4.4 log cfu/g. However, after 48h, *E. coli* was below the limit of detection of the plating methodology (< 0.7 log cfu/g). Nevertheless, *E. coli* was still detected on the samples after enrichment and streaking in 2 out of 9 samples. *L. innocua* lost their viability quicker, dropping from an initial of 8.2 log cfu/g to a mean density of 1.2 log cfu/g after 24h. After 48h, *L. innocua* was detected in only 1 out of 9 samples.

Peppers were surface-inoculated with *E. coli* or *L. innocua* at a mean population density of 7.3 to 7.8 log cfu/g respectively. The population declined to 3.5-4.2 log cfu/g after 24h; after 48h the bacteria were undetectable by plating although *E. coli* was detected after enrichment in 6 out 14 samples (Table 5a). *L. innocua* on the other hand was undetectable in all samples tested after 48h (Table 5b). Taken together, our findings highlight the differential survival of *E. coli*, a zoonotic bacterium of an intestinal origin, and *L. innocua*, an environmental bacterium that predominantly resides in soil, on the surface of fruits. The relatively poor colonizing abilities of these bacteria as epiphytes could partly be attributed to the waxy cuticle and regular topography (smoothness) of the tomato and pepper exocarp. Guo et al. [4] also mentioned that bacteria can more readily colonize and penetrate fruit tissue in the early stages of fruit development prior to deposition of the waxy materials. Erickson et al. [17] noted that *E. coli* O157:H7 cells had a greater propensity to attach to coarse, porous, or injured surfaces than uninjured smooth surfaces of green peppers. The smooth and topographically uniform surface of peppers is devoid of any microenvironments that can afford protection to the deposited inoculum. Hence, it is not surprising to observe a rapid decline in the bacterial population from an initial 7.8 log cfu/g to 4.2 and < 0.7 log cfu/g after 24h and 48h respectively.

Vegetables can be indirectly contaminated in the field when the soil in which they are cultivated becomes contaminated for e.g. during drip-irrigation with contaminated water. In addition to drip-irrigation, vegetables can also be *directly* contaminated during overhead irrigation with contaminated water through splash dispersal of the bacteria onto the fruit surface [50]. Wei et al. [51] previously demonstrated the survival and growth of *Salmonella* deposited as an aqueous cell suspension on natural openings of the tomato fruit such as the stem scar. Contrary to Wei et al. [51], we noted that the inoculum deposited on the surface did not grow; instead the population declined rapidly to below detectable levels after 48h post-inoculation. Wei et al. [51] mentioned that survival of the bacteria was most likely dependent on the inoculum size; when small populations of *S*. Montevideo of 2.8-3.9 log cfu/ml were placed on the smooth periderm of tomato fruits, none could be detected after overnight storage. However, when the concentration of inoculum was increased to 9.5 log cfu/ml, the bacterium could be detected up to three days later. In our experiment, a volume of 1 ml of the overnight culture having a cell density of ca. 9 log cfu/ml was aliquoted on the fruit resulting in the deposition of ca. 10^9 cells on the fruit. In spite of the high inoculum, the population rapidly declined to 3-4 log cfu/g and to undetectable levels after 24 and 48h respectively. It has also been mentioned elsewhere that better survival of the inocula was observed when the bacterial cells were suspended in a buffer as compared with distilled water. In our experiment, a 10-fold dilution of the culture was effected in distilled water rather than buffer. The use of plain water over buffer could have contributed to the poor viability of the culture. Finally, the disparity between Wei et al. [51] and our results could be due to the different bacterial species used in our inoculum.

Congruent with our observation, Todar [52] also noted that tomato fruits were not the preferred substrate for *Salmonella*.

Another study indicated that *Salmonella* generally survived poorly on tomatoes grown in greenhouses as well as in laboratory microcosms [6]. Hence, although *Salmonella* has been linked to outbreaks associated with the consumption of fresh tomatoes, *Salmonella* is a zoonotic pathogen of an intestinal origin and thus survives poorly outside the enteric environment on the surface of vegetables.

Within the plant production systems, two very different environments are encountered, the rhizosphere (below-ground bacterial habitat) and phyllosphere (above-ground surfaces of a plant as a habitat for microorganisms). This pioneering study examined how introduction of bacterial human pathogens in the rhizosphere and phyllosphere of commercially important food crops affected their microbial safety. Tomatoes and peppers were grown in a Biosafety Level-1 greenhouse

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and inoculated with bacterial human pathogen surrogates *Escherichia coli* and *Listeria innocua.* Our findings revealed that artificial introduction of *E. coli* and *L. innocua* in the rhizosphere of tomato and bell pepper plants did not result in translocation of the bacteria into the fruits 24 h post-inoculation although a relatively high surviving population was noted in the bulk soil and in the roots. Moreover, the presence of plant pathogen *Ralstonia solanacearum* and plant beneficial

bacteria *Pseudomonas fluorescens* did not influence the systemic uptake of human pathogenic bacteria from the soil to the aerial parts of the plants. However, when *E. coli* and *L. innocua* were deposited onto the surface of tomato and pepper fruits, they remained viable for up to 48h. Hence, a preventative approach to minimizing the risks of pre-harvest contamination of tomatoes and peppers is through avoiding contact between mature fruits and environmental sources of human pathogens such as overhead or sprinkler irrigation water.

Overall, the potential for systemic uptake and translocation of human pathogens from soil to the edible plant parts was found to be negligible in tomato and pepper plants. Moreover, overhead (spray or sprinkler) irrigation with contaminated water could create opportunities for the deposition and subsequent persistence of human pathogens on the edible surface of vegetables even after harvest. These findings therefore underscore the need for adoption of Good Agricultural Practices (GAPs) by growers and Good Manufacturing Practices (GMPs) by post-harvest handlers of fresh produce in Mauritius. Indeed, everyone in the vegetable value chain, including producers, wholesalers, retailers, and consumers, should practice good food-safety behavior, and exercise their best judgment based on the best available evidence at the time.

Despite a few technical limitations, this pioneering study carried out in Mauritius may serve as a precursor for more in-depth research in this area. Future studies will need to be conducted with actual human pathogens and include realistic plant growth conditions, along with realistic pathogen contamination levels encountered in open field production systems. Future research should also focus on other biotic and abiotic factors affecting prevalence, uptake and persistence of human pathogens in crop production systems. Ultimately, it is hoped that findings from the current and future studies will not only be of academic interest, but also provide a sound basis for developing guidance documents and shaping policies for improving our agronomic practices.

TABLES

Table 1. Inoculation treatments of plants

TREATMENTS	DETAILS OF INOCULATION OF POTTED VEGETABLE PLANTS
Water	Addition of 100 ml of sterile water to the potted vegetable
E	Inoculation of each potted vegetable type with 200 ml of diluted suspension of overnight culture of *E. coli* with a cell density of ca. 10^8 cfu/ml; twice a week
EC + P	Inoculation of each potted vegetable type with 200 ml of diluted suspension of overnight culture of *E. coli* with cell density of ca. 0^8 cfu/ml & 200 ml of diluted suspension of overnight culture of *P. fluorescens* with cell density of ca. 10^7 cfu/ml on alternate days: twice a week
EC + R	Inoculation of each potted vegetable type with 200 ml of diluted suspension of overnight culture of *E. coli* with cell density of ca. 10^8 cfu/ml & 200 ml of overnight culture of *R. solanacearum* with cell density of ca. 10^7 cfu/ml on alternate days: twice a week
LI	Inoculation of each potted vegetable type with 200 ml of diluted suspension of overnight culture of *L. innocua* with cell density of ca. 10^8 cfu/ml; twice a week
LI + P	Inoculation of each potted vegetable type with 200 ml of diluted suspension of overnight culture of *L. innocua* with cell density of ca. 10^8 cfu/ml & 200 ml of diluted suspension of overnight culture of *P. fluorescens* with cell density of ca. 10^7 cfu/ml on alternate days: twice a week
LI + R	Inoculation of each potted vegetable type with 200 ml of diluted suspension of overnight culture of *L. innocua* with cell density of ca. 10^8 cfu/ml & 200 ml of diluted suspension of overnight culture of *R. solanacearum* with cell density of ca. 10^7 cfu/ml on alternate days; twice a week

Table 2. Internalization rate of *E. coli* (EC) and *L. innocua* (LI) in tomato fruits via soil

Bacterial Human Pathogen Surrogates (BHPS)	Inoculum Level of BHPS (log cfu/ml)	Plant Commensal Bacteria (PCB)	Inoculum Level of PCB (log cfu/ml)	BHPS Population in fruits (log cfu/g)	# Presumptive Positive Samples/ Total Samples
------	0	------	0	< 1.7	0/22
EC	8	------	0	< 1.7	**2/34**
EC	8	RS	7	< 1.7	0/24

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EC	8	PF	7	< 1.7	**1/35**
LI	8	------	7	< 1.7	0/18
LI	8	RS	7	< 1.7	0/15
LI	8	PF	7	< 1.7	0/17

Table 3. Internalization rate of *E. coli* (EC) and *L. innocua* (LI) in pepper fruits via soil

Bacterial Human Pathogen Surrogates (BHPS)	Inoculum Level of BHPS (log cfu/ml)	Plant Commensal Bacteria (PCB)	Inoculum Level of PCB (log cfu/ml)	BHPS Population in fruits (log cfu/g)	# Presumptive Positive Samples/ Total Samples
------	0	------	0	< 2.2	0/9
EC	8	------	0	< 2.2	0/17
EC	8	RS	7	< 2.2	0/12
EC	8	PF	7	< 2.2	1/18
LI	8	------	7	< 2.2	0/11
LI	8	RS	7	< 2.2	0/12
LI	8	PF	7	< 2.2	0/20

Table 4a. Survival of *E. coli* spot-inoculated on tomatoes

	Population density (log cfu/g) of *E. coli* on the surface of tomatoes		
Sample ID	Day 0	Day 1	Day 2
Sample 1	7.8	3.6	< 0.7 (-)
Sample 2	7.7	3.1	< 0.7 (-)
Sample 3	8.4	3.7	< 0.7 (-)
Sample 4	8.6	4.3	< 0.7 (+)
Sample 5	7.2	4.4	< 0.7 (+)
Sample 6	8.8	3.7	< 0.7 (-)
Sample 7	8.2	3.0	< 0.7 (-)
Sample 8	7.7	3.0	< 0.7 (-)
Sample 9	8.3	4.1	< 0.7 (-)
Mean	8.1 ± 0.48	3.6 ± 0.51	< 0.7 (2/9)

Table 4b. Survival of *L. innocua* spot-inoculated on tomatoes

	Population density (log cfu/g) of *L. innocua* on the surface of tomatoes		
Sample ID	Day 0	Day 1	Day 2
Sample 1	7.4	< 0.7	< 0.7 (-)
Sample 2	7.7	< 0.7	< 0.7 (-)
Sample 3	8.3	< 0.7	< 0.7 (-)
Sample 4	7.6	< 0.7	< 0.7 (-)
Sample 5	7.2	< 0.7	< 0.7 (-)
Sample 6	8.1	4.96	< 0.7 (-)
Sample 7	7.4	< 0.7	< 0.7 (+)
Sample 8	8.8	< 0.7	< 0.7 (-)
Sample 9	8.2	< 0.7	< 0.7 (-)
Mean	8.2 ± 0.49	1.2 ± 0.00	< 0.7 (1/9)

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Table 5a. Survival of *E. coli* spot-inoculated on peppers

Population density (log cfu/g) of *E. coli* on the surface of peppers			
	Day 0	Day 1	Day 2
Sample 1	8.5	4.8	< 0.7 (-)
Sample 2	8.2	4.7	< 0.7 (+)
Sample 3	7.9	4.6	< 0.7 (+)
Sample 4	8.0	4.6	< 0.7 (+)
Sample 5	7.4	3.9	< 0.7 (-)
Sample 6	7.6	4.2	< 0.7 (-)
Sample 7	8.4	4.0	< 0.7 (-)
Sample 8	7.2	4.1	< 0.7 (+)
Sample 9	8.1	4.5	< 0.7 (-)
Sample 10	7.4	4.4	< 0.7 (+)
Sample 11	7.6	< 0.7	< 0.7 (-)
Sample 12	7.8	2.8	< 0.7 (-)
Sample 13	8.0	4.2	< 0.7 (+)
Sample 14	7.5	3.5	< 0.7 (-)
Overall	7.8 ± 0.40	4.2 ± 0.54	< 0.7 (6/14)

Table 5b. Survival of *L. innocua* spot-inoculated on peppers

Population density (log cfu/g) of *L. innocua* on the surface of peppers			
	Day 0	Day 1	Day 2
Sample 1	7.9	4.1	< 0.7 (-)
Sample 2	6.8	3.0	< 0.7 (-)
Sample 3	7.4	3.0	< 0.7 (-)
Sample 4	7.2	4.9	< 0.7 (-)
Sample 5	6.9	3.6	< 0.7 (-)
Sample 6	6.7	5.2	< 0.7 (-)
Sample 7	7.6	2.0	< 0.7 (-)
Sample 8	7.5	3.0	< 0.7 (-)
Sample 9	6.9	2.0	< 0.7 (-)
Sample 10	7.9	< 0.7	< 0.7 (-)
Sample 11	7.6	< 0.7	< 0.7 (-)
Sample 12	6.8	< 0.7	< 0.7 (-)
Sample 13	7.9	3.2	< 0.7 (-)
Sample 14	7.2	2.8	< 0.7 (-)
Sample 15	6.9	4.2	< 0.7 (-)
Sample 16	7.1	4.8	< 0.7 (-)
Overall	7.3 ± 0.42	3.5 ± 1.10	< 0.7 (0/16)

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FIGURES/CAPTIONS

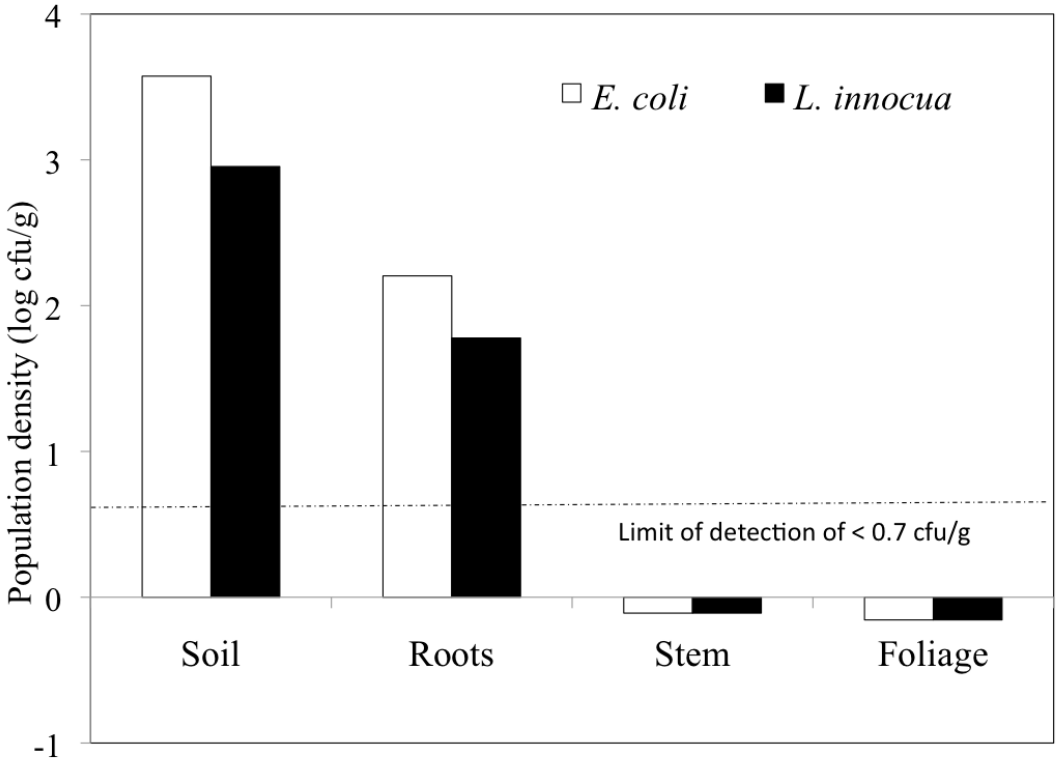

Fig 1: Distribution of *E. coli* and *L. innocua* in different parts of the tomato plant

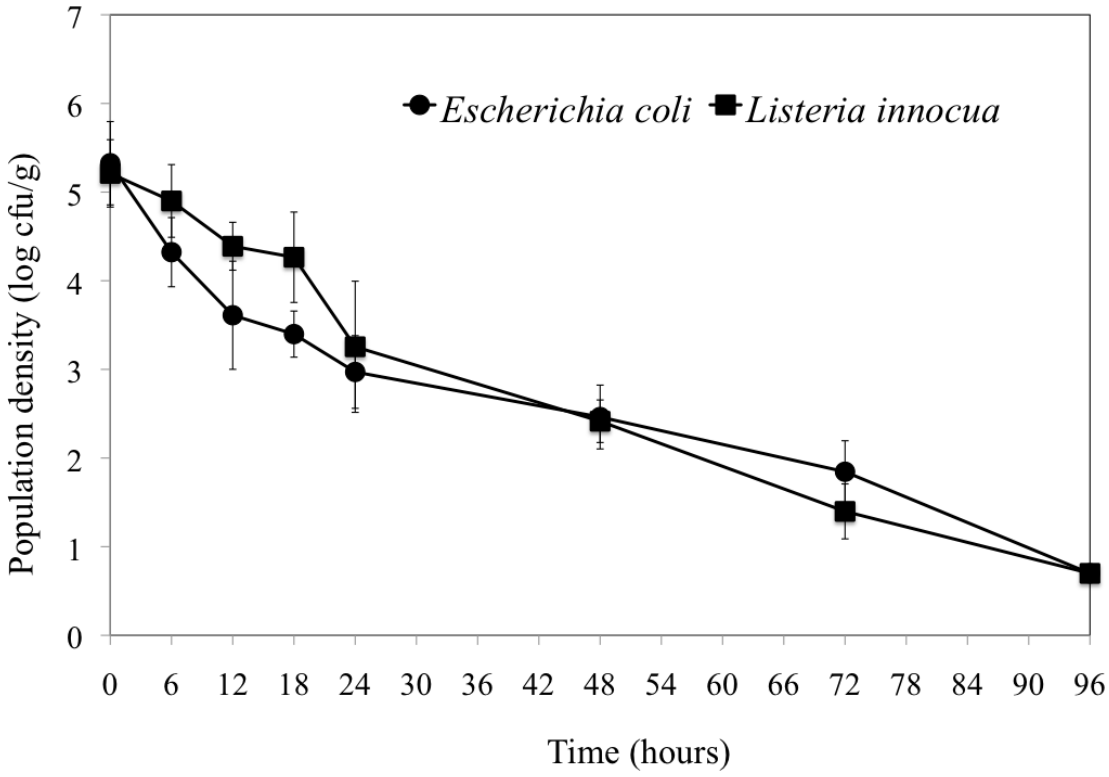

Fig 2: Survival curves of *E. coli* and *L. innocua* in soil-roots microcosm

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REFERENCES

[1] Garrow, J.S., Ralph, A., James, W.P.T., 2000. Human Nutrition and Dietetics, Elsevier, Amsterdam.

[2] Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and Infection, 4: 413-423.

[3] Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., Phatak, S.C., Silvoy, J.J., Davey, L., Payton, A.S., Liao, J., Doyle, M.P., 2010. Surface and internalized *Escherichia coli* O157:H7 into field-grown spinach and lettuce treated with spray-contaminated irrigation water. J. Food Prot. 73:1023–1029.

[4] Guo, X., Chen, J., Brackett, R.E., Beuchat, L.R., 2001. Survival of *Salmonella* on tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Appl. Environ. Microbiol. 67, 4760–4764.

[5] Orozco, L., Rico-Romero, L., Escartin, E.F., 2008. Microbiological profile of greenhouses in a farm producing hydroponic tomatoes. J. Food Prot. 71, 60–65.

[6] Stine, S.W., Song, I., Choi, C.Y., 2005. Effect of relative humidity on pre-harvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce, and bell peppers. J. Food Prot. 68, 1352–1358.

[7] Burke, G., 2008. Mexican peppers posed problem long before outbreak. Assoc. Press. Yahoo News, 08/18/2008.

[8] Beuchat, L.R., 1996. Pathogenic microorganisms associated with fresh produce. Journal of Food Protection, 59(2): 204–216.

[9] Islam, M., Morgan, J., Doyle, M., Phatak, S., Millner, P., Jiang, X., 2004. Fate of *Salmonella* enterica serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. Applied and Environmental Microbiology, 70(4): 2497–2502.

[10] Gurtler, J.B., Douds, D.D., Dirks,B., Quinlan, J.J., Nicholson, A.M., Phillips, J.G., Niemira, B.A., 2013. *Salmonella* and *Escherichia coli* O157:H7 survival in soil and translocation into leeks (*Allium porrum*) as influenced by an arbuscular Mycorrhizal Fungus (Glomus intraradices)Applied and Environmental Microbiology 79(6): 1813-1820.

[11] Neetoo, H., Lu, Y., Wu, C., Chen, H., 2012. Use of high hydrostatic pressure to inactivate preharvest contaminated green onions. Applied and Environmental Microbiology 78(6):

[12] Solomon, E.B., Yaron, S., Matthews, K.R., 2002. Transmission and internalization of *Escherichia coli* O157:H7 from contaminated manure and irrigation water into lettuce plant tissue. Appl. Environ. Microbiol. 68, 397–400.

[13] Cooley, M.B., Miller, W.G., Mandrell, R.E., 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae.* Appl. Environ. Microbiol. 69:4915–4926.

[14] Critzer, F., Doyle, M., 2010. Microbial ecology of foodborne pathogens associated with produce. Current Opinion in Biotechnology, 21(2): 125–130.

[15] Ingham, S.C., Losinski, J.A., Andrews, M.P., Breuer, J.E., Breuer, J.R., Wood, T.M., Wright, T. H., 2004. *Escherichia coli* contamination of vegetables grown in soils fertilized with non-composted bovine manure: Garden-scale studies. Applied and Environmental Microbiology 70, 6420 – 6427.

[16] Wood, J.D., Bezanson, G.S., Gordon, R.J., Jamieson, R., 2010. Population dynamics of *E. coli* inoculated by irrigation into the phyllosphere of spinach grown under commercial production conditions. International Journal of Food Microbiology 143, 198-204.

[17] Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., Phatak, S.C., Silvoy, J.J., Davey, L., Payton, A.S., Liao, J., Doyle, M.P., 2010. Infrequent *internalization* of *Escherichia coli* O157:H7 into field-grown leafy greens. J. Food Prot. 73, 500–506.

[18] Girardin, H., Morris, C.E., Albagnac, C., Dreux, N., Glaux, C., Nguyen-The, C., 2005. Behaviour of the pathogen surrogates *Listeria innocua* and *Clostridium sporogenes* during production of parsley in fields fertilized with contaminated amendments. FEMS Microbiology and Ecology 54, 287-295.

[19] Guo, X., van Iersel, M. W., Chen, J., 2002. Evidence of association of salmonellae with tomato plants grown hydroponically in inoculated nutrient solution. Applied and Environmental Microbiology 68, 3639–3643.

[20] Hintz, L.D., Boyer R.R., Ponder, M.A., Williams, R.C., Rideout, S.L., 2010. Recovery of *Salmonella* enterica Newport introduced through irrigation water from tomato (*Lycopersicum esculentum*) fruit, roots, stems, and leaves. Hort Science 45:675–67

[21] Zheng, J, Allard, S., Reynolds A., Millner, P., Arcea, G., Blodgetta, R.J., Brown, E.W., 2013. Colonization and internalization of *Salmonella enterica* in tomato plants. Appl Environ Microbiol. 79(8): 2494-2502

[22] Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F., Kloepper, J.W., 1997. Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43:895–91

[23] Bell, R.L., Cao, G., Meng, J., Allard, M.W., Keys, C., Hill, T., Ottesen, A., Brown, E.W., 2012. *Salmonella* Newport contamination of produce: ecological, genetic, and epidemiological aspects. In: AS Monte, PED Santos (eds), *Salmonella*: classification, genetics and disease outbreaks. Nova Science Publishers, Inc., Hauppauge, NY.

[24] Shi, X., Namvar, A., Kostrzynska, M., Hora, R., Warriner, K., 2007. Persistence and growth of different *Salmonella* serovars on pre- and postharvest tomatoes. J. Food Prot.70:2725–2731.

[25] Conner, D.E., Kotrola, J.S., 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. Appl. Environ. Microbiol. 61:382–385.

[26] Upreti, R., Thomas, P., 2015. Root-associated bacterial endophytes from *Ralstonia solanacearum* resistant and susceptible tomato cultivars and their pathogen antagonistic effects. Front. Microbiol. 6:255. Doi: 10.3389/fmicb.2015.00255

[27] Pollard, S., Barak, J., Boyer, R., Reiter, M., Gu, G., Rideout, S., 2014. Potential interactions between *Salmonella enterica* and *Ralstonia solanacearum* in tomato plants. Journal of Food Protection 77, 320–324

[28] Van der Schoot, C., van Bel, A.J.E., 1989. Architecture of the intermodal xylem of tomato (*Solanum lycopersicum*) with reference to longitudinal and lateral transfer. Amer. J. Bot. 76, 487–503.

[29] Goodman, R.N., Kira´ly, Z., Zaitlin, M., 1967. The biochemistry and physiology of infectious plant disease. Van Nostrand, Princeton, NJ.

[30] Prior, P. H., Beramis, M., Chillet, M., and Schmit, J. (1990). Preliminary studies for tomato bacterial wilt (Pseudomonas solanacearum E.F.Sm.) resistance mechanisms. Symbiosis 9, 393–400.

[31] Wells, J.M., Butterfield, J.E., 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Disease, 81, 867–872.

[32] Barak, J.D., Chu, B., Mills, D.C., 2003. Recovery of surface bacteria from and surface sanitization of cantaloupes. J. Food Prot. 66, 1805–1810.

[33] Rangarajan, A., Bihn, E.A., Pritts, M.P., Gravani, R.V., 2000. Food safety begins on the farm. Cornell University, Ithaca, NY.

[34] Nicholson, F.A., Groves, S.J., Chambers, B.J., 2005. Pathogen survival during livestock manure storage and following land application. Bioresour. Technol. 96, 135-143.

[35] Jablasone, J., Brovko, L.Y., Griffiths, M.W., 2004. A research note: the potential for transfer of *Salmonella* from irrigation water to tomatoes. J. Sci. Food Agric. 84:287–289

[36] Semenov, A.V., van Bruggen, A.H.C., van Overbeek, L., Termorshuizen, A.J., Semenov, A.M., 2007. Influence of temperature fluctuations on *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cow manure. FEMS Microbiol. Ecol. 60, 419-428.

[37] Habteselassie, M.Y., Bischoff, M., Applegate, B., Reuhs, B., Turco, R.F., 2010. Understanding the role of agricultural practices in the potential colonization and contamination by *Escherichia coli* in the rhizosphere of fresh produce. J. Food Prot. 73:2001–2009.

[38] Feeney, D.S., Crawford, J.W., Daniell, T., Hallett, P.D., Nunan, N., Ritz, K., Rivers, M., Young, I.M., 2006.Three-dimensional microorganization of the soil-root-microbe system. Microbial Ecol. 52:151–158.

[39] Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T., Achouak, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2:1221–1230.

[40] Ibekwe, A.M., Watt, P.M., Shouse, P.J., Grieve, C.M., 2004. Fate of *Escherichia coli* O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR. Can. J. Microbiol. 50:1007–1014.

[41] Fremaux, B., Delignette - Muller M.L., Prigent – Combaret, C., Gleizal, A., Vernozy – Rozand, C., 2007. Growth and survival of non - O157:H7 Shiga- toxin- producing *Escherichia coli* in cow manure. J Appl Microbiol. 102 (1): 89 – 99.

[42] Chandler, D.S., Craven, J.A., 1980. Relationship of soil moisture to survival of *Escherichia coli* and *Salmonella* Typhimurium in soils. Aust. J. Agric. Res. 31: 547-555.

[43] Dowe, M.J., Jackson, E.D., Mori, J.G., Bell, C.R, 1997. *Listeria monocytogenes* survival in soil and incidence in agricultural soils. Journal of Food Protection 60(10): 1201 –1207 .

[44] Palacios M.P., Lupiola P., Fernandez-Vera F., Mendoza V., Tejedor M.T., 2007. *Salmonella* transport and persistence in soil and plant irrigated with artificially inoculated reclaimed water: climatic effects. In: Lamaddalena N. (ed.), Bogliotti C. (ed.), Todorovic M. (ed.), Scardigno A. (ed.). Water saving in Mediterranean agriculture and future research needs [Vol. 2], pp. 199- 205.

[45] Barak, J.D., Liang, A.S., 2008. Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. Plos One. 3:1657-1659.

[46] Micallef, S.A., Rosenberg, R.E., Goldstein, A., George, L., Kleinfelter, M.S., Boyer, C.R., Mclaughlin, A., Estrin, L., Ewing, J., Beaubrun, D.E., Hanes, MH. Kothary, B.D., Tall, J.H., Razeq, S.W., Joseph, A.R., Sapkota, A.R., 2012. Occurrence and antibiotic resistance of multiple *Salmonella* serotypes recovered from water, sediment and soil on mid-Atlantic tomato farms. Environ. Res. 114:31–3922406288

[47] Bernstein, N., Sela, S., Neder-Lavon, S., 2007. Effect of irrigation regimes on persistence of *Salmonella enterica* serovar Newport in small experimental pots designed for plant cultivation. Irrig. Sci. 26:1–8

[48] Franz, E., Visser, A.A., Van Diepeningen, A.D., Klerks, M.M., Termorshuizen, A.J., van Fröder, H., Martins, G., De Souza, L., Landgraf, M., Franco, B., Destro, T., 2007. Minimally processed vegetable salads: microbial quality evaluation. Journal of Food Protection,

[49] Fenlon, D.R., 1985. Wild birds and silage as reservoirs of *Listeria* in the agricultural environment. J Appl Bacteriol. 59, 537–543.

[50] Jablasone, J., Warriner, K., Griffiths, M., 2005. Interactions of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. International Journal Food Microbiology 99, 7–18.

[51] Wei, C.I., Huang, J.M., Lin, W.F., Tamplin, M.L., Bartz, J.A., 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. J. Food Prot. 8, 829–836.

[52] Todar, K., 2005. *Salmonella* and salmonellosis. In: Todar's online textbook of bacteriology.

Author' biography with Photo

Hudaa Neetoo joined the Faculty of Agriculture of the University of Mauritius in August of 2013. Her area of specialization is Microbiology and Food Safety. Prior to joining academia, she worked as Microbiologist and Head of Laboratories in a tuna processing industry, Thon des Mascareignes Ltee. As a budding researcher, Hudaa wants to initiate intra and inter-faculty collaborations with various colleagues but she also wants to maintain industrial ties. Hudaa hopes that her academic career will enable her to give back to the community through outreach activities, student mentoring and graduate empowerment programs.