USDA Agricultural Research Service

Characterization and Mitigation of Bacterial Pathogens in the Fresh Produce Production and Processing Continuum

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Objectives

- **Objective 1**: Contamination of enterohemorrhagic *Escherichia coli* (EHEC), *Salmonella,* and *Listeria* to fresh produce at the farm level.
- **1a.** Population dynamics of non-pathogenic *E. coli* and non-O157 EHEC in soils amended with biological soil amendments (BSA).
- ***1b.*** Persistence of EHEC, *Salmonella* and *Listeria* in soils amended with BSA.

- **Objective 2:** Multispecies biofilm formation, and dissemination of pathogens in processing environments and on contamination of fresh produce.
- **2a, b.** Biofilm formation of pathogens; environmental bacterial strains/species that promote multispecies biofilm formation.
- **2c.** Biofilm formation of non-O157 shiga-toxigenic *E. coli* (STEC) on abiotic and biotic surfaces.

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Objectives (2)

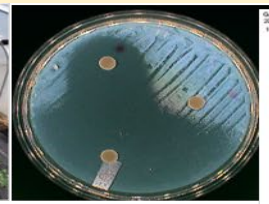
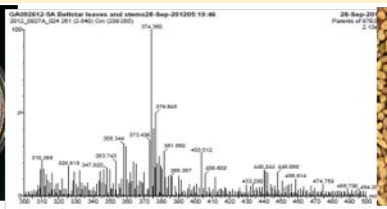
- **Objective 3:** Intervention strategies to minimize contamination of EHEC, *Salmonella* and *Listeria* on fresh produce at the farm level.
- **3a.** *Brassica* vegetables in controlling enteric pathogens in soil.
- **3b.** Control of *Listeria* and *Salmonella* in cantaloupe at the farm.

- **Objective 4**: Intervention technologies to reduce pathogen survival and growth during processing and retail operations.
- **4a.** Identify and validate food safety preventive controls for water application during fresh-cut processing.
- **4b.** Novel antimicrobials to control enteric pathogens on fresh produce.

- **Objective 5:** Microbial safety of fresh produce grown under non-conventional farming practices.
- **5a.** Reclaimed water on microbial safety of fresh produce.

3a. *Brassica* vegetables for controlling pathogens



Studies:

1. Antimicrobial activity of glucosinolate-hydrolysis compounds (GHC)
2. *E. coli* O157:H12 in soil treated with Benzyl isothiocyanate (BIT)
3. *E. coli* O157:H12 survival in soil tilled over with broccoli cultivars (Arcadia, Bellstar, Diplomat, Green Magic, Imperial, Marathon) – Field study
4. BIT for controlling *Salmonella* on alfalfa seeds

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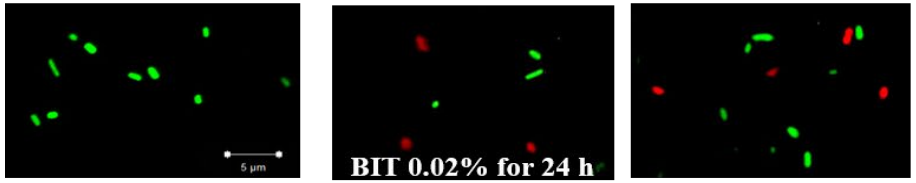
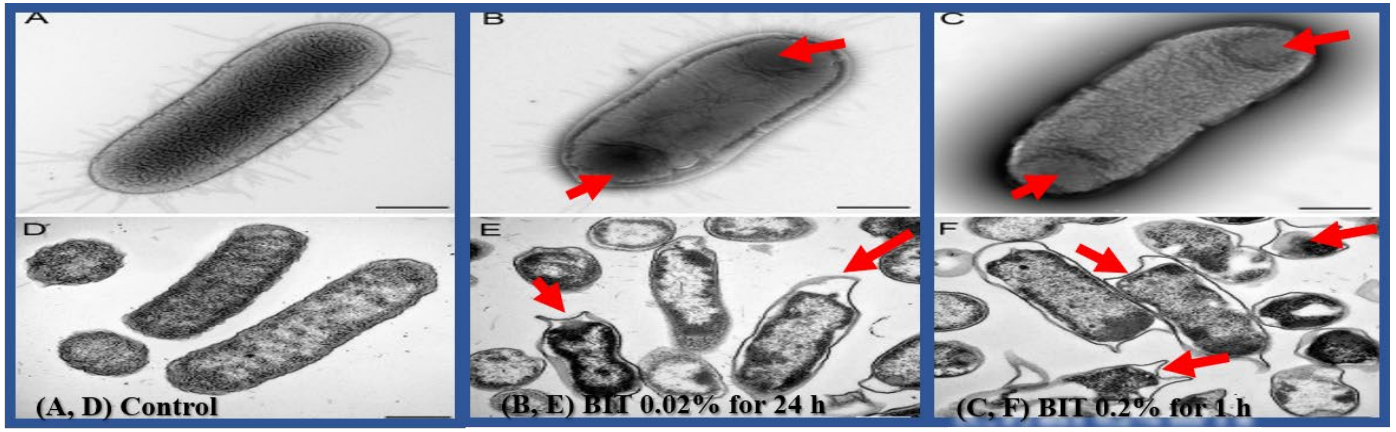
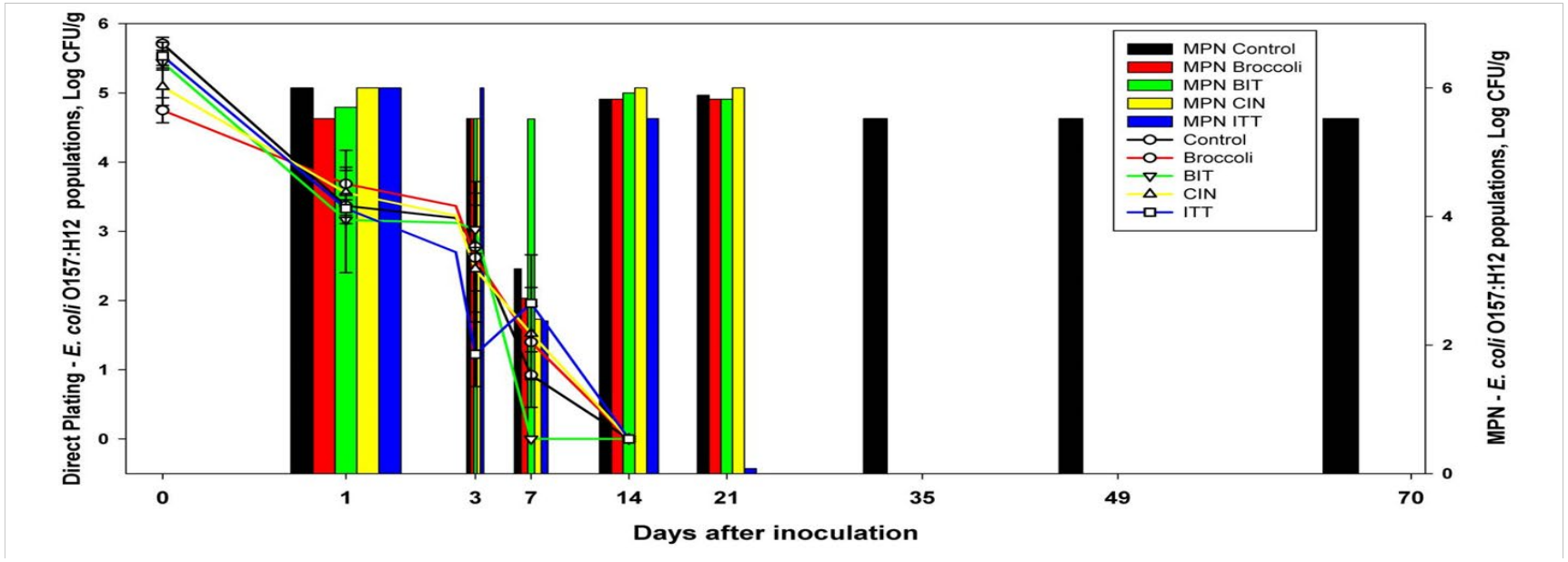
BIT microscopy

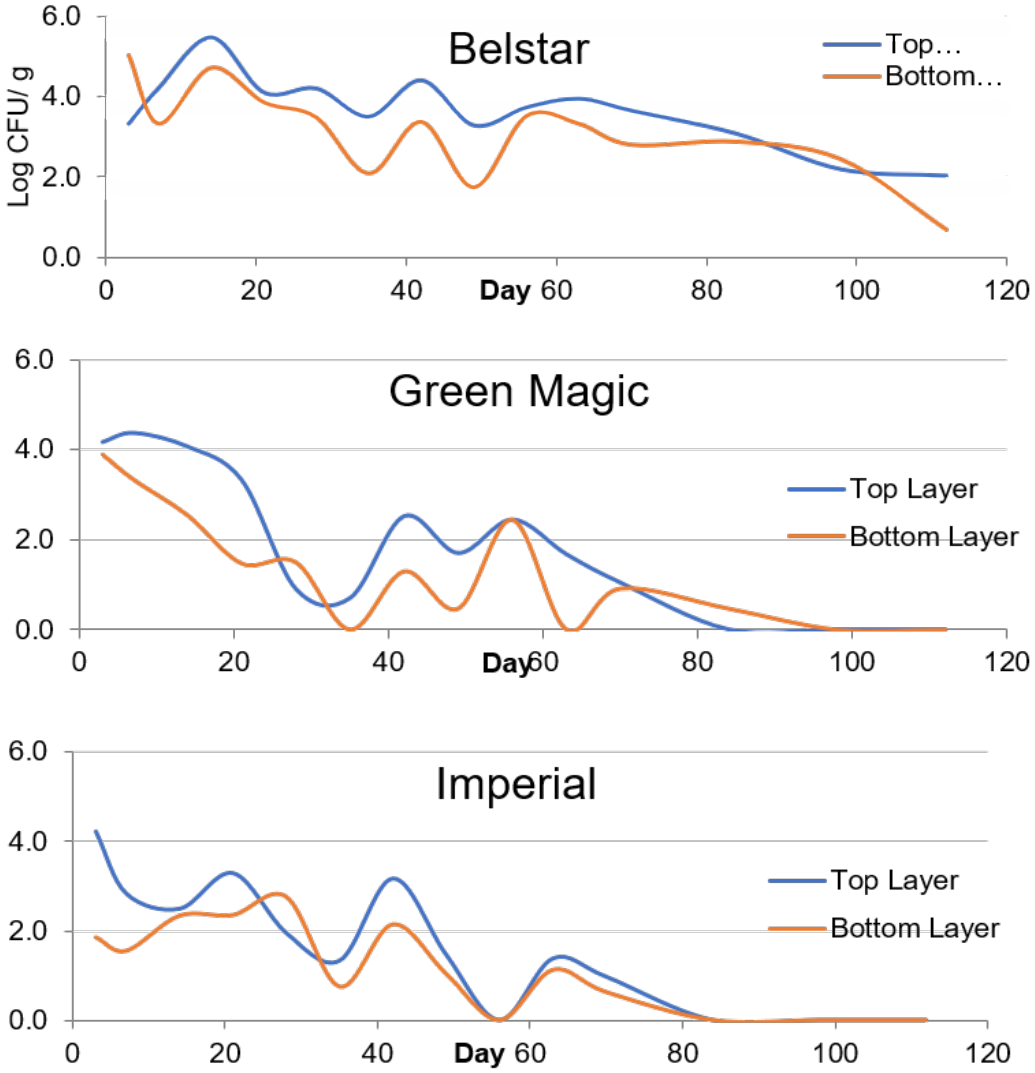




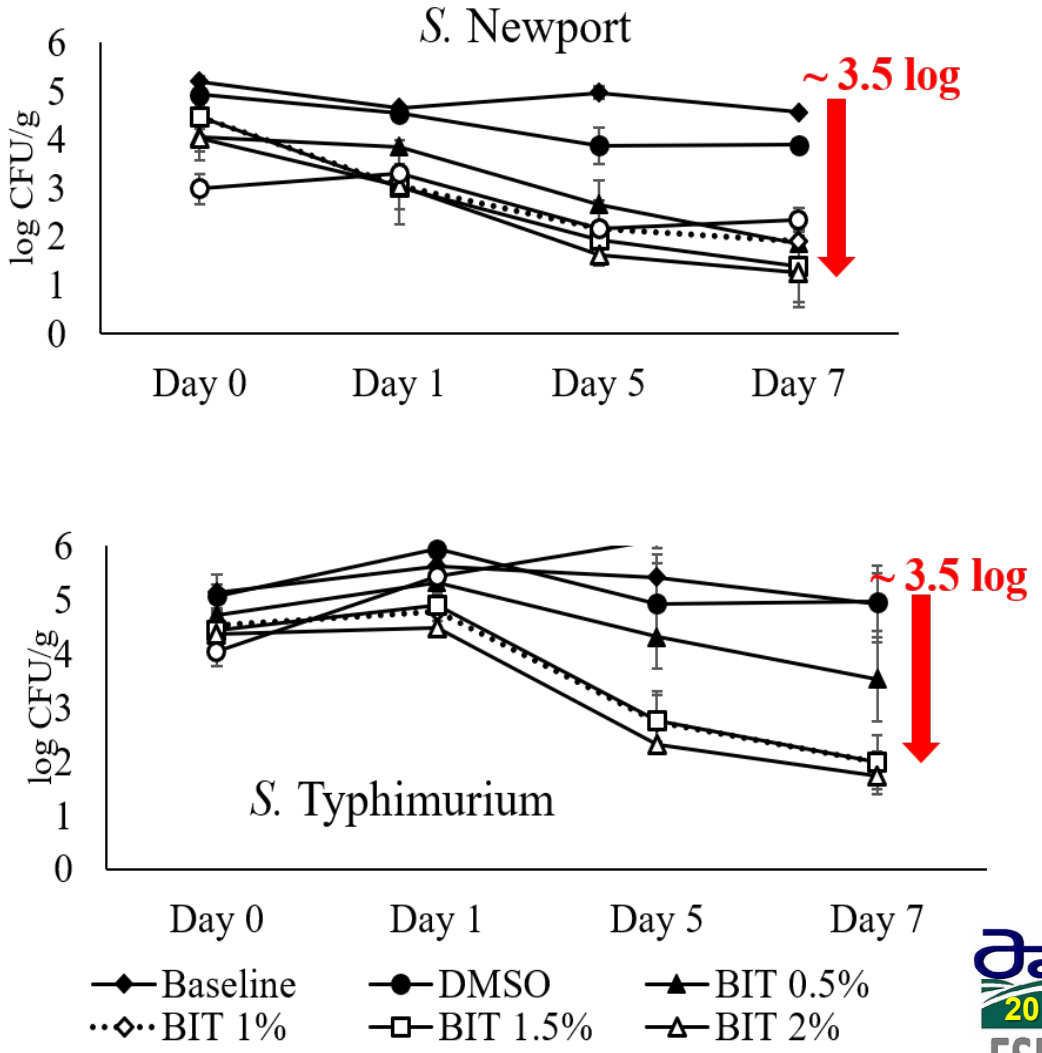
- ***E. coli* O157:H12 survival in soil inoculated with GHC**



E. coli O157:H12 survival in soil tilled over with broccoli



Salmonella populations on BIT-treated alfalfa seeds



Summary

- BIT was superior to other GHC in antimicrobial activity against *Salmonella* and *E. coli* O157:H7
- GHC content of broccoli varied with cultivar and part (root, stem, floret) of the plant
- Antimicrobial effect of Green Magic, Imperial, and Marathon cultivars of broccoli was more pronounced than the effect of Arcadia, Bellstar, or Diplomat cultivars.
- BIT at 1.5% and 2% exerted comparable anti-*Salmonella* activities to 20,000 ppm chlorine on alfalfa seeds at days 5 and 7.
- Germination rate of 0.5%-2% BIT treated alfalfa seeds (~85-87%) was not significantly different from control or chlorine-treated seeds (~90%).

5. Microbial safety of fresh produce irrigated with alternative (reclaimed, primary-, roof) waters

Studies:

1. Novel procedure for microbial analysis of irrigation water (Concentrator®)
2. Spinach irrigation with alternative waters and indicator bacteria – growth chamber study
3. Field study with alternative irrigation waters – seasonal effect
4. Lettuce cultivar effect on persistence of surrogate bacteria in alternative waters

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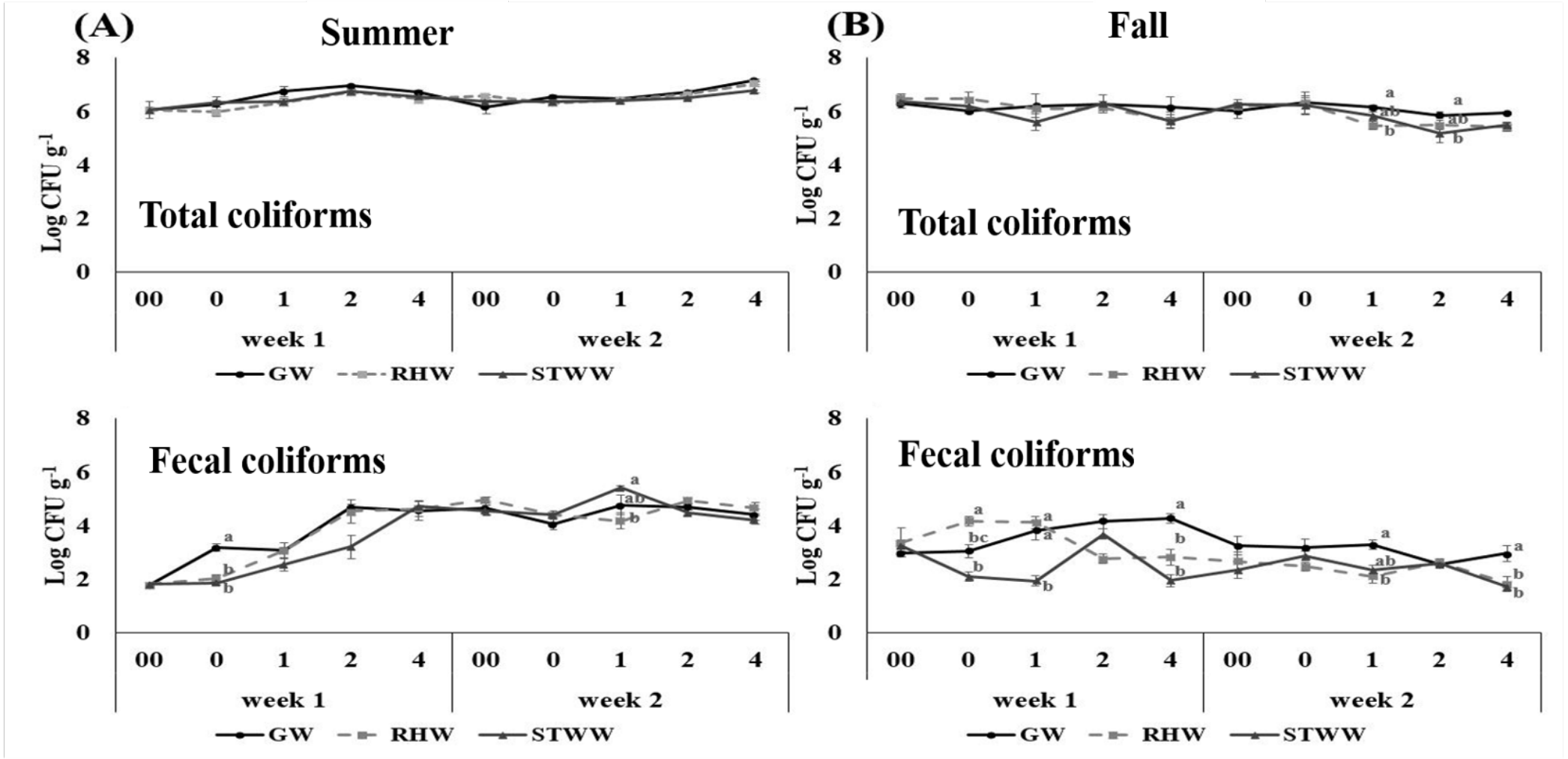
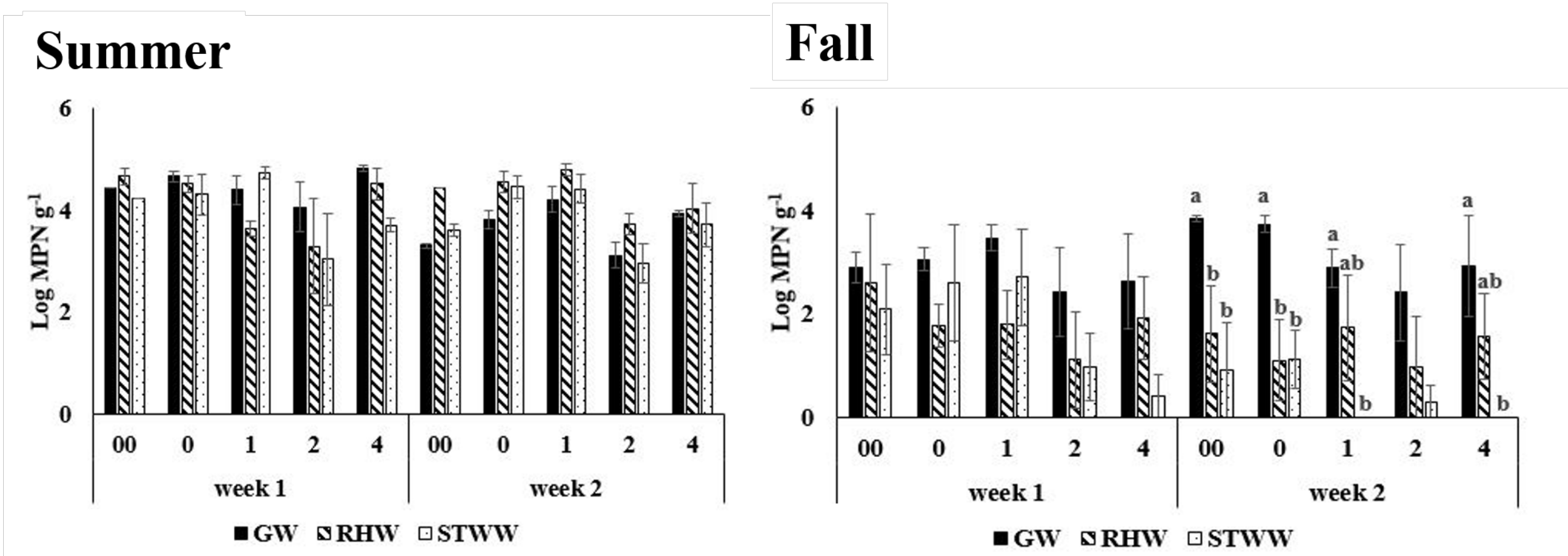
Total and fecal coliforms on spinach irrigated with alternative irrigation waters



GW: ground water; RHW: roof-harvest water; STWW: secondary-treated wastewater

Generic *E. coli* on spinach irrigated with alternative irrigation waters



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Lettuce cultivars irrigated with alternative irrigation waters

***E. coli* H12**

Cultivar	Water source	Day 0	Day 1	Day 3	Day 7	Day 10
Annapolis	Control	4.3±0.0	2.8±0.2	2.1±0.1	1.2±0.0	1.0±0.0
	RHW	4.1±0.1	2.9±0.2	2.0±0.0	1.9±0.1	1.1±0.4
	STWW	4.2±0.0	3.1±0.2	2.0±0.1	1.7±0.3	1.0±0.0
Celinet	Control	4.4±0.0	3.4±0.1	1.7±0.3	1.6±0.0	1.4±0.2
	RHW	4.6±0.0	4.0±0.1	2.9±0.1	1.0±0.0	1.3±0.4
	STWW	4.1±0.0	3.5±0.1	1.8±0.4	1.8±0.2	1.4±0.2
Coastline	Control	4.5±0.0	3.6±0.2	2.2±0.2	1.9±0.2	0.8±0.1
	RHW	4.4±0.0	3.9±0.1	1.2±0.1	2.1±0.2	1.0±0.1
	STWW	4.3±0.0	3.2±0.1	1.5±0.2	1.6±0.3	1.0±0.0

***E. coli* K12**

Cultivar	Water source	Day 0	Day 1	Day 3	Day 7	Day 10
Annapolis	Control	3.3±0.0	2.5±0.3	1.6±0.5	<1	<1
	RHW	3.2±0.1	2.2±0.2	0.7±0.4	<1	<1
	STWW	3.4±0.0	2.4±0.3	1.8±0.5	<1	<1
Celinet	Control	3.5±0.0	2.6±0.1	<1	<1	<1
	RHW	3.7±0.0	2.6±0.1	1.1±0.5	<1	<1
	STWW	3.3±0.0	2.5±0.1	1.2±0.3	<1	<1
Coastline	Control	3.6±0.0	2.7±0.2	1.1±0.2	<1	<1
	RHW	3.8±0.1	2.7±0.2	0.5±0.3	<1	<1
	STWW	3.4±0.1	2.6±0.1	2.5±0.2	<1	<1

Summary (1)

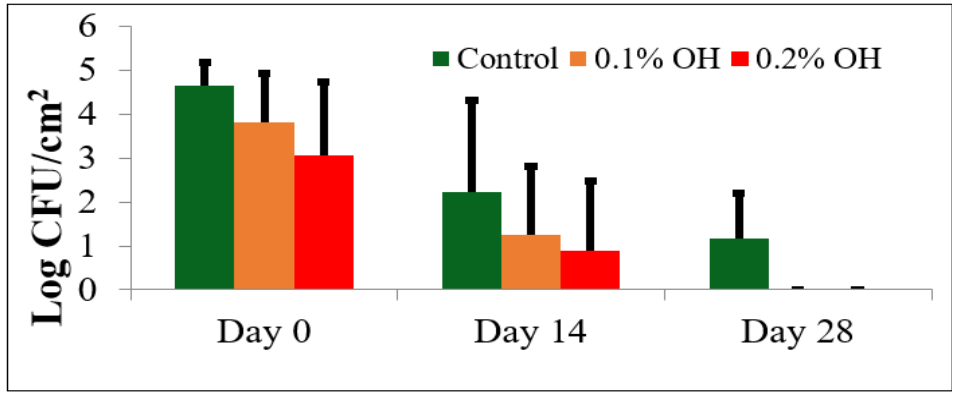
- *Salmonella* and *E. coli* O157:H7 were detected by membrane filtration and concentrator procedures
- Generic *E. coli* in secondary-treated waste water were above FSMA limit (> 3.0 log CFU/100 ml)
- Bacterial indicators were lower in roof-harvest water
- *E. coli* positive spinach samples increased when irrigated with STWW; repeat irrigation resulted in higher number of positive spinach leaves.
- Fecal coliforms and *E. coli* populations were higher in alternative water used for irrigation.
- E. coli H12 persisted at higher level on Celinet lettuce than Annapolis or coastline cultivar
- No significant correlation between *E. coli* in water and transfer on spinach leaves

Pre-harvest control of pathogens on cantaloupe

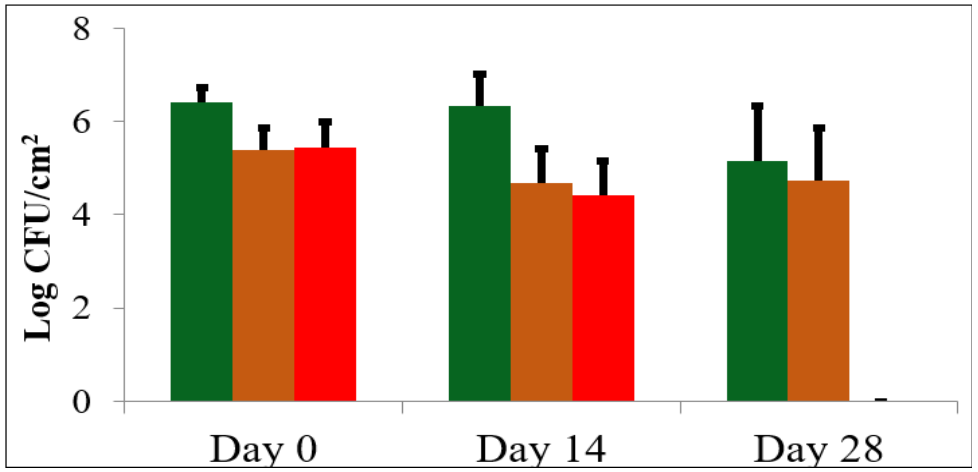
- Cantaloupes grown in High Tunnel and open field
- Inoculation of surrogate bacteria (*L. innocua, E. coli* O157:H12) on cantaloupes followed by spraying with antimicrobial
  - -Octenidine dihydrochloride (OH)
  - -Lactic acid bacteria
- Cantaloupe harvest and analysis

Cantaloupe studies at farm (Beltsville, Chambersburg)

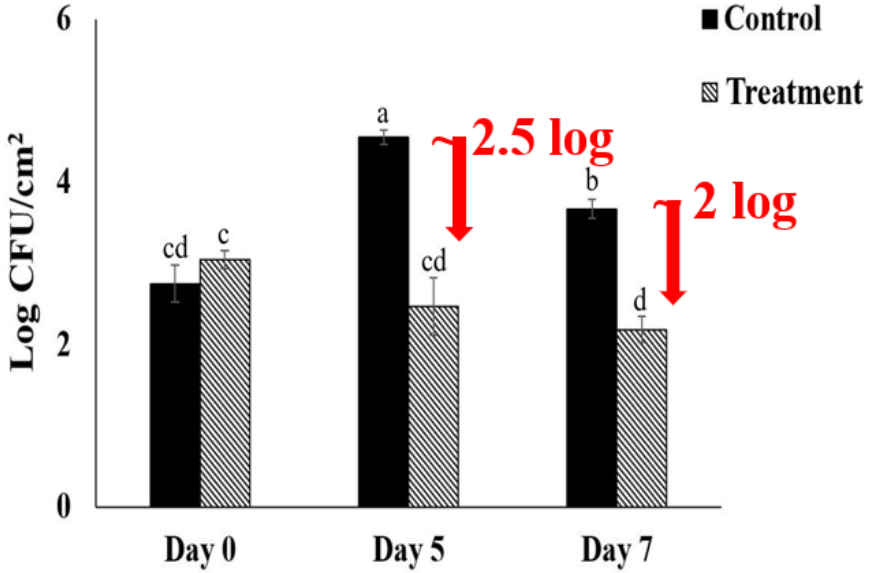
Effect of OH on *E. coli* O157:H12



Effect of OH on *L. innocua*



LAB reduces *L. innocua*



Summary (2)

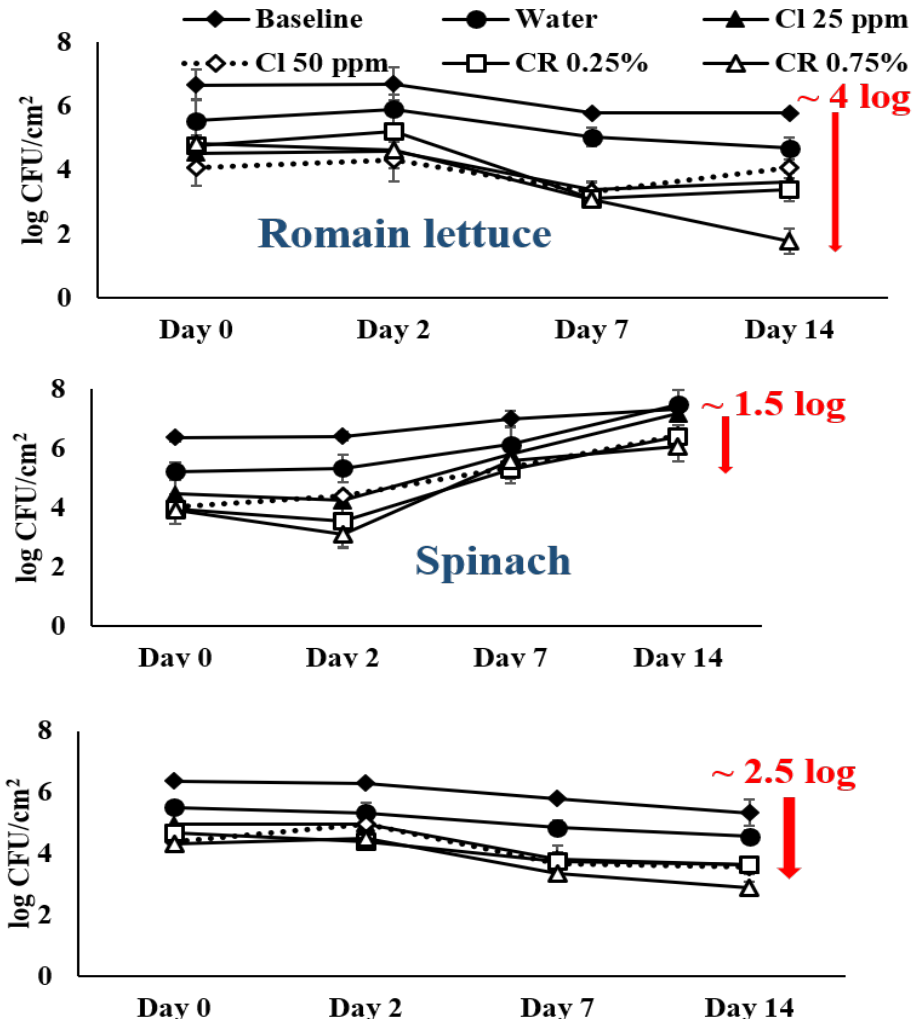
- *Listeria* and *E. coli* were reduced by 1.0 and 2.0 log CFU/cm^2, respectively on cantaloupe following OH treatment
- *Listeria* was ~2 log CFU/cm^2 lower on OH-treated cantaloupes harvested after 28 days compared to control; *E. coli* were undetectable in OH-treated cantaloupes
- *L. plantarum* and *P. pentosaceus* reduced *Listeria* by 2-2.5 log CFU/cm2 on cantaloupes harvested at 7 days

4b. Novel antimicrobials

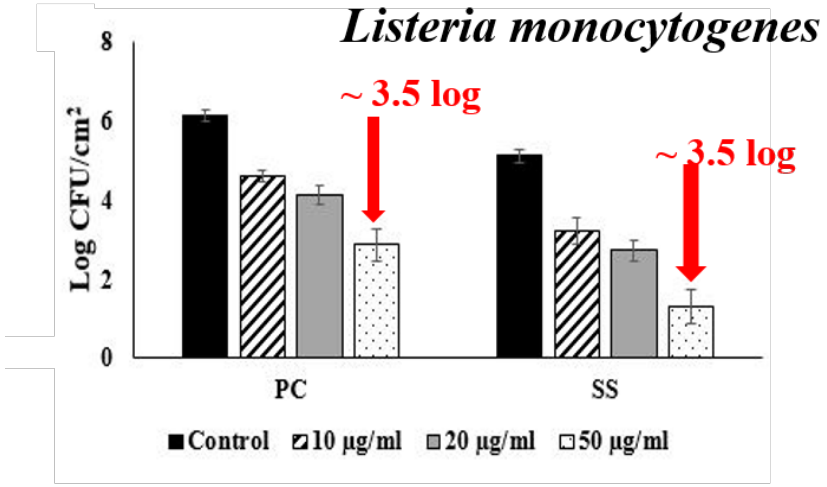
Studies:

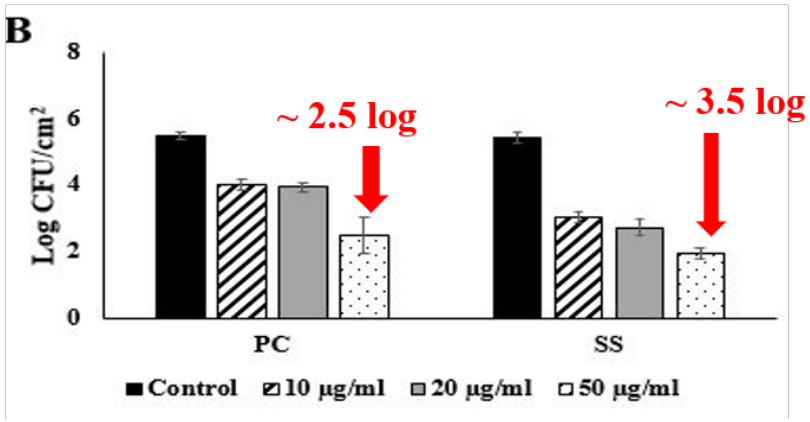
1. Plant-based essential oils on fresh herbs
2. Lactic acid bacteria on strawberries
3. Nano-emulsion on fresh produce
4. Peptides on equipment surfaces

Nano-emulsified carvacrol on EHEC



Biofilm removal by peptide on equipment surface





Summary (3)

- A wash treatment with 0.3% carvacrol or 0.5% cinnamaldehyde reduced *Salmonella* and *E. coli* O157:H7 by ~5 log CFU/g on fresh herbs (Basil, Parsley, Cilantro, Dill, Tarragon). The recovery of these pathogens were lower in treated herbs during storage.
- Fresh herbs treated with 0.1% carvacrol or 0.3-0.5% cinnamaldehyde were not visually different ($P > 0.05$) from control.
- LAB reduced *L. monocytogenes* by ~ 2.0 and ~ 2.5 log CFU/g in strawberries at 3 and 7 days storage, respectively at 4 and 10°C.
- LAB also reduced *Salmonella* by 2.5-3.0 log CFU/g in strawberries stored at 4°C; *S.* Thompson was the most sensitive serovar among the three pathogens used in the study.

Summary (4)

- Nanoemulsified carvacrol reduced ~2 log EHEC on lettuce and spinach following treatment. Up to 4 log reduction was observed in Romaine lettuce treated with carvacrol after 14 days storage
- The carvacrol at 0.75% against EHEC were superior to 25 and 50 ppm chlorine on both lettuces on day 14
- Peptide reduced 2.5-3.5 log CFU/cm^2 *L. monocytogenes* and EHEC in biofilms formed on polycarbonate and st. steel surfaces

2c. Biofilm Formation of Non-O157 STEC

Studies

- STEC (O26, O45, O103, O121, and O145) on St. steel, polycarbonate and PTFE Surfaces
- Curli and cellulose assays, hydrophobicity, MBEC assay
- Persistence on Cabbage, romaine and spinach during storage at 4°, 10° and 22°C

Summary (5)

- Curli producing non-O157 strains formed strong biofilm on equipment and leaf surfaces
- Most strains formed stronger biofilm on PTFE and polycarbonate surfaces than the stainless steel surface
- Persistence of non-O157 serotypes on fresh produce varied among STEC serotypes, produce surface, and incubation temperature
- Leaf topography influenced biofilm formation on fresh produce
- Weak biofilm formation was observed on fresh produce at 22°C

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