Antimicrobial Mitigation via Saponin Intervention on *Escherichia coli* and Growth and Development of Hydroponic Lettuce

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Summary. **Various saponins have demonstrated allelochemical effects such as bactericidal impacts as well as antimycotic activity against some plant pathogenic fungi, thereby acting to benefit plant growth and development. A commercial saponin solution was evaluated for bactericidal effects against *Escherichia coli* and growth of lettuce (*Lactuca sativa*) in a hydroponic system. *E. coli* (P4, P13, and P68) inoculum at final concentration of 10^8 colony-forming units (cfu)/mL was added to 130 L of a fertilized solution recirculating in a nutrient film technique (NFT) system used to grow 'Rex' lettuce. After 5 weeks in the NFT system, *E. coli* populations were lowest in the inoculated treatment that did not contain any saponin addition (0.89 log cfu/mL) when compared with all other inoculated treatments ($P < 0.001$). The treatment containing 100 $\mu g \cdot mL^{-1}$ saponin extract had an *E. coli* population of 4.61 log cfu/mL after 5 weeks that was higher than treatments containing 25 $\mu g \cdot mL^{-1}$ or less ($P < 0.0001$). Thus, higher *E. coli* populations were observed at higher saponin concentrations. Plant growth was also inhibited by increasing saponin concentrations. Fresh and dry shoot weight were both higher in the inoculated and uninoculated treatments without the saponin addition after 5 weeks in the NFT system ($P < 0.0001$). Lettuce head diameter was smaller when exposed to saponin treatments with concentrations of 50 and 100 $\mu g \cdot mL^{-1}$ ($P < 0.0001$). Lettuce leaves were also tested for the potential of *E. coli* to travel systemically to the edible portions of the plant. No *E. coli* was found to travel in this manner. It was concluded that steroidal saponins extracted from mojave yucca (*Yucca schidigera*) are not an acceptable compound for use in mitigation of *E. coli* in hydroponic fertilizer solution due to its ineffectiveness as a bactericide and its negative impact on lettuce growth.**

Every year, 48 million Americans become infected from a foodborne disease; 128,000 of whom require hospitalization resulting in 3000 deaths [Centers for Disease Control and Prevention (CDC), 2019b]. Although healthy foods are an important part of a well-rounded diet, there are concerns, as fruits, vegetables, and nuts accounted for 23% of reported human foodborne illness outbreaks between 2009 and 2015 (CDC, 2017a, 2017b). *Escherichia coli* is one of the most prominent causes of foodborne diarrheal disease in humans. It is also a leading contributor to bacterial infections and extraintestinal infections in humans and animals alike (Njage and Buys, 2014). A primary source of *E. coli* infection in the United States is through contaminated agricultural products. In 2019, the CDC reported two multistate outbreaks related to *E. coli* O157:H7 in leafy greens (CDC, 2019a).

Controlled environment agriculture (CEA) production facilities, such as greenhouses and plant factories, present advantages over traditional field production, such as crop space efficiency and year-round growth, and they also provide a reduced risk for food safety issues (Holvoet et al., 2015). Although contamination risks are reduced in CEA, they are not eliminated (Orozco et al., 2008) and therefore water quality used for irrigating crops is a concern, as contaminated source water (municipal, holding pond, or well) or fertilizer solution can splash onto crops during production and harvesting, leading to *E. coli* infection in humans (Solomon et al., 2003). Additional risks within CEA pre- and postharvest production include pest and wild animal management, planting substrates, unsanitary equipment and buildings, and human handling (Holvoet et al., 2015).

Within CEA, an operational choice of production system establishes the likelihood of microbial spread in the event of contamination. Hydroponic production is a popular practice among CEA producers, adding greater efficiency and control to their cultivation processes over traditional farming practices. *E. coli* exhibits the ability to thrive in fertilizer solutions in a hydroponic system (Shaw et al., 2016). This presents a unique problem for growers who use a recirculating hydroponic system. Because a fertilizer solution is collected and recycled through irrigation lines, a microbial contaminant has the potential to infect not some, but the entire crop, continually recycling a solution of pathogens (Premuzic et al., 2007).

Although contamination potential remains low in CEA, preventive measures to disinfest source water must be a focal point for food safety and public health. Cultural practices, such as personal hygiene and sick employee protocols, will always remain an important area in produce production, but further disinfestation

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	µL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
1	mmho/cm	$dS \cdot m^{-1}$	1
1	ppm	$mg \cdot L^{-1}$	1
1	ppm	$\mu g \cdot mL^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

measures will help safeguard a crop to be free of microbial pathogens like *E. coli*. Current techniques used to mitigate microbial pathogens can be effective but have cost and complexity limitations that prohibit their use to many farmers. Ultraviolet radiation is effective and widely used; however, small pathogens may pass by the light waves in the shadow of debris and remain active, therefore filtration of the water and cleaning of the ultraviolet lamp (Garibaldi et al., 2004) are paramount to this method's usefulness. Biofiltration may reduce pathogens, but does not eliminate them (Belbahri et al., 2007; Wohanka, 1995). Chlorination may cause phytotoxic symptoms to plants (Premuzic et al., 2007) and produce the byproduct trihalomethane, which is classified by the U.S. Environmental Protection Agency as a potential human carcinogen (Symons et al., 1981).

Natural antimicrobials are becoming more prevalent among microbial disinfestation methods in the food industry (Zhu et al., 2015). Plant-based isolated compounds contain secondary metabolites that are known to retard or inhibit the growth of bacteria, yeasts, and molds (Tiwari et al., 2009). Saponins are secondary metabolites widely distributed throughout the plant kingdom and have been documented to exhibit natural antibacterial properties (Lokesh et al., 2016; Wallace, 2004). Saponins are nonionic detergents that have an assortment of biological properties. Their structure is composed of a steroidal or triterpenoid aglycone skeleton attached to one or more sugar chains (Arabski et al., 2011). This diversity in structure is what leads to the great diversity in biological properties. Beyond their bactericidal functions, saponins also display antifungal, hemolytic, membrane-depolarizing, ammonia-binding, antiyeast, antimold (Arabski et al., 2011; Oleszek, 1996), and many other natural biological properties. Their effects are generally credited to their ability to permeate cellular membranes (Francis et al., 2002).

The following study was conducted to investigate the antimicrobial properties of steroidal saponins extracted from mojave yucca (*Yucca schidigera*) on gram-negative *E. coli* in a hydroponic fertilizer solution over time. In addition, the growth and development of lettuce (*Lactuca sativa*) grown in an NFT hydroponic system was evaluated for yield parameters.

Materials and methods

Sterility of components. Before each replication, all materials (lettuce seedlings, municipal tap water, and hydroponic equipment) used in this study were analyzed (using the same enumeration protocol in the section "Data Collection and Bacterial Enumeration") and found to be negative for the presence of *E. coli* cfu (data not shown; detection limit was 100 cfu/mL).

Bacterial cultures. Individual isolates of nonpathogenic *E. coli* (P4, P13, and P68) were obtained from the culture collection of the Microbial Food Safety Laboratory, Iowa State University, Ames, IA. Isolate selections were based on behavioral similarities to *E. coli* O157:H7 (Marshall et al., 2005). All strains were adapted to grow in the presence of 80 $\mu g \cdot mL^{-1}$ rifampicin (Thermo Fisher Scientific, Waltham, MA), through stepwise exposure (Parnell et al., 2005). Parnell procedure: Briefly, 100 µL of an overnight culture was spread onto plate count agar containing antibiotic. After incubation for 24 h at 37 °C, isolated colonies were selected from the plate containing the highest level of antibiotic and cultured overnight in nutrient broth. This procedure was repeated until a variant resistant to 80 $\mu g \cdot mL^{-1}$ rifampicin was obtained. Growth curves of the parent and variant strains were similar in tryptic soy broth (Difco Laboratories, Detroit, MI) (data not shown). Bacterial strains were subsequently combined into a cocktail suspended in a cryoprotective glycerol solution and stored at –21 °C. Frozen cultures were thawed in cold water, diluted 1:10 in buffered peptone water (BPW), and incubated at 37 °C for 24 h to yield a population of $\approx 10^8$ cfu/mL. The resulting solution was used to inoculate irrigation water in the following experiment.

Lettuce growing conditions, inoculation, and saponin solution. Under ambient greenhouse light, foam hydroponic seed germination media (276-cell count Horticubes; Smithers Oasis, Kent, OH) was placed in sub-irrigated hydroponic propagation trays (American Hydroponics, Arcata, CA), where they were leached, and seeded with 'Rex' lettuce (Johnny's Selected Seeds, Winslow, ME). Greenhouse temperature setpoints were set to cool at 21 °C and heat at 18 °C. Average recorded daily light integral across all experimental replications was 18 $mol \cdot m^{-2} \cdot d^{-1}$. Before seedling transplantation, six separate NFT systems were filled with 130 L tap water and allowed to recirculate for 24 h. At this point, selected systems were inoculated with 20 mL of *E. coli* cocktail to obtain a population of $\approx 10^4$ cfu/mL. Dissolved fertilizer salts and 1 M sulfuric acid (H_2SO_4) were added to create 130 L of fertilizer solution with an electrical conductivity of 1.4 $dS \cdot m^{-1}$ and a pH of 5.9, which was maintained daily in all systems throughout the study. Subsequently, a saponin intervention was added. The saponin product used in this study was supplied in a premixed solution (Micro-Aid Liquid 50; DPI Global, Porterville, CA), and contained sapogenin with a steroidal aglycone structure extracted from mojave yucca. The solution was certified by the Organic Materials Review Institute and contained 14% saponins. It was supplied to each 130-L irrigation reservoir at treatment rates of 0, 12.5, 25, 50, or 100 $\mu g \cdot mL^{-1}$ a.i. Saponin treatments were randomly assigned to each NFT system. The methods reported here were repeated in three experimental replications. System water loss due to transpiration and evaporation was replenished weekly with a commensurate amount of saponin solution. Seedlings were transplanted into the NFT systems at the four-true-leaf stage.

Data collection and bacterial enumeration. Fertilizer solution samples of 25 mL were taken at 1 h after inoculation and then 1, 168, 336, 504, 672, and 840 h after saponin intervention. To ensure a homogeneous sample, 5-mL aliquots were taken from five separate locations within each system: top half of the nutrient reservoir, lower half of the nutrient reservoir, drain collector, NFT channel, and dripper emitter. These samples were used to evaluate viable *E. coli* populations in each NFT system.

Enumeration of *E. coli* populations was determined by serial dilutions using BPW as the dilution

solution. Dilutions were plated on MacConkey agar (0.1% rifampicin) containing a tryptic soy agar (TSA) overlay using a spread plate technique. Plates were incubated at 37 °C for 24 h before manual counts. Presence of rifampicin was to ensure bacteria were accurately detected in the presence of high natural flora.

Five lettuce plants were selected using a random number generator and collected for analysis from each treatment after 5 weeks (840 h) post-transplant into the NFT systems. The first three plants selected were evaluated for growth characteristics and the subsequent two plants were tested for the presence of *E. coli*.

Plants that had been designated for measuring growth characteristics were weighed immediately to determine fresh shoot weight on a digital balance (AP250D; Ohaus, Parsippany, NJ). Lettuce plant diameter was measured at the widest point before plants were placed inside a paper bag and into an oven. Plants were heated at 70 °C for 2 d to fully desiccate before obtaining dry weights.

Plants that had been designated to be tested for *E. coli* presence were harvested as described earlier and then immediately transferred into 14 × 19-inch sterile sample bags (Nasco, Fort Atkinson, WI). Plant weight was determined using a digital balance (AP250D) to create a 1:10 (w/v) dilution with deionized water. The bag contents were then manually stomached to suspend internal microorganisms. The resulting solution was used to determine presence or absence of *E. coli* on or within the edible portions of the lettuce leaves by the enumeration techniques described previously.

Statistical analysis. Quantification of *E. coli* concentration samples were log ($\log_{10}$) transformed before analysis. Each sample was unilaterally increased by one to prevent syntax error to any zero counts. The noninoculated treatment without saponin addition was removed from the analysis due to lack of variability and influence on the remaining dataset. The factorial analysis was performed as a repeated measure using a Student's *t* test least significant difference to examine mean separation. Plant growth and development data were normalized by examining each measurement as a percentage of the noninoculated treatment without saponin addition mean for that block. A one-way analysis of variance was performed at each time point to evaluate mean differences. Mean separation was determined using a Tukey's honestly significant difference. All analyses were performed using JMP Pro (version 14.0.0; SAS Institute, Cary, NC).

Results

Effects of saponins on growth of *E. coli*. Lettuce plants that were evaluated for the presence of *E. coli* every week were not found to have any recoverable populations compartmentalized within the edible portions of the plant (data not shown). The timing of treatment and treatment itself were significant effects in the analysis ($P < 0.0001$, $P < 0.0001$). Table 1 displays the effects of saponin on *E. coli* at the various time periods. Through the experiment, the treatment with no inoculum and no saponins remained with no recoverable *E. coli* at all time periods. After 1 h from inoculation of the recirculating tap water in the NFT systems, all inoculated treatments had no saponin addition and contained *E. coli* at 0.6 to 0.83 log cfu/mL ($P = 0.74$).

After 2 h from inoculation (1 h from the saponin addition), all inoculated treatments containing a saponin addition had similar amounts of *E. coli* between the treatment levels [<0.001–1.11 log cfu/mL ($P = 0.57$)].

After 168 h (1 week) from the saponin addition, all treatments increased *E. coli* cfus by at least 2 logs (2.49–5.28 log cfu/mL) and were different ($P = 0.001$). The inoculated treatment without saponin (2.49 log cfu/mL) was similar to the treatments with saponin concentrations of 12.5 and 25 $\mu g \cdot mL^{-1}$, which had 3.57 and 3.7 log cfu/mL, respectively. Similarly, the treatments with the highest concentrations of saponin (50 and 100 $\mu g \cdot mL^{-1}$) yielded the highest amount of *E. coli* ranging from 5.08 to 5.28 log cfu/mL, which were similar results.

Treatment differences persisted after 336 h (2 weeks) from the saponin addition ($P = 0.0001$). The inoculated treatment without saponin had 2.17 log cfu/mL, which was similar to the other treatments with saponin concentrations of 12.5 and 25 $\mu g \cdot mL^{-1}$ (3.3 and 3.45 log cfu/mL). The inoculated treatments containing saponin concentrations of 50 and 100 $\mu g \cdot mL^{-1}$ had the highest yields of *E. coli* populations with 5.02 and 5.61 log cfu/mL, respectively, and were different from all other treatments.

After 504 h (3 weeks) from the saponin addition, differences remained ($P = 0.0001$). The inoculated treatment without saponin experienced a reduction in *E. coli* population from the previous week to 1.53 log cfu/mL, which was lower than all other inoculated treatments. The inoculated treatment containing a saponin concentration of 12.5 $\mu g \cdot mL^{-1}$ had 3.48 log cfu/mL and was similar to the inoculated treatments with saponin additions of 25 and 50 $\mu g \cdot mL^{-1}$, but not to the treatment with a saponin concentration of 100 $\mu g \cdot mL^{-1}$. The inoculated treatment with a saponin concentration of 100 $\mu g \cdot mL^{-1}$ had a slight reduction in *E. coli* population from the previous week, yet still carried the highest amount at the 3-week time period with 5.23 log cfu/mL.

After 672 h (4 weeks) from the saponin addition, treatment differences persisted ($P = 0.0001$). The inoculated treatment without saponin reduced in population to 0.89 log cfu/mL, which was lower and different from all other treatments. The inoculated treatments with saponin additions of 12.5 and 25 $\mu g \cdot mL^{-1}$ contained *E. coli* populations of 2.84 and 2.91 log cfu/mL, respectively. These treatments experienced a reduction for the first time and were also similar to each other. The inoculated treatments with saponin additions of 50 and 100 $\mu g \cdot mL^{-1}$ had populations of *E. coli* at 4.39 and 4.77 log cfu/mL, which were similar to one another and different from treatments containing saponins at concentrations of 25 $\mu g \cdot mL^{-1}$ or lower.

At the final time point, 840 h (5 weeks) from the saponin addition, treatment differences remained ($P = 0.0001$). The inoculated treatment without saponin maintained a low population (0.89 log cfu/mL), whereas inoculated treatments containing lower saponin concentrations of 12.5 and 25 $\mu g \cdot mL^{-1}$ reduced in recoverable *E. coli* populations and were similar to the inoculated

Table 1. Comparison of *Escherichia coli* colony-forming unit (cfu) grown in hydroponic fertilizer solution with 0, 12.5, 25, 50, and 100 μg·mL^{-1} (ppm) saponin treatment over time.

Treatment		Time (h)						
		1[z]	2[y]	168	336	504	672	840
E. coli[x]	Saponin (μg·mL^{-1})	Mean (log cfu/mL + 1)[w]						
–	0	0	0	0	0	0	0	0
+	0	<0.001 a[v]	<0.001 a	2.49 a	2.17 a	1.53 a	0.89 a	0.89 a
+	12.5	0.16 a	0.42 a	3.57 a	3.30 a	3.48 b	2.84 b	2.32 ab
+	25	0.32 a	0.79 a	3.70 ab	3.45 a	4.01 bc	2.91 b	2.15 ab
+	50	0.83 a	0.88 a	5.08 bc	5.02 b	4.70 bc	4.39 c	3.21 bc
+	100	0.72 a	1.11 a	5.28 c	5.61 b	5.23 c	4.77 c	4.61 c

[z]Saponin treatment intervention not yet applied.
[y]Enumeration occurred 1 h post saponin treatment intervention.
[x]Positive (+) indicates presence of *E. coli* inoculated at an initial population of 10^3 cfu/mL, negative (–) indicates *E. coli* not present.[w]
[w]1 cfu/mL = 29.5735 cfu/fl oz.
[v]Means with different letter(s) are significantly different using a Student's *t* test at $P \leq 0.05$. All data were pooled from three replications with two subsamples each (n = 6).

treatment without saponin and contained populations that ranged from 2.15 to 2.32 log cfu/mL. The inoculated treatments with saponin additions of 50 and 100 μg·mL^{-1} also contained lower amounts of *E. coli*, yet were higher than other treatments with 3.21 and 4.61 log cfu/mL and were similar to one another.

Effects of saponins on plant growth and development. After 840 h (5 weeks) in the NFT system, the noninoculated treatment that did not contain saponins and the inoculated treatment that did not contain saponins were the highest in terms of fresh shoot weight and dry shoot weight (Table 2) and were only similar to the inoculated treatment with a saponin addition of 25 μg·mL^{-1} ($P < 0.0001$ and $P < 0.0001$, respectively). The inoculated treatment containing a saponin concentration of 12.5 μg·mL^{-1} was similar in fresh and dry shoot weight to the treatment with 25 μg·mL^{-1} of saponins. The inoculated treatment with a saponin addition of 50 μg·mL^{-1} had lower fresh and dry shoot weight than the lower concentrations of saponins; however, it had a higher fresh and dry shoot weight than the inoculated treatment with a saponin addition of 100 μg·mL^{-1}.

Lettuce head diameter after 840 h (5 weeks) was larger among the noninoculated treatment without saponins and the inoculated treatment without saponins (Table 2). However, they were similar ($P < 0.0001$) to the two inoculated treatments with the lower levels of saponins (12.5 and 25 μg·mL^{-1}). The inoculated treatment with a saponin addition of 50 μg·mL^{-1} had a smaller head diameter than the treatments with no saponins and lower-level saponins. The inoculated treatment with a saponin addition of 100 μg·mL^{-1} had a head diameter that was smaller than all other treatments evaluated.

Discussion

Saponin concentration had the most influential effect on growth of *E. coli*. Over time, all treatments exhibited growth and decline (Fig. 1). The rate of growth and decline were affected by the presence and level of the saponin treatment. The experimental hypothesis was that saponins would have an antibacterial effect on *E. coli*. This would suggest that more saponins would equate to less *E. coli*. The resulting outcome of the experiment was the opposite. The greatest population of *E. coli* was consistently found in the inoculated treatment containing the highest concentration of saponins. At its highest population (336 h), this treatment produced 3 log increases over treatment 2, which contained no saponin addition. This result was reliably seen at every time point beyond the initial first hours of the experiment.

These results were consistent with those found in the work of Arabski et al. (2011) on triterpenoid saponins who also observed an enhancement of *E. coli* growth when exposed to saponins. The current experiment was conducted using steroidal saponins extracted from mojave yucca found in the southwest United States and northwest Mexico. As discussed previously in this article, the aglycone structure of each saponin compound determines its biological properties. Using the results from this study and those found by Arabski et al. (2011), both steroid and triterpenoid saponins react similarly to stimulate the growth of *E. coli*. The leading postulate to the reasoning of increased bacterial growth is that saponins increase cell permeability and the influx of nutrients (Arabski et al., 2011). Instead of opening intercellular space to potentially harmful

Table 2. Mean fresh and dry shoot weight and plant diameter of lettuce reported as a percentage of the control grown in a hydroponic fertilizer solution with 0, 12.5, 25, 50, and 100 μg·mL^{-1} (ppm) saponin treatment at time of harvest (5 weeks).

Treatment		Plant characteristic		
		Fresh wt	Dry wt	Diam
E. coli[z]	Saponin (μg·mL^{-1})	(% control)[y]		
–	0	100 a[x]	100 a	100 a
+	0	101 a	95 a	98 a
+	12.5	75 b	70 b	89 a
+	25	80 ab	87 ab	88 a
+	50	44 c	46 c	74 b
+	100	14 d	16 d	48 c

[z]Positive (+) indicates presence of *Escherichia coli* inoculated at an initial population of 10^3 colony-forming units (cfu)/mL, negative (–) indicates *E. coli* not present; 1 cfu/mL = 29.5735 cfu/fl oz.
[y]Mean responses displayed as percent of noninoculated treatment without saponin (without *E. coli*, without saponin addition). All data were pooled from three replications with three subsamples each (n = 9).
[x]Means with different letter(s) are significantly different using a Tukey's honestly significance difference test at $P \leq 0.05$.

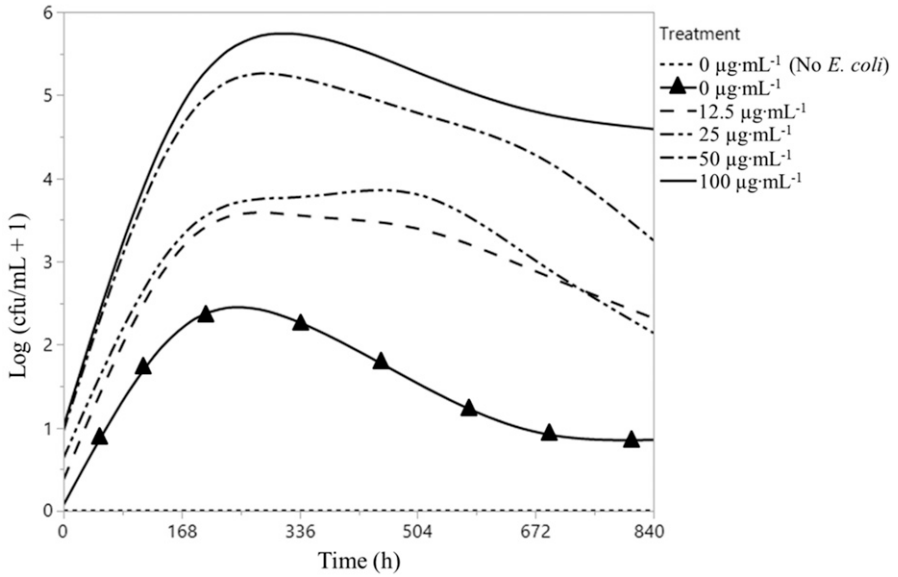

Fig. 1. Growth curves of *Escherichia coli* populations grown in hydroponic fertilizer solution with 0, 12.5, 25, 50, and 100 µg·mL^{-1} saponin treatment over time. All data were pooled from three replications with two subsamples each (n = 6); 1 µg·mL^{-1} = 1 ppm, 1 cfu/mL = 29.5735 cfu/fl oz.

extracellular conditions, newly formed pores in bacterial membranes allow the passage of nutrients to flow into the cell, allowing *E. coli* to prosper.

Another possible explanation to the higher populations of *E. coli* in higher concentrations of saponins revolves around bacterial structure. *E. coli* are gram-negative bacteria, and in research are harder to kill than gram-positive bacteria with a peptidoglycan layer. Previous experimenters have elucidated the antibacterial effects of saponins against other gram-negative bacterium (Khan et al., 2018; Mandal et al., 2005). A large difference between those experiments and this experiment is the addition of plants into the system ecology. A plant's rhizosphere can contain up to 100 times the amount of microorganisms found in soil without plants (Haas et al., 2002). This rich biodiversity of microbes is home to a group known as rhizobacteria, which produce beneficial secondary metabolites that enhance plant growth through a variety of mechanisms (Sturz and Christie, 2003). A few notable rhizobacteria are found within the genera *Pseudomonas*, *Streptomyces*, and *Bacillus* (Emmert and Handelsman, 1999; Haas et al., 2002). Brown et al. (1976) were able to isolate naturally occurring sulfur-containing carboxylic acids from strains of *Streptomyces*, which are very potent inhibitors of *E. coli*. Soetan et al. (2006) reported that saponins only produced inhibitory effects on gram-positive bacteria, contrary to Khan et al. (2018) and Mandal et al. (2005). As previously noted, *E. coli* are gram-negative; however, *Streptomyces* is a gram-positive bacterium, leading the investigator in the current study to postulate that higher saponin concentrations inhibited beneficial rhizobacteria like *Streptomyces,* which allowed *E. coli* to survive in a less-competitive environment.

It is important to note that early time points in this experiment had very low populations of *E. coli* to report. A study by Cooper et al. (2001) involving *E. coli* thermal dependence also indicated that most bacterial loss was seen in early stages of the experiment, when adaptation is the most rapid. Bacterial injury was observed on a great deal of the TSA plates. Typical colony morphology appeared circular, convex, and smooth. *E. coli* that were recovered and cultured at early time points were irregular in shape and size. Initially this experiment used MacConkey agar without the TSA overlay. Recovery became increasingly lower as water temperatures dropped in the nutrient reservoirs due to changing seasons. *E. coli* will grow across the temperature range of 10 to 49 °C, but it will grow at a progressively slower rate when temperature is raised above 40 °C or below 20 °C (Cooper et al., 2001; Jones et al., 1987). Water temperature readings were below 20 °C for the early stages of the first two replications. *E. coli* was present (indicated by subsequent aliquots), but in low numbers and in some cases undetectable. A pre-enrichment step was deemed necessary to facilitate bacterial recovery (McKillip, 2001). In this case, it was the addition of TSA to the MacConkey plates in the form of an overlay. This gave injured bacteria an opportunity to repair themselves in the nutrient-rich environment and increased laboratory success in proper enumeration of *E. coli* (Smith et al., 2013).

Results for *E. coli* presence within the edible portions of lettuce were omitted from the statistical analysis due to the simplicity of the findings. An *E. coli* presence or absence screening was conducted on a total of 172 lettuce plants throughout the duration of the experiment. No contaminated plants were found, indicating that *E. coli* cannot be internalized from the rhizosphere into the root system growing in a hydroponic system. This evidence is contrary to that found by Solomon et al. (2002). The discrepancy of the before-mentioned study and this study could be the result of differing identification techniques. Solomon et al. (2002) used sophisticated microscopy for detection of bacterial internalization. They also grew plants in soil rather than a hydroponic system. However, this study is supported by Hora et al. (2005) who did not find internalization in aerial plant portions of spinach (*Spinacia oleracea*) when roots had been inoculated in soil containers.

Although mean separations were found at individual time points for lettuce growth and development parameters, the most important time point to address is 840 h (week 5). This time point reflects the most accurate time of maturation for 'Rex' lettuce and therefore conveys the most fundamental information to a grower considering the use of saponins in a recirculating hydroponic NFT system. Under every growth measurement, the noninoculated treatment without saponin addition and the inoculated treatment without saponin addition produced the highest yields on average. The fact that these treatments were the only

treatments tested that did not include the saponin intervention indicates the economic impracticality of this treatment as a mitigation tool for *E. coli* or any other microbe when growing lettuce in an NFT system.

Reduced growth of lettuce was clearly related to an increase in saponin solution. It is difficult to say whether this reduced growth pattern was due to the active ingredient (steroid saponins) or other ingredients within the solution or a combination of these factors. The provided saponin solution used in this experiment is not currently on the market; however, there are similar products available to consumers from the manufacturer. These similar products are used as supplements for livestock feed to control ammonia and other noxious gasses in the immediate environment, conveying air-quality improvements. The formulation of the tested saponin extract product is not necessarily engineered for plant growth in a hydroponic NFT system.

The most likely cause of limited plant growth at higher concentrations of saponins is an increase in damaged plant cell membranes. Saponins are nonionic surfactants, which have phytotoxic effects on plant membranes by increasing permeability (Riechers et al., 1994). The damage caused to the root zone may have inhibited nutrient uptake and retarded the growth cycle.

Another postulate worth considering encompasses dissolved oxygen (DO) in the nutrient water. Unfortunately, due to equipment failures, DO was not measured across all replications of the experiment and therefore not included in the statistical analysis. Lettuce grows sufficiently at DO levels of at least 4 ppm (4 $mg \cdot L^{-1}$) (Brechner et al., 2013). Using the limited measurements recorded and averaged over time in this study, DO levels are lower as higher saponin concentrations are added to the NFT system (Fig. 2). Levels did not fall below 4 $mg \cdot L^{-1}$ until saponins were added at a concentration of 50 $\mu g \cdot mL^{-1}$ and above. Saponins are well-known for their ability to foam in aqueous solutions (Francis et al., 2002) as detergent-like compounds. Increased amounts of foam were observed at increasing saponin levels in this experiment. The amounts of foam were large enough to obstruct gas exchange between the nutrient reservoir of the NFT system and the atmosphere. A correlation cannot be stated, but appears to be consistent with DO levels, saponin treatment, and plant growth.

Conclusions

The primary objectives of this research study were to identify whether steroidal saponins from mojave yucca could be used as a natural bactericide for *E. coli* and what, if any, effects that would have on plant growth and development of 'Rex' lettuce grown in a hydroponic NFT system. The fact of the matter is that increasing saponin levels not only failed to elicit a bactericidal effect but promoted the growth of *E. coli*. All the while, plant health and vigor suffered in the presence of increasing amounts of saponin levels. Based on these results, this chemical intervention technique would not be recommended for the intended use of bacterial mitigation in hydroponic irrigation water.

Although data were not taken, another observation was that the saponin solution was rather unpleasant to work with because of a foul odor and equipment-clogging issues. Pumps and irrigation lines required extensive cleaning between experimental replications to prevent occlusions from manifesting.

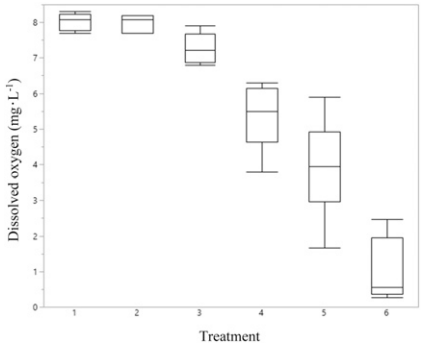

Fig. 2. Box and whisker plots of dissolved oxygen for individual saponin treatments in the nutrient film technique (NFT) system (unofficial). Treatment 1 = no *Escherichia coli*, no saponin addition; Treatment 2 = *E. coli*, no saponin addition; Treatment 3 = *E. coli*, 12.5 $\mu g \cdot mL^{-1}$ saponin; Treatment 4 = *E. coli*, 25 $\mu g \cdot mL^{-1}$ saponin; Treatment 5 = *E. coli*, 50 $\mu g \cdot mL^{-1}$ saponin; Treatment 6 = *E. coli*, 100 $\mu g \cdot mL^{-1}$ saponin; 1 $mg \cdot L^{-1}$ = 1 ppm, 1 $\mu g \cdot mL^{-1}$ = 1 ppm.

Another important takeaway was that *E. coli* does not appear to travel from the rhizosphere into the edible portions of lettuce plants by systemic means. *E. coli* also did not affect lettuce growth. In this study, the noninoculated treatment without saponin was juxtaposed to the inoculated treatment without saponin and found no differences in fresh shoot weight ($P = 0.74$). This indicates that *E. coli* living in the fertilizer solution and interacting with the vast community of microorganisms surrounding the root zone do not negatively impact the growth and development of 'Rex' lettuce in a hydroponic NFT system.

E. coli recovery was inadequate when using MacConkey agar growth media. Due to sublethal bacterial injury, a pre-enrichment step should be implemented in future research to ensure proper recovery and enumeration of bacteria. A TSA overlay on MacConkey agar was used in this experiment and is recommended for future study.

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