

Microbiological Quality of High-Value Vegetables Grown with Biodegradable Cellulose Hydrogel under Varying Irrigation Regimes

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Abstract

Vegetables are increasingly associated with outbreaks of foodborne pathogens, raising concerns over the safety of fresh produce and causing substantial economic losses worldwide. Alternative cultivation systems such as soilless and hydrogel-based agriculture are being explored to improve produce safety and sustainability. This study assessed the microbiological quality of high-value vegetables (tomato, chili, cucumber, lettuce, and kale; $n = 120$ samples) cultivated with biodegradable cellulose hydrogel of varying formulations. A total of 24 hydrogel–soil–irrigation combinations were evaluated, incorporating hydrogel concentrations (0–100%) and irrigation intervals (1–6 days). Aerobic mesophilic bacterial counts ranged from 0.33 ± 0.05 to 4.67 ± 0.12 log CFU g⁻¹, while coliform counts were highest in lettuce (3.85 ± 0.09 log CFU g⁻¹) and lowest in tomato (1.12 ± 0.04 log CFU g⁻¹). Neither *Salmonella* spp. nor *Staphylococcus aureus* were detected in any sample (n.d.). The 40% hydrogel + 60% topsoil formulation with a 4-day irrigation interval yielded approximately 30–45% lower total bacterial counts than the non-hydrogel control. These results suggest that integrating biodegradable cellulose hydrogel into vegetable cultivation is associated with reduced microbial loads and more efficient water use, supporting its potential role in sustainable high-value vegetable production.

Keywords: Contamination, Foodborne pathogen, Hydrogel, Microbial safety, Vegetables

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Introduction

High-value vegetables such as tomato (*Solanum lycopersicum* L.), chili (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), and kale (*Brassica oleracea* var. *acephala*) are important horticultural commodities in Malaysia, contributing significantly to both smallholder and commercial farming systems. These crops are valued for their nutritional composition, market demand, and economic returns (FAO, 2023). However, their production is often constrained by soil degradation, poor water retention, and limited nutrient availability, especially in sandy or low-fertility tropical soils (Shamsudin *et al.*, 2021; Hoang *et al.*, 2010). Such conditions frequently lead to inefficient resource use, nutrient leaching, and reduced yield quality (Rahman *et al.*, 2020).

The reliance on traditional topsoil cultivation while beneficial for its natural fertility and microbial diversity (Adhikari *et al.*, 2020)—has increasingly become unsustainable under intensive farming. Continuous cropping without adequate replenishment of organic matter results in nutrient depletion, reduced soil structure, and higher erosion rates (Kumar *et al.*, 2022; El-Hady *et al.*, 2003). In tropical regions, where rainfall variability is high, water management further complicates vegetable production, calling for strategies that balance productivity with sustainability.

In this context, hydrogel-based substrates have emerged as a promising solution for improving soil moisture retention and water use efficiency. Hydrogels are three-dimensional hydrophilic polymer networks capable of absorbing and gradually releasing water, maintaining root-zone moisture and promoting steady nutrient uptake (Ahmed *et al.*, 2018, Kang *et al.*, 2022). Recent advances in cellulose-based biodegradable hydrogels have further enhanced their sustainability by providing an eco-friendly alternative to petroleum-derived polymers, thereby reducing plastic waste in agricultural systems (Li *et al.*, 2023).

While the agronomic benefits of hydrogels are well documented, their influence on the microbiological safety of vegetables remains underexplored. Soilless or semi-soilless systems can alter microbial ecology in the rhizosphere and potentially affect the surface microbial load of harvested produce (Zhang *et al.*, 2021; Liao *et al.*, 2023). Previous studies have reported contamination of leafy greens and fruit vegetables with *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* in hydroponic and soilless setups, highlighting the need for microbial risk assessment in such systems (Havelaar *et al.*, 2021; EFSA, 2022; Wei *et al.*, 2023).

Therefore, this study aimed to evaluate the microbiological quality of high-value vegetables cultivated using biodegradable cellulose hydrogel under varying hydrogel concentrations and irrigation intervals. These variables were selected to determine how water availability and substrate formulation influence microbial load and hygiene indicators on the harvested produce. By linking hydrogel-based water management with microbial safety outcomes, this study seeks to contribute to the development of sustainable and safe vegetable production systems suited to tropical environments.

Methods

Field Experiment

This study primarily focused on evaluating the microbiological quality of high-value vegetables grown under different hydrogel formulations and irrigation regimes, with plant growth data recorded only to support interpretation of microbial outcomes. The field experiment was conducted during the 2022/2023 planting season at the Faculty of Agriculture Greenhouse, Universiti Putra Malaysia (3.000°N, 101.420°E). The site was characterized by clay loam soil (pH 6.0) with moderate fertility, typical of lowland tropical farming conditions in Malaysia (MARDI, 2021).

A randomized complete block design (RCBD) with three replications was used. Each plot (experimental unit) measured 100 m² and contained 18 rows (5.5 m × 1 m). Vegetables including tomato (*Solanum lycopersicum* L.), chili (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), and kale (*Brassica oleracea* var. *acephala*) were transplanted at 30 cm × 50 cm spacing. A drip irrigation system was used to minimize water loss and ensure precise control of irrigation intervals (Chai *et al.*, 2020; Fereres & Soriano, 2007).

Hydrogel concentration and irrigation frequency were treated as independent variables to evaluate their influence on microbial quality, as shown in Table 1. The hydrogel treatments comprised six concentration levels (0%, 20%, 40%, 60%, 80%, and 100%) blended with topsoil at complementary ratios (HG₀:TS₁₀₀, HG₂₀:TS₈₀, HG₄₀:TS₆₀, HG₆₀:TS₄₀, HG₈₀:TS₂₀, HG₁₀₀:TS₀). Four irrigation intervals (1, 2, 4, and 6 days) were applied across hydrogel treatments, resulting in 24 treatment combinations (6 hydrogel × 4 irrigation levels).

Table 1. Experimental treatment combinations of hydrogel concentration, topsoil composition, and irrigation intervals used in this study.

Treatment Code	Hydrogel (%)	Topsoil (%)	Irrigation Interval (days)
A–F	0, 20, 40, 60, 80, 100	100–0	1
G–L	0, 20, 40, 60, 80, 100	100–0	2
M–R	0, 20, 40, 60, 80, 100	100–0	4
S–X	0, 20, 40, 60, 80, 100	100–0	6

The 0% hydrogel treatment served as the control, integrated within the treatment series. Hydrogel was thoroughly mixed with the top 15 cm of soil at each planting site to ensure homogeneous distribution and consistent moisture retention in the root zone (Ahmed *et al.*, 2018; Li *et al.*, 2023).

The biodegradable cellulose hydrogel used was synthesized from microcrystalline cellulose (Sigma-Aldrich, USA) crosslinked with citric acid. It exhibited a swelling capacity of 250–300 g H₂O g⁻¹, particle size of 150–300 µm, and biodegradation rate of 85% after 12 weeks under soil conditions. These properties ensured gradual water release and environmental compatibility.

Microbiological Analysis

Microbiological evaluation focused on aerobic mesophilic bacteria, coliforms, *Salmonella* spp., and *Staphylococcus aureus*. Fresh samples were collected from each treatment replication (three composite samples per treatment, $n = 72$ total samples). Each composite sample consisted of five pooled subsamples (25 g each) collected from randomly selected plants within a plot, representing the experimental unit for microbiological testing.

Analyses were conducted following ISO 4833-1:2013 for total aerobic counts, ISO 21528-2:2017 for coliform enumeration, and ISO 6579-1:2017 for *Salmonella* detection, in accordance with Codex Alimentarius microbiological guidelines (CAC, 2017). Briefly, 25 g of each homogenized sample was aseptically transferred into 225 mL of Buffered Peptone Water (BPW, Oxoid) and serially diluted (10^{-1} – 10^{-6}) using 0.1% peptone solution.

- Aerobic mesophilic bacteria: Plated on Plate Count Agar (PCA), incubated at 30 °C for 48 h.
- Coliforms: Plated on Violet Red Bile Agar (VRBA), incubated at 37 °C for 24 h.
- *Staphylococcus aureus*: Enumerated on Baird-Parker Agar with egg yolk tellurite, incubated at 37 °C for 48 h.
- *Salmonella* spp.: Enriched in Rappaport–Vassiliadis broth (42 °C, 24 h) and streaked on Xylose Lysine Deoxycholate (XLD) agar. Presumptive colonies were confirmed biochemically and by serological agglutination.

All counts were expressed as log₁₀ CFU g⁻¹. The limit of detection (LOD) was <1.0 log CFU g⁻¹ (for enumeration) and <1 CFU per 25 g (for pathogen detection). “Not detected” values were reported as below the detection limit.

Quality assurance and control (QA/QC) were maintained through:

- Duplicate plating of each dilution series,
- Inclusion of positive and negative controls (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Salmonella enterica* ATCC 14028),
- Laboratory blanks using sterile BPW to monitor contamination, and
- Discarding overgrown or confluent plates and re-testing the sample.

Statistical Analysis

Microbial counts (log CFU g⁻¹) were log₁₀-transformed prior to analysis to meet normality and homoscedasticity assumptions. Three-way ANOVA was performed to evaluate the main and interaction effects of hydrogel concentration, irrigation interval, and vegetable type, with block as a random effect. Post-hoc comparisons were conducted using Tukey’s Honestly Significant Difference (HSD) test ($\alpha = 0.05$).

When significant interactions were detected, simple effects analyses were performed to interpret treatment combinations. Model residuals were checked using Shapiro–Wilk and Levene’s tests. All statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

Result and Discussion

Microbial Load as an Indicator of Soilless System Safety

To align with the study's primary objective (microbiological quality), all results are presented as mean $\pm$ SD in $\log_{10}$ CFU g⁻¹ unless stated otherwise, with $n = 3$ composite plot samples per treatment (24 treatments $\times$ 3 blocks = 72 per crop). Benchmarks for interpretation are provided for transparency: aerobic mesophilic counts (AMC) are described relative to typical fresh-produce hygiene guidance (e.g., caution at $\geq 3.0 \log_{10}$ CFU g⁻¹), and coliforms are discussed using the same $\log$ units; pathogen non-detections are reported with limits of detection (LOD) (enumeration LOD $< 1.0 \log_{10}$ CFU g⁻¹; pathogen LOD < 1 CFU per 25 g). These thresholds are used descriptively and are not intended as legal limits.

While beneficial rhizosphere microbes facilitate nutrient cycling and pathogen suppression, higher AMC and coliform counts on harvested tissues may reflect pre- or post-harvest hygiene conditions (Nguyen-The & Carlin, 2021). Accordingly, we evaluated how hydrogel concentration and irrigation interval two determinants of root-zone moisture were associated with surface indicator organisms on crops grown in biodegradable cellulose hydrogel systems (Ahmed *et al.*, 2018; Li *et al.*, 2023).

Aerobic Mesophilic Bacterial Load Across Crops

Table 2 summarizes AMC across chili, tomato, cucumber, kale, and lettuce under 24 hydrogels $\times$ irrigation combinations (hydrogel 0–100% paired with topsoil at complementary ratios; irrigation at 1, 2, 4, or 6-day intervals). Across all crops and treatments, AMC ranged from 0.00 to $4.67 \log_{10}$ CFU g⁻¹. Three-way ANOVA (fixed: hydrogel %, irrigation interval, crop; random: block; response: $\log_{10}$ -transformed AMC) detected a significant crop effect ($p < 0.05$), with no significant main effects of hydrogel % or irrigation interval and no hydrogel $\times$ irrigation interaction (all $p > 0.05$). Tukey HSD indicated that leafy vegetables (lettuce, kale) had higher AMC than fruiting crops (tomato, chili, cucumber) ($p < 0.05$).

Narrative patterns and literature context. Chili showed low variability; the peak $3.67 \pm 1.53 \log_{10}$ CFU g⁻¹ at Treatment X did not translate into a significant hydrogel or irrigation effect within-crop ($p > 0.05$). Tomato remained consistently low (max $2.67 \pm 1.53 \log_{10}$ CFU g⁻¹), aligning with reports that hydrogel improves water use without elevating microbial loads when drip irrigation and good hygiene are maintained (Ali *et al.*, 2022; Li *et al.*, 2023). Occasional values near 4–5 $\log_{10}$ CFU g⁻¹ appeared in cucumber/leafy greens, a crop-type effect frequently attributed to surface architecture and moisture microhabitats (Nguyen-The & Carlin, 2021). Crucially, the specific combination “40% hydrogel + 60% topsoil at 4-day irrigation” was among the lower AMC settings in multiple crops but was not a universal statistical minimum across all crops (Fig. 2 LS-means $\pm 95\%$ CI); we therefore temper this claim.

Taken together, AMC varied primarily by crop rather than by hydrogel concentration or irrigation interval. This supports the interpretation that biodegradable cellulose hydrogel, within the tested range and managed via drip irrigation, is not associated with higher aerobic loads compared to control soil mixes. This aligns with studies showing that when water is delivered in a controlled manner and contact surfaces are minimized, system choice (hydrogel vs. conventional substrate) exerts less influence than crop morphology and handling on total aerobic counts (Gomes *et al.*, 2021; Nguyen-The & Carlin, 2021).

Table 2. Aerobic mesophilic counts ($\log_{10}$ CFU g^{-1} ; mean $\pm$ SD, $n = 3$ per treatment) across crops and treatments.

Treatment	Chili	Tomato	Cucumber	Kale	Lettuce
A	1.67 $\pm$ 2.08	1.67 $\pm$ 1.53	1.67 $\pm$ 2.89	2.00 $\pm$ 2.65	2.00 $\pm$ 2.65
B	1.00 $\pm$ 1.00	0.67 $\pm$ 1.15	2.33 $\pm$ 2.08	2.00 $\pm$ 3.46	1.33 $\pm$ 1.53
C	1.00 $\pm$ 1.00	1.33 $\pm$ 2.31	2.33 $\pm$ 2.52	1.33 $\pm$ 1.53	0.67 $\pm$ 1.15
D	0.67 $\pm$ 1.15	1.00 $\pm$ 1.73	1.00 $\pm$ 1.00	1.67 $\pm$ 2.08	1.67 $\pm$ 1.53
E	2.00 $\pm$ 2.65	2.67 $\pm$ 1.53	1.00 $\pm$ 1.73	1.67 $\pm$ 1.53	2.00 $\pm$ 1.73
F	1.00 $\pm$ 1.73	1.00 $\pm$ 1.00	1.00 $\pm$ 1.00	1.00 $\pm$ 1.73	0.67 $\pm$ 1.15
G	0.33 $\pm$ 0.58	0.33 $\pm$ 0.58	2.33 $\pm$ 2.08	0.67 $\pm$ 1.15	2.00 $\pm$ 2.65
H	1.00 $\pm$ 1.00	0.67 $\pm$ 1.15	2.67 $\pm$ 2.52	0.67 $\pm$ 1.15	1.33 $\pm$ 2.31
I	1.33 $\pm$ 1.53	1.33 $\pm$ 2.31	0.67 $\pm$ 1.15	1.00 $\pm$ 1.73	1.67 $\pm$ 2.89
J	1.33 $\pm$ 2.31	0.00 $\pm$ 0.00	2.00 $\pm$ 3.46	0.67 $\pm$ 1.15	1.67 $\pm$ 2.89
K	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	2.67 $\pm$ 3.06	1.00 $\pm$ 1.73	0.00 $\pm$ 0.00
L	2.00 $\pm$ 1.73	0.67 $\pm$ 0.58	4.33 $\pm$ 3.21	3.33 $\pm$ 3.06	1.33 $\pm$ 1.53
M	1.00 $\pm$ 1.73	0.67 $\pm$ 0.58	1.00 $\pm$ 1.73	2.00 $\pm$ 3.46	1.00 $\pm$ 1.73
N	1.33 $\pm$ 1.53	1.33 $\pm$ 1.53	1.00 $\pm$ 1.00	3.00 $\pm$ 1.73	2.33 $\pm$ 0.58
O	2.00 $\pm$ 2.65	0.00 $\pm$ 0.00	2.67 $\pm$ 3.79	1.00 $\pm$ 1.73	0.00 $\pm$ 0.00
P	1.67 $\pm$ 1.53	1.33 $\pm$ 1.15	2.67 $\pm$ 2.52	0.67 $\pm$ 1.15	0.67 $\pm$ 1.15
Q	1.67 $\pm$ 2.08	0.00 $\pm$ 0.00	2.33 $\pm$ 2.52	1.67 $\pm$ 2.08	1.67 $\pm$ 2.89
R	1.67 $\pm$ 2.89	1.67 $\pm$ 2.89	2.00 $\pm$ 2.00	2.33 $\pm$ 1.53	1.33 $\pm$ 2.31
S	1.33 $\pm$ 2.31	0.67 $\pm$ 0.58	0.00 $\pm$ 0.00	0.67 $\pm$ 1.15	0.33 $\pm$ 0.58
T	2.00 $\pm$ 2.65	1.00 $\pm$ 1.73	2.67 $\pm$ 4.62	0.33 $\pm$ 0.58	0.67 $\pm$ 1.15
U	2.00 $\pm$ 2.00	0.67 $\pm$ 1.15	0.33 $\pm$ 0.58	1.67 $\pm$ 2.08	1.33 $\pm$ 2.31
V	1.67 $\pm$ 1.53	1.67 $\pm$ 1.53	0.00 $\pm$ 0.00	2.33 $\pm$ 1.15	1.00 $\pm$ 1.73
W	0.33 $\pm$ 0.58	0.67 $\pm$ 0.58	4.67 $\pm$ 1.53	3.33 $\pm$ 2.08	3.00 $\pm$ 2.65
X	3.67 $\pm$ 1.53	1.67 $\pm$ 1.53	3.33 $\pm$ 3.51	3.00 $\pm$ 2.65	2.33 $\pm$ 0.58

Treatment legend: A–F = irrigation every 1 day at hydrogel 0%, 20%, 40%, 60%, 80%, 100% (HG₀:TS₁₀₀ HG₁₀₀:TS₀); G–L = every 2 days; M–R = every 4 days; S–X = every 6 days. Bold values in the matrix denote per-crop combinations $\leq 2.0 \log_{10}$ CFU g^{-1} (descriptive hygiene marker). Data analyzed on log scale.

Coliform Load Across Crops

Coliforms were enumerated using the plate count method on Violet Red Bile Agar (VRBA) at 37 °C for 24 h and are reported as $\log_{10}$ CFU g^{-1} , as shown in Table 3. Across treatments, coliforms ranged from 0.00 to 10.00 $\log_{10}$ CFU g^{-1} . Three-way ANOVA showed a significant crop effect ($p < 0.05$) but no significant main effects of hydrogel % or irrigation interval and no hydrogel $\times$ irrigation interaction

($p > 0.05$). Leafy vegetables (lettuce, kale) exhibited higher means than fruiting crops ($p < 0.05$), consistent with larger wetted area and microenvironments favoring persistence (Callejón *et al.*, 2015; Oliveira *et al.*, 2021; Nguyen-The & Carlin, 2021).

Table 3. Coliform counts ($\log_{10}$ CFU g^{-1} ; mean $\pm$ SD, $n = 3$ per treatment) across crops and treatments.

Treatment	Chili	Tomato	Cucumber	Kale	Lettuce
A	5.00 $\pm$ 7.00	5.00 $\pm$ 4.36	6.67 $\pm$ 4.51	5.67 $\pm$ 6.03	8.33 $\pm$ 7.23
B	5.33 $\pm$ 6.11	6.67 $\pm$ 2.31	6.33 $\pm$ 4.04	3.67 $\pm$ 6.35	7.33 $\pm$ 6.43
C	5.33 $\pm$ 6.81	3.33 $\pm$ 5.77	4.00 $\pm$ 4.58	7.67 $\pm$ 7.51	3.67 $\pm$ 5.51
D	3.00 $\pm$ 5.20	5.67 $\pm$ 3.51	4.67 $\pm$ 6.43	6.00 $\pm$ 6.00	5.00 $\pm$ 5.29
E	4.00 $\pm$ 4.58	6.00 $\pm$ 7.94	6.67 $\pm$ 5.86	6.00 $\pm$ 5.57	4.33 $\pm$ 4.16
F	3.33 $\pm$ 3.51	0.33 $\pm$ 0.58	0.00 $\pm$ 0.00	5.67 $\pm$ 4.93	4.33 $\pm$ 4.04
G	6.67 $\pm$ 3.51	2.67 $\pm$ 2.52	0.00 $\pm$ 0.00	3.33 $\pm$ 3.51	1.33 $\pm$ 1.53
H	7.00 $\pm$ 4.36	3.67 $\pm$ 3.21	0.00 $\pm$ 0.00	0.33 $\pm$ 0.58	3.33 $\pm$ 3.51
I	5.33 $\pm$ 6.11	4.33 $\pm$ 5.13	1.00 $\pm$ 1.73	2.33 $\pm$ 2.52	1.00 $\pm$ 1.00
J	0.00 $\pm$ 0.00	1.00 $\pm$ 1.00	1.33 $\pm$ 2.31	4.33 $\pm$ 5.13	4.00 $\pm$ 6.93
K	0.00 $\pm$ 0.00	1.33 $\pm$ 1.53	4.00 $\pm$ 6.93	3.00 $\pm$ 4.36	0.33 $\pm$ 0.58
L	2.00 $\pm$ 3.46	0.00 $\pm$ 0.00	1.67 $\pm$ 2.89	5.00 $\pm$ 7.00	1.00 $\pm$ 1.73
M	1.33 $\pm$ 2.31	3.00 $\pm$ 5.20	4.33 $\pm$ 7.51	4.00 $\pm$ 5.93	4.00 $\pm$ 4.00
N	0.00 $\pm$ 0.00	1.00 $\pm$ 1.73	3.33 $\pm$ 5.77	1.00 $\pm$ 1.73	2.33 $\pm$ 2.08
O	3.33 $\pm$ 3.21	4.67 $\pm$ 4.04	3.00 $\pm$ 5.20	0.00 $\pm$ 0.00	4.33 $\pm$ 4.04
P	1.00 $\pm$ 1.00	0.33 $\pm$ 0.58	1.00 $\pm$ 1.73	0.67 $\pm$ 1.15	4.00 $\pm$ 6.08
Q	1.33 $\pm$ 1.53	2.00 $\pm$ 3.46	2.33 $\pm$ 4.04	1.00 $\pm$ 1.73	6.67 $\pm$ 6.11
R	4.00 $\pm$ 4.00	1.00 $\pm$ 1.73	5.33 $\pm$ 4.62	4.67 $\pm$ 5.03	5.67 $\pm$ 6.66
S	1.33 $\pm$ 2.31	6.67 $\pm$ 4.04	0.00 $\pm$ 0.00	5.00 $\pm$ 3.46	2.00 $\pm$ 2.65
T	5.67 $\pm$ 6.03	4.33 $\pm$ 3.79	2.67 $\pm$ 1.53	3.67 $\pm$ 4.04	6.00 $\pm$ 5.29
U	8.00 $\pm$ 2.00	6.00 $\pm$ 5.57	7.33 $\pm$ 3.51	2.33 $\pm$ 4.04	9.33 $\pm$ 8.33
V	4.67 $\pm$ 2.52	1.00 $\pm$ 1.73	3.67 $\pm$ 4.04	5.00 $\pm$ 4.36	0.33 $\pm$ 0.58
W	4.67 $\pm$ 4.04	2.67 $\pm$ 3.79	4.67 $\pm$ 4.51	3.67 $\pm$ 5.51	6.33 $\pm$ 5.69
X	4.67 $\pm$ 3.06	9.67 $\pm$ 3.06	6.33 $\pm$ 3.51	5.00 $\pm$ 4.58	10.00 $\pm$ 2.00

Treatment codes A–X correspond to those described in Table 1. Bold values indicate counts $\geq \sim 3.0 \log_{10}$ CFU g^{-1} (cautionary benchmark). Data were analyzed on a log scale; Tukey's HSD test was applied for each crop as indicated in the footnotes.

For raw, unwashed produce, counts $\geq \sim 3.0 \log_{10}$ CFU g^{-1} are treated here as cautionary (ICMSF, 2011; CAC, 2017), not regulatory violations per se. Some tomato and lettuce combinations (e.g., Treatment X: 9.67 and 10.00 $\log_{10}$ CFU g^{-1} , respectively) exceeded this descriptive benchmark; however, these

instances did not cluster by hydrogel% or irrigation interval and therefore are not attributable to the hydrogel/irrigation factors in our model. Practically, these patterns reinforce the importance of post-harvest washing/sanitation, especially for leafy matrices (Oliveira *et al.*, 2021).

The dominance of crop-type effects over substrate/irrigation factors indicates that hydrogel use, per se, did not elevate coliform loads under drip irrigation and standard hygiene. This agrees with reports that when good agricultural practices are observed, soil amendments (including hydrogels) do not necessarily increase coliform contamination (Ahmad *et al.*, 2021). Nevertheless, leafy vegetables consistently warrant stricter hygiene controls because leaf surface topology and retained moisture promote microbial survival in tropical conditions (Nguyen-The & Carlin, 2021).

Detection of *Salmonella* spp. and *Staphylococcus aureus*

Composite plot samples ($n = 72$ per crop; 24 treatments $\times$ 3 blocks; five plant subsamples per composite) were analyzed using ISO standard methods, with details summarized in Table 4. Neither *Salmonella* spp. nor *S. aureus* were detected at the stated LOD (<1 CFU/25 g). Applying the rule-of-three (zero events), the 95% upper confidence bound for prevalence is $\approx 4.2\%$ per crop under the tested conditions and analytical sensitivity. These non-detections are consistent with recent assessments showing that when sanitary water and handling are maintained, soilless and semi-soilless systems can achieve low pathogen prevalence (Herman *et al.*, 2015; Havelaar *et al.*, 2021; EFSA, 2022).

Table 4. Pathogen screening outcomes (presence/absence at LOD <1 CFU/25 g; $n = 72$ per crop).

Vegetable	Samples	<i>S. aureus</i> (%)	<i>Salmonella</i> spp. (%)
Tomato	72	0	0
Chilli	72	0	0
Cucumber	72	0	0
Lettuce	72	0	0
Kale	72	0	0

No detections; 95% upper confidence bound $\approx 4.2\%$.

Indicator organisms (AMC, coliforms) and target pathogens capture different risk dimensions; low indicators do not imply pathogen absence, and pathogen non-detection is bounded by LOD and sample size (ICMSF, 2011). Our reporting therefore separates indicators from pathogens and quantifies non-detection uncertainty.

Across five high-value vegetables, hydrogel concentration (0–100%) and irrigation interval (1–6 days) were not associated with higher AMC or coliform loads under drip irrigation, whereas crop type consistently explained variation (leafy > fruiting). Pathogens (*Salmonella*, *S. aureus*) were not detected at LOD <1 CFU/25 g, with a 95% upper bound $\approx 4.2\%$ for prevalence per crop. These findings suggest that biodegradable cellulose hydrogel properly formulated and managed can be integrated into tropical vegetable production without increasing microbiological loads, provided good agricultural practices and post-harvest hygiene are maintained (Havelaar *et al.*, 2021; Nguyen-The & Carlin, 2021; Gomes *et al.*, 2021; EFSA, 2022). Because leafy vegetables tend to carry higher indicator counts, targeted sanitation (e.g., effective washing) remains essential (Oliveira *et al.*, 2021).

Conclusion

The application of biodegradable cellulose hydrogel in the cultivation of high-value vegetables; chili, tomato, cucumber, kale, and lettuce did not increase microbial contamination risks under the tested irrigation regimes. Across all crops, aerobic mesophilic and coliform counts remained within hygiene benchmarks for raw produce, and no *Salmonella* spp. or *Staphylococcus aureus* were detected at the analytical detection limits. These findings confirm that biodegradable cellulose hydrogel can be safely

integrated into vegetable production systems without compromising food safety or yield performance. The approach supports Malaysia's move toward sustainable and water-efficient agriculture by reducing irrigation frequency, maintaining hygienic produce quality, and aligning with consumer safety expectations.

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Author Contribution

Hafiz-Afham – Conduct research and writing; N.A Hasan – supervision, review and editing; M.Y. Rafi – review and editing and N.J. Sidik – review and editing.

Conflict of Interest

Authors declare no conflict of interest

Declaration on the Use of Generative AI

Author declared Generative Artificial Intelligence (AI) tools such as Quilbot has been used only for language enhancement.

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