

Final Report

Pre-harvest sanitisation of leafy vegetables

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Potential for in-field pre-harvest control of foodborne human pathogens in leafy vegetables: Identification of research gaps and opportunities

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ABSTRACT

Background: Leafy vegetables (LVs) used as raw ingredients in salads have become a crucial part of our healthy diets. However, they are considered to be high-risk foods due to the lack of reliable measures to fully mitigate food safety risks in the absence of cooking prior to consumption. Indeed, outbreaks of foodborne illnesses and recalls associated with LVs continue to occur. This highlights the potential for additional strategies, such as pre-harvest sanitization, to better address the risks.

Scope and approach: This review undertook a comprehensive analysis of the current state of pre-harvest technologies that apply chemical sanitisers via treated irrigation water or via sanitization sprays of the field crop.

Key findings and conclusions: Several potential chemical sanitisers were shown to be effective against various foodborne pathogens when applied pre-harvest to crops. The review identified significant knowledge gaps concerning the efficacy of chemical sanitisers including their effect on the ecosystem health such as plant health, soil health, impacts on the natural leaf and soil microbiome. Addressing these gaps will provide a better understanding of the feasibility of these sanitization methods, including cost-benefit analyses. It is proposed that a risk framework, tailored to specific crops, soil types and weather conditions, should be developed to provide a science-based justification for the implementation of pre-harvest sanitization to improve the safety of LVs.

1. Introduction

Leafy vegetables (LVs) are considered an important component of a healthy diet, providing nutrients that can help prevent chronic diseases (Blekkenhorst et al., 2018). They are often produced ready-to-eat with no, or minimal, processing, and consumed raw. Therefore, with the possible exception of ionising radiation, which has been approved in some countries there are no fully reliable kill steps from sowing to farm gate to prevent transmission of any contaminating pathogens (Gil et al., 2015). Consequently, LVs are considered high-risk foods in terms of food safety to the general public and particularly to immunocompromised people including cancer patients, the elderly, and during pregnancy (Gomez et al., 2023). Internationally there have been numerous outbreaks associated with consumption of LVs. Most of these have been linked to *Salmonella enterica*, pathogenic *Escherichia coli*, Norovirus and *Listeria monocytogenes* (Food Standards Australia New Zealand, FSANZ,

2020; Mogren et al., 2018).

Contamination of LVs with pathogenic microorganisms can occur at any point throughout the production chain from in the field, at harvest, and in post-harvest processing (Rosberg et al., 2021). Moreover, LVs cultivated in the field environment are susceptible to contamination from soil, irrigation water, airborne pathogens and animals, amongst other sources. They have a large surface area to volume ratios allowing for more pathogen attachment site (Gil et al., 2015). Once pathogens contaminate the plant, they can survive on its surface, including through the formation of biofilms and multiply rapidly in injured tissues. Laboratory studies suggest that pathogens can also become internalized within the plant tissue via stomatal pores (Li et al., 2024; Lim et al., 2014).

To address the microbial risks associated with LVs, the industry currently relies on preventative controls proposed by voluntary Global Food Safety Initiative (GFSI) Recognised Food Safety Management

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Schemes (e.g. Global G.A.P.; FSSC 22000; SQF Food Safety and Quality Codes) as set out by the Code of Hygienic Practice for Fresh Fruit and Vegetables (Food and Agriculture Organisation, 2003). This helps growers and processors with on-farm and post-harvest food safety, quality and environmental certification (Allende & Monaghan, 2015; Frankish et al., 2021). In terms of food safety, clearly the first risk reduction step in the supply chain from farm to consumer is to prevent microbial contamination of the crop by managing sources of contamination on the farm such as contaminated water and animal manures. These pre-harvest best practices aim to ensure that the product, farm and harvesting machinery are at reduced risk of pathogen contamination and cross-contamination. However, they do not address the risks from pathogen contamination that may already have occurred.

Following harvesting, the industry utilises post-harvest washing of the produce, for removal of gross soil, debris and dust. Sanitizers are often added to the wash water to reduce microbial load and the potential for cross contamination from the wash water (Gombas et al., 2017; Du et al., 2024). Nevertheless, not all LV crops will be washed post-harvest, and post-harvest washing will not completely eliminate or prevent cross contamination that occurred during the pre-harvest period. Post-harvest wash water has also been identified as a risk factor for pathogen cross-contamination from samples of LV that are contaminated to the bulk crop that was previously free of contamination (Murray et al., 2017; Rosberg et al., 2021). *E. coli* and *Salmonella* Typhimurium can be reduced but not removed completely from lettuce leaf surfaces once attached after inoculation (i.e., during pre-harvest), even after several post-harvest wash treatments with a chlorine-based sanitizer (Banach et al., 2017). Some foodborne pathogens can also enter a temporary 'viable but not culturable state' after being exposed to a sanitizer (Ferro et al., 2018; Highmore et al., 2018) though whether this is relevant to field conditions remains to be determined (López-Gálvez et al., 2017). The fact that outbreaks associated with consumption of LVs continues to occur arguably suggests that reliance on post-harvest sanitization alone to manage food safety risks in the modern LV industry may not be adequate (Banach & van der Fels-Klerx, 2020; FDA, 2017; Frankish et al., 2021).

The LV industry could potentially further reduce risk by implementing additional safety interventions such as including the pre-harvest application of a chemical sanitizer to the crop. In concept, effective sanitizers could be applied to crops via irrigation water or via boom sprayers. This is in addition to any chemical disinfection of the irrigation water and system, which is already commonly practiced, killing any pathogens in the irrigation water as well as preventing bio-film fouling of irrigation pipes, and consequential blocking of low-pressure drippers (Dandie et al., 2020).

Industry experts, particularly in Australia, noted that applying water treated with sanitizers directly to the crop at preharvest stage, is currently not widely used by the LV industry (Hort Innovation, 2024). There remain many questions on whether a pre-harvest crop sanitization strategy would be feasible, cost-effective, or even environmentally advisable for widespread commercial use.

Recent peer-reviewed reviews have focused on the feasibility of various disinfection technologies to disinfect irrigation water only (Dandie et al., 2020; van Asselt et al., 2021; Gurtler & Gibson, 2022). Public sources suggest that ozone systems (for example <https://ingenieriadeloazono.es/en/agricultural-ozone/>) are already commercially available for agricultural irrigation high flow capacity (50–150 m³/ha) to both 'reduce biofilm of the pipes' and potentially to 'sanitize your crop'. However, published scientific evidence to support these claims, or determine potential ecological impacts, is lacking.

This review was, therefore, undertaken to provide: (i) an overview of current farm management practices to reduce contamination prior to harvest, (ii) an evaluation of the potential for chemical sanitization of the crops in the field via treated irrigation water or sanitization sprays, (iii) a summary of our current knowledge of the efficacy of chemical sanitizers in disinfecting irrigation water, and (iv) identify the

knowledge gaps and barriers, as well as explore the future prospects to industrially applying pre-harvest sanitization technologies. Given the scarcity of evidence-based research on the viability, benefits and risks of pre-harvest sanitization strategies on managing microbial load, the review considers both peer-reviewed and publicly accessible information.

2. Farm management to reduce food safety risks in the field

Foodborne pathogens are reported to survive and persist in the environment for years (Jacobsen & Bech, 2012). *L. monocytogenes*, as a pathogen of high concern for LV's contamination, is able to grow in a wide variety of ecological habitats from soil and decaying vegetable matter, to also infecting animals, birds and people (Sauders & Wiedmann, 2007). This widespread occurrence and persistence of pathogens allows contamination to occur through multiple pathways, such as contact with contaminated dust, soil, soil amendments, irrigation water, animal incursions, and human contact during harvesting, harvesting/processing equipment, and wash water (Mogren et al., 2018).

Farm management and agronomic practices are key factors that contribute to reducing the microbial hazards associated with LVs. They have already been extensively reviewed (Devarajan et al., 2023; Gil et al., 2015; Gutierrez-Rodriguez & Adhikari, 2018). Among these practices, irrigation water, soil management and proximity to animal production are considered the most effective targets (Devarajan et al., 2023) with approaches including animal exclusion, water treatment, manure treatments or bans, and soil solarisation (Mogren et al., 2018). Other practices that reduce the risk of microbial contamination of LVs include regular cleaning (e.g., premises, facilities, harvesting equipment, tools, machinery) along with food safety instructions and training for staff (Frankish et al., 2021), wild and domestic animal management (e.g., fencing, buffer areas, exclusion periods) (Patterson et al., 2018), and general agronomic practices that improve the overall resilience of the agrosystem (e.g., short term cropping, vegetated filter strips, pesticide application) (Gutierrez-Rodriguez & Adhikari, 2018; Lenzi et al., 2021).

A reduction in the microbial risks associated with LVs could potentially be achieved through the selection of varieties or cultivars with different susceptibilities to pathogen attachment and persistence of the pathogen (Melotto et al., 2020). Plant susceptibility is due to the involvement of leaf morphology (e.g., wax, veins, stomata, trichomes, roughness and wettability) in pathogen attachment and further persistence, which varies among different leafy crop species, types of the same species and even cultivars (Frankish et al., 2021). Indeed, it has frequently been reported that pathogen attachment and persistence positively correlate with stomatal density, stomata size and surface roughness (Jacob & Melotto, 2019; Palma-Salgado et al., 2020). From a practical perspective, if conditions are less than optimal, producers may potentially choose to grow more robust varieties/cultivars (e.g., lamb's lettuce and wild lettuce) rather than those that may have higher risks of microbial contamination (e.g., rocket and Swiss chard) (Truschi et al., 2023) though this may be dictated by market requirements.

Good agricultural practices reduce risks of contamination, but most do not actively treat existing contamination if it occurs. Many farm management practises also remain understudied, making it difficult to accurately evaluate their efficacy in minimising microbial risks (Devarajan et al., 2023). Controlling food safety risks through farm management alone cannot be solely relied upon given the complexity of different production systems combined with many additional interacting variables, including season, weather conditions, and growing regions (see Fig. 1) (Devarajan et al., 2023). It has been concluded that a multi-faceted approach of preventing and treating pathogen contamination is required to effectively manage pre-harvest safety of LVs (Devarajan et al., 2023; Mogren et al., 2018).

2.1. Water management

Contamination of LVs via irrigation water is considered a major

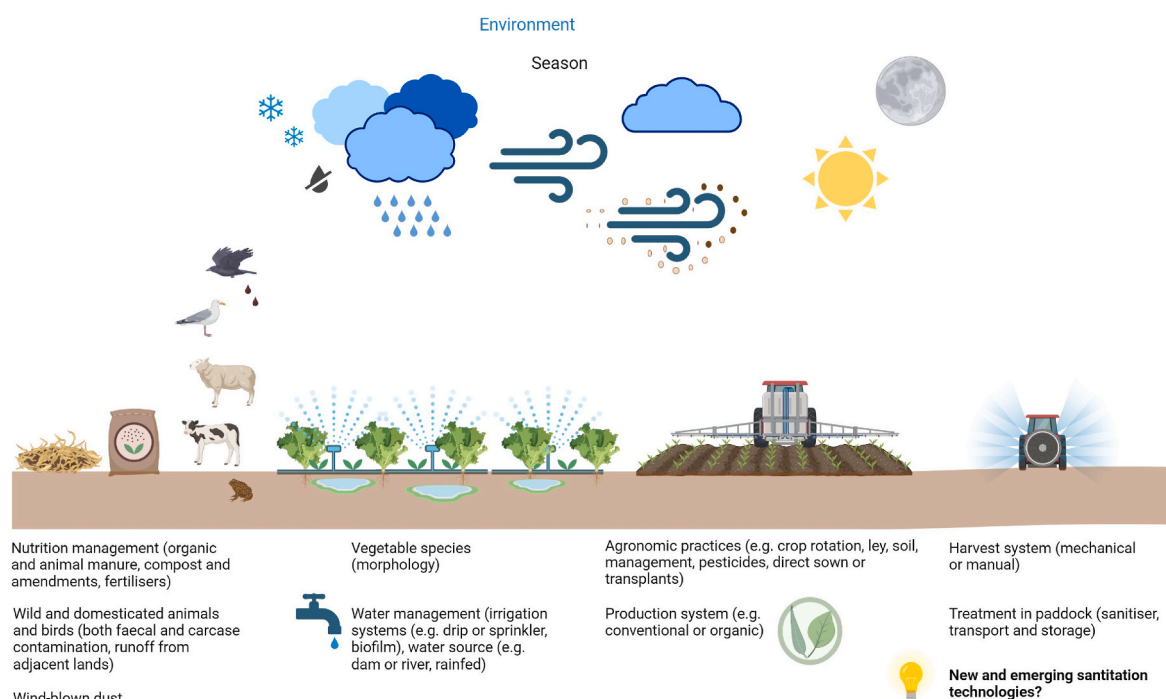


Fig. 1. Pre-harvest factors influencing contamination risk of foodborne pathogens in field production of leafy green vegetables that need to be considered separately and for interactions. Created with [BioRender.com](https://www.biorender.com). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

potential risk factor and has been directly linked to multiple outbreaks associated with LVs. A review by [Devarajan et al. \(2023\)](#) identified eight studies that directly showed sources of irrigation water affecting food safety risks. Surface water (i.e., irrigation channels, rivers, creeks, lakes, and on-farm reservoirs) posed greater risks compared to municipal, and ground water. Surface water is inherently susceptible to faecal contamination, run-off and seepage. The prevalence of pathogenic bacteria in irrigation water depends on a number of factors that include distribution system, season, geographical location, exposure to animal activity and animal manure-based fertilisers, and weather conditions ([Gil et al., 2015](#)).

Regardless of the source of water, testing the quality of the irrigation water is mostly self-regulated and relies on industry-lead certification guidance rather than legislated rules to achieve food safety ([White, 2022](#)). For example, in Australia, growers are required to test the microbial quality of their water supplies monthly during the period of use, or annually following four consecutive tests below specified microbial limits (i.e., < 100 *E. coli* cells per 100 mL if applied within 48 h of harvest to the edible portion of a crop usually eaten raw and/or minimally processed) ([Freshcare, 2020](#)). In comparison, in USA, there is no irrigation water testing requirement for *E. coli* ([FDA, 2023](#)). Instead, the Food Safety Modernisation Act Produce Safety Rules was recently revised in May 2024 to allow for systems-based water assessments for hazard identification and risk management decisions-making purposes ([FDA, 2023](#)).

The withholding period between irrigation and harvesting (see Section 2.2 for more details) are recommended to be of sufficient length for possible pathogens on the LV crop to 'die-off' (inactivation of the pathogen) via exposure to potentially harsh, dry conditions in the field ([Gutierrez-Rodriguez & Adhikari, 2018](#)). However, the rate of pathogen die-off depends on crop types, potential leaf damage, and environmental conditions, especially the dynamic changes in temperature, relative humidity, sunlight UV intensity, soil type and the existing soil microbial community ([Gutierrez-Rodriguez & Adhikari, 2018](#)).

The method of irrigation is an important farm management practice that needs to be considered in reducing microbial load in LV production

([Fonseca et al., 2011](#); [Gil, 2021](#)). The review by [Devarajan et al. \(2023\)](#) concluded that the use of sprinkler, spray, and foliar irrigation typically generated higher food-safety risks compared to furrow and drip irrigation systems. This is most likely due to direct contact of the water with the fresh product when the former irrigation systems are applied. For example, *E. coli* levels on lettuce were found to be higher (4–6 versus <1 log CFU/g) when irrigated with inoculated water via sprinkler systems compared to furrow and drip irrigation systems ([Fonseca et al., 2011](#)). This is because splash events (e.g., from sprinklers or rain) can transfer pathogens present in the irrigation water and soil to the surface of the fresh produce ([Lee et al., 2019](#); [Weller et al., 2017](#)). Irrigation methods that avoid or minimise contact between the irrigation water and the edible portion of a crop are, therefore, considered preferable for reducing contamination risk ([Allende & Monaghan, 2015](#); [Gil, 2021](#)) but may not be practical for all crops due to differences in cropping systems and soil types.

2.2. Soil management

Ground moisture and exposure to soil constitutes a food safety risk for LVs ([Devarajan et al., 2023](#); [Gutierrez-Rodriguez & Adhikari, 2018](#)). As reviewed by [Doren et al. \(2022\)](#), the role of mulch in the survival and spread of food borne pathogens has shown to be variable – it may promote pathogen persistence in the soil or it may also hinder direct contamination from soil by acting as a barrier. Soil and mulch can become contaminated through amendments such as manures and bio-solids as fertilisers ([Phan-Thien et al., 2020](#)), as well as via contaminated water, previous land use including animal presence, and other anthropogenic activities ([Gutierrez-Rodriguez & Adhikari, 2018](#)). Composting of soil amendments has been associated with improved food safety outcomes compared to using raw manures ([Devarajan et al., 2023](#)). Further, pathogen die-off in the field could be enhanced by maximising the time interval between application of soil amendments and time of harvest ([Devarajan et al., 2023](#); [Gil et al., 2015](#)).

Guidelines on the length of withholding periods to allow for pathogen die-off vary between standards and countries. For example, a

minimum of 60 days is required for [GlobalG.A.P.](#) Integrated Farm Assurance Smart (2024), 120 days for high-risk products is advised by the U.S. Department of Agriculture ([USDA, 2024](#)) and Canada ([CanadaG.A.P 2021](#)) and 45–280 days (depending of air temperature) for the Harmonised Australian Retailer Produce Scheme ([HARPS, 2022](#)) and 365 days for Californian Leafy Greens Marketing Agreement ([Products Handler Marketing Agreement, LGMA, 2023](#)). It is important to note that these exclusion periods do not necessarily align with the scientific evidence on the survival and persistence of bacterial pathogen in the field, which has shown to be up to one year or longer ([You et al., 2006](#)). For instance, *E. coli* O157:H7 and *S. Typhimurium* were found to persist for more than 100 days in soils amended with untreated manure ([Ramos et al., 2021](#)) while *E. coli* O157:H7 was shown to survive over 226 days in manure-amended soil stored at 15 °C ([Jiang et al., 2002](#)).

3. Potential antimicrobials for pre-harvest application on LV crop

3.1. Chemical sanitizers for application on crops

Conventional horticultural practice typically involves the implementation of a chemical program to manage plant bacterial and fungal pathogens that can damage crop yields and reduce shelf life. For example, in Australia, all chemical usage is regulated by the Australian Pesticides and Veterinary Medicines Authority (APVMA) under a permit approval system (<https://www.apvma.gov.au/>). This includes the use of sanitizers for water, soil or equipment used in the field. There are also

many chlorine-based products that are listed by APVMA as approved for post-harvest washing. However, it is worthy to note that there are currently no sanitizers that have been approved specifically as a microbiocide for pre-harvest application on food crops by the APVMA. The process to obtain approval by APVMA can be challenging and costly, and it highly likely that this situation is not unique to Australia. In the European Union, sanitizers used in agriculture to reduce microbial contamination before harvest are considered biocides, as regulated by the European Chemicals Agency (ECHA). Any biocidal product intended for pre-harvest use must go through a rigorous approval process, which includes an evaluation of the active substances to ensure they meet safety standards for human health, the environment, and food safety (as outlined in Regulation (EU) No 528/2012; [Eur-Lex, 2024](#)).

Emerging technologies, potentially suitable for in-field pre-harvest sanitization of crops via irrigation water, have garnered increasing attention. These are either as oxidising chemical concentrates (e.g., hypochlorite, peroxyacetic acid, hydrogen peroxide, 1-chloro-3-bromo-5,5-dimethylhydantoin, chlorine dioxide) or from on-site manufacturing units such as for producing electrolyzed oxidising water (EOW) ([Ogunniyi et al., 2021](#); [Shang, et al., 2023a, 2023b](#)). These technologies draw from current fertigation and spraying systems for plant pathogen management. However, to our knowledge, very few studies have examined the efficacy of pre-harvest sanitization of LVs by directly assessing the microbial reduction on the crop itself, and most of them were conducted over the past three years ([Table 1](#)).

These studies have shown promising results confirming the efficacy of the various sanitizers to reduce microbial load and foodborne

Table 1
Selected examples on the efficacy of pre-harvest sanitizer application to reduce foodborne pathogens on crops.

Study	Application method	Inoculated bacterial load on crop	Sanitizer	Crop	Log reduction on plants ^{a,b}	Sensory effects
López-Gálvez	Field trial (no inoculum)	<i>E. coli</i> field population of the water <i>Enterobacteriaceae</i> of the plant	Hypochlorite (<1 mg/L)	Baby spinach	<i>E. coli</i> counts (0.2–0.3 log reductions) of the water only <i>Enterobacteriaceae</i> : NS	Reduction in photosynthesis Build-up of chlorate (up to 0.99 mg/kg)
Ogunniyi et al. (2021)	Glasshouse trial (25 L of solution over 10 min; equivalent to a 6 mm irrigation event; harvested 1–2 h later)	<i>E. coli</i> , <i>S. Enteritidis</i> and <i>L. innocua</i> (~1 × 10 ⁹ CFU per plant) Air-dried for 2–3 h before application of sanitizers	Neutral electrolyzed oxidising water (50 ppm free chlorine)	Cos lettuce Baby spinach	<i>E. coli</i> : 0.7 <i>S. Enteritidis</i> : NS <i>L. innocua</i> : 0.8 <i>E. coli</i> : NS <i>S. nEnteritidis</i> : 1.4 <i>L. innocua</i> : 1.4	No visual differences pre- or post-harvest
Shang, Zheng Tan, et al. (2023)	Field trial (inoculum sprayed evenly with shoulder spray). Harvested 24 h later	<i>L. innocua</i> (~1 × 10 ⁸ CFU/mL) Air-dried for 30 min before application of sanitizers	Sodium hypochlorite (50 ppm free chlorine)	Cos lettuce	Only reduced <i>L. innocua</i> by 0.8	Negative effects on leaf appearance for both varieties Sensory quality and shelf life similar to control
			Neutral electrolyzed oxidising water (20 ppm)	Baby spinach Mizuna, rocket, red chard, spinach	No effect on all species 0.8–2.5	
			Peroxyacetic acid (80 ppm)	Mizuna, rocket, red chard, spinach	2.4–5.5	
Shang, Huang, et al. (2023)	Glasshouse trial (harvested 120 min later.	<i>E. coli</i> and <i>L. innocua</i> (~1 × 10 ⁸ CFU/mL) Air-dried for 30 min before application of sanitizers	1-bromo-3-chloro-5,5-dimethylhydantoin (20 ppm)	Mizuna, rocket, red chard, spinach	0.2–1.5	NA
			Neutral electrolyzed oxidising water (5 and 20 ppm)	Mizuna, rocket, red chard	<i>E. coli</i> : 1.7–2.7 <i>L. innocua</i> : 1.5–2.8	
			Peroxyacetic acid (80 and 150 ppm)	Mizuna, rocket, red chard	<i>E. coli</i> : 1.6–3.8 <i>L. innocua</i> : 2.1–2.7	
			1-bromo-3-chloro-5,5-dimethylhydantoin (5 and 20 ppm)	Mizuna, rocket, red chard	<i>E. coli</i> : 1.1–2.2* <i>L. innocua</i> : 1.1 - and 2.3	

^a Compared to water controls.

^b Where multiple concentrations were tested, results reported with lower concentration first followed by higher concentration.

NS = not significant.

NA = not assessed.

pathogens on LV crops in the field (López-Gálvez et al., 2018; Shang, Zheng Tan, et al., 2023) and glasshouses/hydroponic systems (Ogunniyi et al., 2021; Shang, Huang, et al., 2023). Across all three studies, neutral EOW (hypochlorous acid, 20–50 ppm) was found to be effective against *E. coli* and *Listeria innocua*, resulting in a reduction of at least 0.7 to 3.8 log CFU/g depending on the crop type (Ogunniyi et al., 2021; Shang, et al., 2023a, 2023b). Peroxyacetic acid or PAA (80–150 ppm) was typically more effective, where reductions in *E. coli* and *L. innocua* ranged between 2.5 and 5.5 log CFU/g (Shang, et al., 2023a, 2023b). Furthermore, out of the tested sanitizers, there appeared to be little negative impact on the sensory qualities of the produce pre- or post-harvest, with the exception of sodium hypochlorite (50 ppm) where severe necrotic zones and yellowing/browning of leaves occurred prematurely (Table 1). In addition, application of 1 mg/L of hypochlorite in the irrigation water was found to reduce photosynthetic efficiency of baby spinach and also resulted in the build-up of chlorate residue (López-Gálvez et al., 2018). We found only one other study that examined pre-harvest sanitation in another horticultural crop (Zhao et al., 2021). found pre-harvest sanitation with the bactericide comprised of levulinic acid (0.5%) and sodium dodecyl sulphate (0.5% SDS) reduced foodborne pathogens (*E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*) and microbial loads of pot-grown tomato grown under full sun by an average of 1.7 log CFU/fruit. These studies applied sprays pre-harvest up to 2 h prior to assessment but these short timeframes may not accurately reflect field conditions, given that longer times would be required by a grower to apply sanitizer treatments over extensive crop areas (Shang, Zheng Tan, et al., 2023).

It is important to note that the high inoculum levels (>5 log CFU/g) of specific pathogens used in these studies are not likely to frequently occur in the field. For example, Holvoet et al. (2014) found that in most cases the natural *E. coli* contamination levels on lettuce were below <0.7 log CFU/g across four open field farms, with one farm having slightly higher levels of between 2 and 3 log CFU/g in less than 10% of samples (Holvoet et al., 2014). Therefore, from a practical perspective, a 1–2 log reduction is likely to be sufficient to provide appropriate levels of food safety under most natural contamination levels. However, in-field trials, are warranted and would need to consider a number of factors including sanitizer type, concentration of sanitizer, volume/coverage of spray and sanitizer contact time (Shang, Zheng Tan, et al., 2023).

3.2. Other antimicrobials for application on crops

Apart from chemical sanitizers, other antimicrobials have recently emerged as potential options for pre-harvest application to reduce foodborne pathogens in LV crops. These include bacteriophage and lactic acid bacteria (LAB) treatments, which are considered safe, effective, and natural antimicrobials for fresh produce. Furthermore, these approaches are thought to have low impact on sensory qualities of the produce (Lenzi et al., 2021). However, most of the research has been done in a post-harvest context and adoption as a spray treatment in a pre-harvest context would arguably be expensive and unfeasible due to the scale required. There are currently limited studies that have investigated the application of phage-based sanitization for pre-harvest control of pathogens on LVs, however, its efficacy is restricted by the high specificity of the phage to the host (Bumunang et al., 2023), and only one for the application of LAB (Yin et al., 2023). Specifically, Yin et al. (2023) highlighted the potential efficacy for LAB treatments as it was evident that after electrostatically spraying lettuce crops with a cocktail of LAB (8 log CFU/ml containing *Lactococcus* spp. and *Lactiplantibacillus* sp.), *L. monocytogenes* and *E. coli* O157:H7 numbers were reduced by ~2 and 1 log units, respectively. Despite these promising results, the practicability and ecological impacts of innovative biological treatments such as LAB and bacteriophage for pre-harvest control of pathogens on LVs must also be considered in relation to associated costs, e.g. of 'scaling up' and ability to withstand environmental fluctuations (Imran et al., 2023).

4. Reducing microbial load from irrigation water

Disinfection technologies could allow growers to broaden their options for irrigation water sources without relying on frequent microbial testing to account for potential changes in water quality (Allende & Monaghan, 2015). Currently there are a range of physical and chemical technologies that have been demonstrated either alone, or in combination, to be effective for disinfection of irrigation water (Meireles et al., 2016; van Asselt et al., 2021; Irakoze et al., 2022; Supplementary Table 1).

While physical/mechanical technologies such as membrane filtration, slow sand filtration, ozone and UV irradiation (Banach et al., 2021) are considered beyond the scope of this review, it is worthy to mention that the main advantage for physical/mechanical treatments is that they do not form potentially hazardous disinfectant by-products and regulatory guidelines around their use are less onerous (Dandie et al., 2020; van Asselt et al., 2021). However, the main disadvantage of physical/mechanical technologies is that there is no residual effect beyond the specific treatment time. Further, many of the physical treatments also reduce turbidity, suspended solids, and organic matter, therefore enhancing the effectiveness of any subsequent physical (e.g., ultraviolet or ionising irradiation) and chemical sanitising treatments (Banach & van der Fels-Klerx, 2020; Dandie et al., 2020).

By comparison to physical/mechanical technologies, implementation of chemical sanitization technology is relatively straight forward, requires low energy input and generally involves low maintenance. Based on knowledge gained in post-harvest processing activities comparatively safe sanitizers tend to be weak acids that are strongly electrophilic and possess a degree of oxidation selectivity, including ClO^- , ClO_2 , HOCl, HOBr, ozone (O_3), peroxides (H_2O_2 , PAA), and natural organic acids (e.g., lactic acid, citric acid) (Feliziani et al., 2016). The reviews by Raffo and Paoletti (2022) and Dandie et al. (2020) have already provided detailed information on the advantages and disadvantages of common chemical sanitizers. However, it should be noted that with these chemicals, safety is a relative term. For example, HBrO (hydrobromous acid) is one of the most powerful known electrophiles but it is this reactivity that makes it environmentally hazardous (Raffo & Paoletti, 2022). As a result, chemical sanitizers that are useful are constrained by many factors including efficacy, cost, safety during use, and ecotoxicity. Further, some chemical sanitizers, such as chlorine and bromo-chloro dimethyl hydantoin, can pose healthy safety concerns due to the potential formation of toxic disinfectant by-products (DBPs) (Raffo & Paoletti, 2022). The exact chemicals used, however require individual appraisal of risk from exposure, during application and the presence of residues (Santos et al., 2023).

Studies to date have primarily focused on evaluating the efficacy of chemical sanitizers to treat wash water in LV post-harvest systems rather than pre-harvest irrigation systems (Supplementary Table 2). This is because these same sanitizers have traditionally not been used to disinfect irrigation water although many have use in disinfection of cooling water towers, swimming pools and spas. However, it may be possible to draw from the knowledge gained from the many post-harvest sanitization studies that examined the key factors influencing their efficacy. Overall, chemical sanitizers are sensitive to the water quality parameters such as turbidity, pH and organic load. Therefore, treatment concentrations need to be optimised and monitored regularly with sensors to ensure accurate dosages, and in some cases, water may require pre-treatments such as filtration or pre-oxidation to reduce organic load (Santos et al., 2022).

Comparison of the efficacy of sanitizers is difficult given the wide range of test variables across studies, along with there being fewer relevant experiments comparing different sanitizers in parallel. Of particular note, Ogunniyi et al. (2019) compared the efficacy of pH-neutral EOW, sodium hypochlorite, and chlorine dioxide against *E. coli*, *L. innocua* and *Salmonella* in simulated irrigation water with increasing organic matter content. The efficacy of EOW containing 5

ppm free chlorine equivalents was unaffected by increasing organic matter (i.e. dissolved organic content of 40 mg/L) and compared favourably with equivalent concentrations of sodium hypochlorite, and chlorine dioxide (Table 1). EOW was more effective, compared to the other sanitizers, in the presence of high dissolved organic carbon (20–100 mg/L) when applied at a higher concentration of 20 ppm free chlorine equivalents.

Murphy et al. (2023) reported the efficacy of chlorine (calcium hypochlorite; 2–4 and 10–12 ppm) and PAA (6 and 10 ppm) against *Salmonella* in pond and river irrigation water with up to 10 min contact time (Supplementary Table 1). Irrespective of all other factors like contact time, temperature, and water quality, the results demonstrated that the reductions of *Salmonella* by chlorine were 3.25 log greater than that of PAA. Different results between studies may be attributed to the target organisms tested (e.g. *Salmonella* vs mesophilic aerobic bacteria), contact time (30 min vs 24 h) and nature of the organic loads in the water samples tested. This suggests that the different scenarios where sanitizers are being applied pre-harvest require investigation. Currently we lack empirical knowledge to accurately predict the efficacy of sanitizers and thus still require ground truthing to arrive at optimal recommendations.

5. Research needs and future perspectives on applying pre-harvest sanitization technologies

Despite considerable research on post-harvest sanitization of LVs, there are relatively few refereed publications on evaluating pre-harvest sanitization approaches. We can assume that chemical sanitation technology that can disinfect irrigation water has provided a base of evidence of net-positive impacts that pre-harvest sanitization could generate. The main challenge is to determine what pathways can best enable industrial pre-harvest sanitization. A review by Van Haute et al. (2015) broke down this to pathways of costs, safety, and environmental impact.

5.1. Developing a risk framework where the need for pre-harvest sanitization could be justified

Pre-harvest safety interventions could be considered under a validated risk framework where risks of pathogen contamination are estimated. This would include the risk of contamination via transfer of water as well as, animal contaminated soil and dust particle deposition onto plants, processes that can be exacerbated by climate events (Miron et al., 2023), such as major rainfall and moderate to high velocity wind events. These processes represent potentially measurable risks, however yet remain to be conceived in a quantitative microbiological risk assessment-like framework. Other possible measurable hazards that may be relevant involve, for example, the movements and distributions of wild animals or adjacent domesticated animals (Jeamsripong et al., 2019). Other sources of microbial contamination are possible, such as the sources of water used for irrigation. The development of a probabilistic risk framework that considers a suite of scenarios that could increase contamination potential in farming systems (Buscaroli et al., 2021) may then better frame the usefulness of pre-harvest interventions described earlier in this review. Until this happens net benefits can only be assumed. Having actual evidence of utility could then allow greater opportunities to quantitatively predict effectiveness of different pre-harvest antimicrobial interventions within the LV supply chain. This is useful since the production phase should not be seen in isolation, but rather considered with other requirements for LV products, such as spoilage mitigation and smart packaging options (Karanth et al., 2023) particularly given that the commercial feasibility of LVs and their inherent health-related value is tied to both safety and quality.

5.2. Challenges of cost-effectively managing large scale disinfection of irrigation water

The availability and cost of irrigation water is often very competitive, and potentially the water is of highly variable quality (Bhagwat, 2019). In dry periods, there is often a need for water storage in ponds and man-made reservoirs thus increasing the risk and level of biological contamination (Jiang et al., 2019). The use of chemical sanitizers or physical technologies (see Section 3) that allow water to be effectively treated to potable standard have long been established for domestic and industrial processing water supplies. Smaller scale decentralised treatment of water, in a world where water scarcity has become a serious issue, is likely to become a normal requirement (Siwila & Brink, 2018). However, the cost efficiency of using existing or newer, more sustainable technologies to treat pre-harvest irrigation water, whether through on-site treatment (Mishra et al., 2023) and/or supplied via boom sprayers on a tractor, is yet unclear for large scale agricultural or horticultural activities under the diverse possible scenarios and variable water quality inputs.

Non-protected, LV agriculture requires water quality systems appropriately engineered to allow producers to be able to access water that could be variable in initial quality. This entails potentially significant capital costs and has already been considered extensively in protected horticultural systems that are moving towards technological sophistication. Even then the actual production efficiency, economic and safety advantages though highly touted have still to be conclusively quantified either economically (Moghimani & Asiabanpour, 2023) or in terms of biological risk control (Hamilton et al., 2023). Overall, available technology would need to be assessed to determine the most effective and affordable options to be integrated into agricultural water deployment systems.

5.3. The efficacy and cost-benefit of applying pre-harvest sanitizers to crops

For implementing pre-harvest interventions, producer costs can include the costs of the chemicals, any additional capital infrastructure needed to implement chemical applications, as well as the labour, training and quality monitoring needed to run the system in a safe manner. However, given the current lack of scientific information on the efficacy of this technology, it may be seen by producers as adding an unnecessary cost and that risks, for example from weather, is simply part of doing business and there are limits on how proactive farmers can practically be (Ricart et al., 2023).

Implementation could also consider a risk assessment to determine if irrigation water disinfection is warranted in the first place and then if required, other factors would need to be considered including the effects of timings, frequency and application rates of treating irrigation water and/or using irrigation water to treat crops pre-harvest. Sanitizers could, ideally, be applied just once prior to harvest as a final 'rinse' either in the irrigation water or delivered as a spray in a similar way to pesticides. This is basically a one-step pre-harvest treatment that could be used selectively to augment post-harvest sanitization processing. Based on recommended commercial dosage rates applied post-harvest, the estimated costs of four commonly used post-harvest sanitizers for a final rinse were calculated and summarised in Table 2. However, the efficacy of sanitizers as listed in Table 1 suggest that meaningful log reductions could be achieved even at much lower concentrations than the values presented in Table 2. Therefore, the approximate cost of the sanitizer may be even less than calculated in Table 2, notwithstanding the initial capital costs for storage, production and application equipment. Cost-benefit analysis should also consider indirect costs such as the additional time that may be required to clean equipment to minimise unknown interactions between sanitisers and any agronomic chemicals that are being mixed in spray booms.

Table 2

Estimated cost of four common sanitizers in Australia if applied in irrigation water for a final crop 'rinse'. This does not factor the cost of water as water is already used for irrigation. All costs are in US dollars.

Sanitizers	Recommended level (ppm) ^a	Cost per hectare ^b	Capital costs ^c
Chlorine	25–80	\$275–\$881	Up to \$23K
Peroxyacetic acid	20–80	\$295–\$1182	Up to \$23K
Electrolysed oxidising water	2–20 (as free chlorine)	\$5–\$50	Up to \$24K for a generator and installation for automated dosing systems
1-bromo-3-chloro-5,5-dimethylhydantoin	5–10 (as free chlorine)	\$128–\$256	\$561–\$792 for an erosion feeder to disperse concentrate plus automated dosing systems

^a Higher end of the recommended level is based on post-harvest conditions.

^b Cost was estimated based on dilution of concentrates and assumed that 300,000 L is used per hectare (i.e., coverage is assumed to be a 3 mm depth purely from a single spray); Cost of each sanitizer was obtained directly from distributors located in Australia.

^c Costs required for equipment and installation for automated dosing systems.

5.4. Effect of pre-harvest sanitizer residuals on ecosystem health

The effects of pre-harvest sanitizer residuals on long-term ecosystem health from multiple applications is currently largely unknown. This includes the effect on soil health, natural leaf and soil microbiome (e.g., rhizosphere) that may in fact act as protection against contamination with plant and human pathogens (Dandie et al., 2020) and on plant physiology and quality (see below). Further, the impact of pre-harvest sanitizers on the accumulation of DBPs in the environment and in the harvested product remains unknown and warrants investigation. The potential effects will depend on many variables, such as the type of sanitizer and its concentration, and the frequency of application. For instance, a recent glasshouse study by Lombi (2021) found that the application of EOW (5 ppm) spray resulted in minimal change in soil properties (pH, electrical conductivity) for all soils tested when applied once a week over a 14 week period. In contrast, under the same conditions, application of sodium hypochlorite significantly increased soil pH for the majority of soils tested. Further, there remains uncertainty on whether or not multiple applications of NaCl enhanced production of EOW would contribute to higher soil salinity, although this is unlikely given that the concentration of NaCl is EOW is 0.26% wt/vol (Environlyte, 2024). More expensive KCl-based EOW could be used to overcome this problem with dual nutrient potential (Ogunniyi et al., 2019). More research is required to evaluate this further in the field under a range of conditions.

Research is also required to assess the effects on microbial ecosystems, including the natural microbial community of the LV phylloplanes (Dakwa et al., 2021) and potential co-benefits to suppress plant pathogen populations (Zheng et al., 2008). However, the possible co-benefits of using water with sufficient residual oxidising activity to treat both plant and human pathogens on LVs are not known. The compatibility of sanitizers for use in conjunction with approved treatments of LVs (e.g., fungicides and pesticides) needs to be also evaluated to inform long-term soil and ecosystem health. While it is known that oxidising sanitizers can affect the efficacy of some pesticides and potentially interact with them, this knowledge is fragmentary and needs to be further detailed with use guidance for producers (Mueller et al., 2003).

5.5. Impact of pre-harvest sanitizers on plant and product quality

The potential effect of pre-harvest sanitizers on plant and product

quality is a significant concern to producers and is not well studied. The levels at which potential negative impacts occur to plant growth and sensory qualities will depend on many variables such as the type of sanitizer and its concentration, and the frequency of application (López-Gálvez et al., 2017). For example, practice evidence exists that single exposures to oxidation sanitizers up to 50 ppm chlorine equivalents damages fresh produce (Nguyen et al., 2019). In contrast, pre-harvest application of PAA (up to 150 ppm), EOW, and 1-bromo-3-chloro-5,5-dimethylhydantoin BCDMH (both up to 20 ppm free chlorine) to LVs had no effect on product appearance, colour, texture or odour but also did not extend shelf-life at 4 °C (Shang, Huang, et al., 2023). This may be due to a rapid deactivation of oxidising ability in the 24-h contact time suppressing bacterial growth during the initial stage of storage (up to 3 days) but otherwise did not influence subsequent bacterial outgrowth. The only extensive information available on sanitizer concentration versus impacts on plant health has been research on the impacts of chlorinated water supply. The chlorine concentration threshold above which damage plant health occurs from repeated regular over-head water is > 1–2 mg/L for many vegetables and seedlings but listed as >18 mg/L for lettuce and >50 mg/L for sweet pepper (Zheng et al., 2008). Furthermore, data on plant health after pre-harvest sanitization of mature LVs prior to harvest is not available but would be species dependent and resistance to oxidative damage may change with age and environmental exposure.

Overall, the limited existing data does not allow robust conclusions to be drawn considering the wide range of crops and scenarios in which sanitizers could be applied. The impacts of sanitizer types, concentration and frequency of applications on the resilience and quality of LVs grown commercially needs to be assessed more thoroughly and measured against the benefits of microbial pathogen load reduction.

5.6. Regulatory limitations in applications for pre-harvest safety

The lack of scientific evidence for pre-harvest sanitization technologies creates a gap in regulatory guidance being available for its use. This is especially for managing potential contamination with foodborne pathogens but recognising that longer term pre-harvest use could impact both the growth of plant pathogens or the effectiveness of plant sprays against plant pathogens. This includes constraints on usage owing to potential issues with residual chemicals in the field and on the crop, as well as user safety in deploying chemicals on a wide scale. Organic farming systems are controlled for pre-harvest sanitizer with restrictions for some sanitizers and approval for others such as EOW being approved as equivalent to chlorine for organic usage (USDA, 2014).

In terms of usage often the regulations are quite specific in that a sanitizer product has a prescribed use. For example, PPA (5% peroxyacetic acid) (Ayoub et al., 2017) is used as an antifungal agent in grapevine but is not approved for other applications. There is a precedence for approval of sanitizers labelled as microcides that can be applied pre-harvest but there is currently no approved advice on field use against foodborne pathogens in Australia, for example (Murphy et al., 2023). In the USA, there are a number of EPA-registered products based on PAA that have been approved for use in foliar sprays in addition to irrigation water systems pre-harvest. However, the majority of these have been approved to "control the growth of non-public health microorganisms that can cause spoilage" or "to cure or prevent bacterial and fungal diseases on growing agricultural crops." In contrast, only a few of these have been approved to reduce and control foodborne bacterial pathogens in preharvest irrigation water (Produce Safety Alliance, 2024). Fortunately, there is an emerging impetus for regulatory amendments, particularly in the US, where the Food and Drug Administration have recently developed a testing protocol to generate data on the efficacy of antimicrobial products to reduce foodborne pathogens, with the aim to support approval of chemical sanitization of irrigation water (FDA, 2023).

6. Conclusion

The increasing number of foodborne outbreaks and recalls associated with LVs highlights the need for additional strategies to reduce the safety risks of crop contamination in the field. The review identified viable pre-harvest interventions for disinfection of irrigation water and/or sanitization of the crop via irrigation water or sprayers. Recent studies confirmed the efficacy of pre-harvest sanitization of the crop to reduce pathogen contamination. There is already approval for specific sanitizers to be used for irrigation water treatment. However, the cost efficiency of these technologies at large scale agricultural activities under different scenarios and variable water quality inputs is unknown. Pre-harvest sanitization could also be used a strategy to mitigate microbial food safety risks associated with extreme weather events (e.g. dust storms and heavy rainfall splash), immediately prior to harvest. The review also identified numerous knowledge gaps and areas for research and development to enable adoption of pre-harvest sanitization technologies. Specifically, further research is needed to evaluate the efficacy of these interventions under different scenarios and how they affect the quality of plant/product and their ecosystem health. A risk framework for specific crops and scenarios should also be developed to justify the need for implementing pre-harvest sanitization.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tifs.2025.104928>.

Data availability

No data was used for the research described in the article.

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