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Efficiency of Domestic Hygienization of Fertirrigated Lettuces with Wastewater from Dairy Cattle Farming

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Abstract

Lettuce is the most consumed vegetable worldwide, which has a great economic importance. Its growth demands a large volume of water, encouraging the search for alternative water sources, such as fertigation with cattle wastewater. However, as it is a fecal-origin material, this practice can increase the initial microbial load of the vegetable and affect the efficiency of the sanitation process. Thus, the objective of this study was to evaluate the efficiency of domestic sanitation with sodium hypochlorite solution of lettuces obtained with different dosages of fertigation with wastewater from dairy cattle. After the sanitization, samples of all treatments showed lower number of total Coliforms, *Escherichia coli* and Aerobic Mesophiles. However, lettuces grown with 200% and 300% of the recommended dose of nitrogen showed microbial counts higher than those declared safe for consumption even after sanitization. There was no development of *Salmonella* spp. Although the productivity of the vegetable has increased with this irrigation technique, the limit of 100% of the recommended dose of nitrogen must be respected, since higher doses caused the persistence of pathogens at unsafe levels for human health even after the sanitation process. However, fertigation proved to be an outstanding alternative for saving water resources and proper disposal of wastewater.

Keywords: effluents, chlorinated solution, food safety, sanitization

1. Introduction

Leafy vegetables are mostly consumed raw, so they do not undergo any heat treatment capable of eliminate or reduce pathogenic microorganisms to safe levels for consumption. If nor properly sanitized, vegetables can be vehicles for these microorganisms, which can cause foodborne illnesses (Mishra et al., 2017).

According to Carstens et al. (2019), the contamination of fresh products may occur throughout the production chain: before, during and after the harvest. The initial microbial population is variable and depends on the cultural management practices and cultivation techniques used, the post-harvest management and even on possible cross-contamination during preparation and consumption.

As the most consumed vegetable in the world, lettuce is constantly associated with foodborne outbreaks caused by vegetable consumption (Elias et al., 2019). According to the US Centers for Disease Control and Prevention, *Salmonella* spp., *Escherichia coli*, and *Listeria* sp. are the microorganisms responsible for most cases of food outbreaks related to the consumption of fresh vegetables in the country, and *Escherichia coli* is the most common bacteria in lettuce (CDC, 2021). A similar scenario is reported in Brazil, where Escherichia coli is the etiological agent related to the highest incidence of foodborne cases (around 24%), along with *Salmonella* spp. and *Staphylococcus aureus* (Brazil, 2020).

Originally from Asia, lettuce is a vegetable remarkably economic important (Demartelaere et al., 2020). China is the main producer of lettuce, while the United States are the largest exporter, and Canada the largest importer (Trigde, 2020). The Brazilian production reached 671,509 tons of the vegetable in 2017, representing approximately R$ 1,200,000.00. São Paulo is the largest Brazilian producer of lettuce, followed by Minas Gerais and Paraná

(IBGE, 2021). Its cultivation has a high demand for water availability, which encourages the use of alternative water sources (Varallo et al., 2011; Demartelaere et al., 2020).

The use of wastewater from cattle farming has been widely discussed and applied throughout the world, especially in arid and semi-arid regions, as well as in regions with limited water availability (Medeiros et al., 2005). This technique is classified as fertigation and has been highlighted for allowing the proper disposal of effluents that could lead to serious environmental damage if improperly discarded, or even impact on extra expenses for the management of these residues. In general terms, it consists of the use of effluents to irrigate crops in order to accelerate the cycle of nutrients used by plants, especially nitrogen, potassium and phosphorus, present in wastewater. Such application makes it possible to reduce the volume of drinking water used in irrigation and minimize the use of synthetic fertilizers in vegetable cultivation (Juchen et al., 2013; Jorge et al., 2017).

However, the use of these effluents can result in a sanitary risk, with serious consequences for human health (Fonseca et al., 2007). As it is a material of fecal origin, coming from animal farms, this practice can lead to food contamination by pathogenic microorganisms, causing foodborne illnesses and outbreaks (Wadamori et al., 2017; Elias et al., 2019), with emphasis on vegetables that are consumed raw, such as lettuce.

Therefore, it is important that special hygiene practices are taken before the consumption of vegetables produced by this technique. According to Banach et al. (2015), the efficiency in reducing pathogenic microorganisms of enteric origin is directly linked to the sanitizer used for the sanitization of the vegetable. Although several studies indicate possible deleterious effects of sanitization performed with chlorine, products based on this element are still the most used for sanitize fresh vegetables, and they are recommended even by official regulatory agencies, such as ANVISA (Brazil, 1988; Brazil, 2004; Brazil, 2016; Tao et al., 2019).

In addition to the sanitizer used, the concentration of the microorganism also impacts the efficiency of cleaning (Pezzuto et al., 2016). Thus, our objective was to evaluate the efficiency of chlorinated solution in the sanitation of lettuces submitted to different fertigation treatments, with wastewater from dairy cattle. The study simulated vegetable hygiene techniques performed in a domestic environment, according to the recommendations described on the sanitizing product label, in order to translate the procedures performed by the consumer before consuming lettuce at home.

2. Material and Methods

2.1 Curly Lettuce

The samples of Vera cultivar lettuce (*Lactuca sativa* var. *Crispa* L.) were obtained from the Integrated System of Agroecological Production – SIPA (Fazendinha Agroecológica do km 47), located in Seropédica, Rio de Janeiro – RJ (220 48 '00" South latitude and 430 41'00" West longitude), from 4 different fertigation treatments with dairy cattle wastewater, with nitrogen as the reference nutrient. The wastewater was previously treated in a pilot plant with an upflow anaerobic filter and a constructed wetland system cultivated with vertiver grass

(*Chrysopogonzizanioides (L.) Roberty*), following the procedures described by Guimarães et al. (2016). Four different concentrations of the reference nutrient were used: 50% of the recommended nitrogen dose – A1; 100% of the recommended nitrogen dose – A2; 200% of the recommended nitrogen dose – A3; 300% of the recommended nitrogen dose – A4. The wastewater application was carried out by fertigation using a drip irrigation system and the reference treatment was A2, with a dosage of 90 kg ha^{-1} – proportion determined from preliminary studies. The management of nitrogen dosage was carried out by controlling the rate of fertigation supplied to the plant, expressed in L h^{-1}.

2.2 Impact of Nitrogen Dosage on Lettuce Development

The impact of nitrogen dosage on lettuce development and growth was investigated comparing leaf number and weight of the plants. The leaves were counted manually, discarding the yellowed and/or dry leaves; and weighing was performed on a scale with a 0.01 g precision (Neto et al., 2005). The results were expressed in number of leaves/plant and grams/plant, respectively.

2.3 Sanitization of the Lettuce

The samples were composed of four plants from each of the fertigation treatments randomly chosen (Neto et al., 2005). The plants were received, manually defoliated, and visually selected to discard the injured leaves. The healthy leaves were pre-washed in potable running water to remove coarse dirt. Then, the leaves were immersed in a sodium hypochlorite solution (200 ppm) for 10 minutes, following the recommendations indicated on the label of the chosen chlorinated product: 10 mL of sanitizer per 1 liter of water. The pH of the chlorinated solution was measured and corrected to 7.00. The proportion used was 25 g of lettuce for 250 mL of chlorinated solution (Gomes Neto et al, 2012). Afterwards, the leaves were rinsed in potable running water and centrifuged in a plastic domestic centrifuge to remove excess water. The leaves were refrigerated (4 °C) for 30 minutes in polypropylene packages sterilized by UV light, for further analysis. The procedure conducted was recommended by Ordinance CVS n°. 05/2013 (São Paulo, 2013) and in Resolution RDC n°. 216/2004 (Brasil, 2004), using of the following necessary personal protective equipment: gloves, masks and disposable caps.

2.4 Evaluation of the Efficiency of Sanitization of Lettuce

To evaluate the efficiency of the sanitization, microbiological analyses were conducted for enumerating *Salmonella* spp., Total Coliforms, *Escherichia coli* and aerobic mesophiles, according to the procedures described by APHA (2001). The Normative Instruction IN n° 161/2022 (Brasil, 2022), determines that vegetables must have the absence of Salmonella spp. and maximum enumerations for Escherichia coli of 10^2 MPN g^{-1} before and 10 MPN g^{-1} after sanitized.

2.5 Statistical Analysis

Assays were performed in triplicate and results presented as means ± standard deviations. The effects of the different treatments were compared by Analysis of variance (ANOVA), F test. And the difference between them, with an error level lower than 0.05, was determined by Tukey's test. Statistical analyzes were performed using the STATISTICA 7.0 program (StatiSoft, Inc., Tulsa, Okla., U.S.A.).

3. Results

3.1 Impact of Nitrogen Dosage on Lettuce Development

A significant difference (p>0.05) was only observed on the number of leaves per plant between A1 and A4. Higher doses of nitrogen supplied to the plants caused an increase of approximately 30% in the number of leaves per plant (Table 1). Considering the lettuce weight, the growth was proportional to the nitrogen dosage supplied. However, the increase was only statistically significant (p > 0.05) comparing A1 with the other treatments, whose increase was 63%, 90% and 96% compared to A2, A3 and A4, respectively (Table 1).

Based on that, we concluded that plants presented greater weight, without great variation in the number of leaves, indicating the leaves presented larger sizes and masses. The increase in weight per plant can be an indicator of higher productivity, since a greater mass will be obtained in a given area.

Table 1. Effect of nitrogen dosage on the development of lettuces produced by the fertigation technique using wastewater from dairy cattle

Treatment	number of leaves per plant *M(SD)*	weight (g) per plant *M(SD)*
A1	22.80(4.66)^{b}	86.76(12.50)^{b}
A2	27.00(2.87)^{ab}	141.59(18.45)^{a}
A3	27.10(3.45)^{ab}	165.31(21.62)^{a}
A4	29.60(1.43)^{a}	170.81(20.39)^{a}

A1 – 50% of the recommended nitrogen dose; A2 –100% of the recommended nitrogen dose; A3 – 200% of the recommended nitrogen dose; A4 – 300% of the recommended nitrogen dose. $^{a-b}$ Different letters in the same column for the same parameter indicate a significant difference (p > 0.05).

3.2 Microbiological Quality

The levels of aerobic mesophiles, total Coliforms (35 ℃), Escherichia coli, and absence/presence of *Salmonella* spp. are shown in Table 2. Biochemical tests (biochemical tests for citrate, indole, methyl red test - VM, and Voges-Proskauer - VP) were performed and all samples (A1, A2, A3 and A4) showed typical colony for *Escherichia coli*.

Table 2. Microbiological quality, before and after sanitizing, of lettuces produced by the fertigation technique with dairy cattle farming wastewater with different nitrogen contents

Microorganism	Condition	Treatment			
		A1	A2	A3	A4
Salmonella spp.	*Before hygienization*	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
	After hygienization	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Aerobic mesophiles in log CFU g^{-1} *M(SD)*	*Before hygienization*	6.96(0.06)^{bA}	7.09(0.10)^{bA}	7.31(0.07)^{aA}	7.44(0.05)^{aA}
	After hygienization	2.32(0.18)^{dB}	3.28(0.20)^{cB}	4.14(0.11)^{bB}	4.68(0.11)^{aB}
Total Coliforms 35 ℃ in log MNP g^{-1} *M(SD)*	*Before hygienization*	3.87(0.00)^{dA}	4.65(0.46)^{cA}	6.17(0.29)^{bA}	6.17(0.29)^{aA}
	After hygienization	0.48(0.00)^{cB}	0.48(0.00)^{cB}	1,36(0.00)^{bB}	2.17(0.29)^{aB}
Escherichia coli in log MNP g^{-1} *M(SD)*	*Before hygienization*	3.71(0.22)^{dA}	4.32(0.00)^{cA}	6.17(0.29)^{bA}	6.17(0.29)^{aA}
	After hygienization	0.48(0.00)^{cB}	0.48(0.00)^{cB}	1,36(0.00)^{bB}	2.17(0.29)^{aB}

A1 – 50% of the recommended nitrogen dose; A2 –100% of the recommended nitrogen dose; A3 – 200% of the recommended nitrogen dose; A4 – 300% of the recommended nitrogen dose. $^{a-d}$ Different letters in the same line for the same parameter indicate a significant difference ($p > 0.05$). $^{A-B}$ Different letters in the same column for the same parameter indicate a significant difference ($p > 0.05$).

To demonstrate the impact of the sanitization on the naturally present microorganisms, Figures 1, 2 and 3 show the reductions observed for aerobic mesophilic microorganisms, Coliforms at 35 ℃ and *Escherichia coli*. after washing and sanitizing the lettuce leaves:

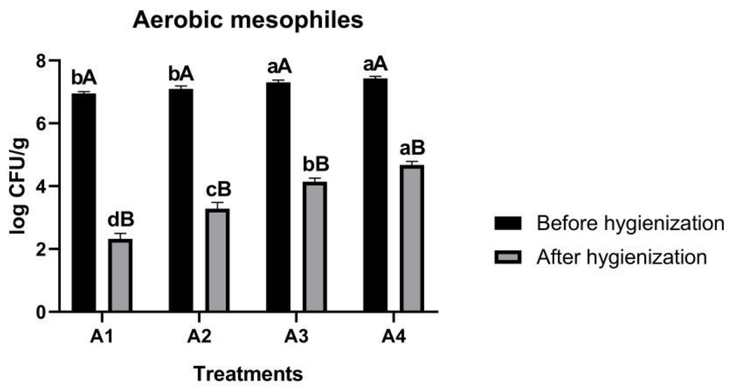

Figure 1. Effectiveness of sanitation process of lettuce produced by the fertigation technique using wastewater from dairy cattle farming with different nitrogen contents against aerobic mesophilic microorganisms

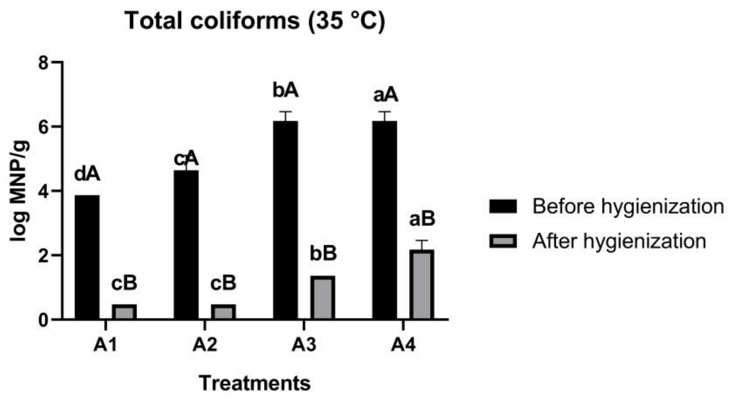

Figure 2. Effectiveness of sanitation process of lettuce produced by the fertigation technique using wastewater from dairy cattle farming with different nitrogen contents against total Coliforms at 35 ℃

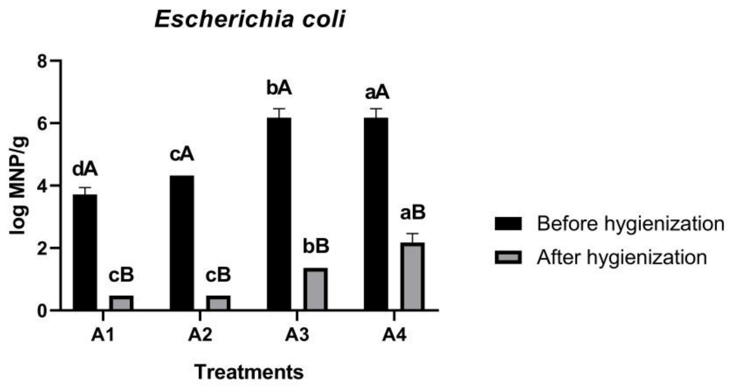

Figure 3. Effectiveness of sanitation process of lettuce produced by the fertigation technique using wastewater from dairy cattle farming with different nitrogen contents against *Escherichia coli*

4. Discussion

4.1 Impact of Nitrogen Dosage on Lettuce Development

As shown in Table 1, the effect of nitrogen content on lettuce yield and development has been the subject of several studies in recent years, with a variety of reported results on the number of leaves/plant and grams/plant. Djidonou & Leskovar (2019) evaluated the effect of six different nitrogen (N) concentrations (100, 150, 200, 250, 300 and 400 mg L^{-1}) on hydroponic lettuce growth. They observed that dosages between 100 and 150 mg L^{-1} maximized the growth and yield of hydroponic lettuce. Gashaw & Haile (2020) determined the optimal level of nitrogen that should be supplied to lettuce for better growth. They studied four different levels (0 kg ha-1, 50 kg ha-1, 100 kg ha-1 and 150 kg ha-1) and observed that the dosage increase caused a positive effect in plant weight and productivity based on mass generated per hectare. On the contrary, Acar et al. (2008) did not observe a significant difference (p >

0.05) between the average weight and number of leaves per plant with increasing nitrogen dosage.

Urbano et al. (2017) studied the effects of irrigation with treated wastewater on soil properties and lettuce yield. They observed that the use of treated domestic effluent significantly increased the lettuce fresh weight. Additionally, they evidenced the microbiological quality of the tested samples, once they had the same microbiological levels of the commercial samples. These findings agree with Vergine *et al.* (2014), who reported higher productivity using treated municipal wastewater in the management of the lettuce.

Lee et al. (2021), evaluated the impact of treated domestic wastewater as nutrient source for hydroponic lettuce cultivation. They observed that samples treated with treated effluents with a high nitrogen content provided similar results to the samples given as a negative control in relation to the size (height) of the plant. They also verified that the heavy metal contamination risk for human health was insignificant. Thus, they stated that the reuse of treated wastewater is a sustainable alternative. Although no differences were observed in plant development, the use of an effluent –domestic or industrial –, cause an reduction of treated water used and, besides the guarantee of an adequate destination for the wastewater. Cáceres et al. (2015) corroborated this perspective when evaluated the efficiency of leachate from manure composting. The authors observed that the lettuce yield of lettuces treated with nitrified effluents was similar to the yield of lettuces treated with a standard synthetic nutrient solution. The viability of these nutritive effluents is justified by the similar plant growth characteristics, reducing the dependence on synthetic fertilizers.

4.2 Microbiological Quality

None of the samples was contaminated with *Salmonella* spp. before and/or after cleaning. On the other hand, the other groups of microorganisms were detected even after washing and sanitizing the lettuce leaves. Still, a significant reduction, at a level of 5%, was observed in microbial counts after cleaning the lettuces for all applied treatments. The sanitation applied for the samples A1 and A2 was efficient once the levels of total Coliforms (35 °C) and E. coli were undetectable by the used techniques. This result meets the requirements of Normative Instruction IN n°161/2022 (Brasil, 2022), which determines that vegetables after sanitization must have a maximum count of 10 MNP g^{-1} (Tresseler et al., 2009).

On the contrary, the results for total Coliforms and *Escherichia coli* in A3 and A4 samples were greater than 10 NMP g^{-1}, indicating that the lettuces were unsuitable for human consumption (Brasil, 2022). The efficacy of the sanitizing process may have been affected by the higher initial microbial load observed for A3 and A4, comparing with A1 and A2. Bases on this, two of the four dosages proposed in the present study for the application of fertigation: 200% of the recommended nitrogen dose (A3) and 300% of the recommended nitrogen dose (A4) can be a risk for the food safety (Lima et al., 2017).

The microbial load of the not-sanitized lettuces was directly proportional to the organic load used during its cultivation. It may be caused by the contamination by the fertigation microorganisms or by the higher amount of nutrients provided to the naturally present microflora, once the soil is a natural source of pathogenic microorganisms, including E. coli (Luna-Guevara et al., 2019; Chen et al., 2021).

For all of the samples, the cleaning process was satisfactory, once the microbial concentration of the analyzed microorganisms significantly reduced ($p > 0.05$) (Table 2 and Figures 1, 2 and 3).

Total Coliforms and aerobic mesophilic microorganisms are considered hygienic-sanitary indicators of unit operations and processes. Large reductions to minimal and/or undetectable levels of these microorganisms reflect the correct application of some of the good food handling practices protocols and the efficiency of the applied procedure (Aycicek et al., 2006; Olaimat; Holley, 2012; Lima et al., 2017). The population of mesophilic microorganisms decreased in 4.64; 3.81; 3.17 and 2.76 logarithmic cycles for treatments A1, A2, A3 and A4, respectively. Additionally, the number of total Coliforms and E. coli reduced by 3.39; 4.28; 3.86 and 4.00, and 3.26; 3.84; 4.51 and 4.00 in samples A1, A2, A3 and A4 for, respectively. Gomes Neto et al. (2012) obtained similar results when evaluated the bacterial count and the occurrence of parasites in lettuce from three cropping systems in Brazil (traditional, organic and hydroponic). The sanitation process with sodium hypochlorite solution was also used and proved to be efficient once reduced the number of Coliforms (total and thermotolerant) by 2.00 logarithmic cycles and aerobic mesophilic microorganisms by 5.00 logarithmic cycles. Nascimento & Alencar (2014) evaluated the antimicrobial and antiparasitic efficiency of different sanitizers used in vegetables, including sodium hypochlorite, which reduced the number of Coliforms to acceptable levels for human consumption. Serra et al. (2020) evaluated the efficiency of different conventional sanitizers in 60 samples of vegetables grown in a conventional and hydroponic way. In this study, 95% of the samples had E. coli before cleaning. They found that the chlorinated solutions reduced the population of this microorganism, showing inhibitory potential. Pan & Nakano (2014) evaluated the disinfection effectiveness of sodium hypochlorite (NaClO) and chlorine dioxide (ClO2) alone and combined with microbubbling, ultrasonication or moderate heat (50 ℃), at different times and concentrations, in three leafy vegetables (lettuce, spinach and Chinese cabbage). The authors observed that there was no statistical variation ($p > 0.05$) between the different treatments, indicating that the sanitization with chlorinated solutions is sufficient to promote the reduction of pathogenic microorganisms.

Although the development of *Salmonella* spp. in the samples was not detected, there is a possible efficiency of hypochlorite against a possible growth of this microorganism. Pezzuto et al. (2016) evaluated the efficiency of five different chemical agents (peracetic acid, percitric acid, sodium bicarbonate and sodium hypochlorite) and vinegar in cleaning arugula. Only the solution prepared with sodium hypochlorite significantly reduced the number of Salmonella.

By the treatment, the number of *E. Coli* on the samples A3 and A4 reduced, reaching 1,36 log NMP g-1 and 2,22 log NMP g-1. However, it remained above the quantification limit of the Brazilian legislation (IN MS No. 60/2019), which is 1 log NMP g-1 qualifying these samples as unsuitable for consumption. Souza et al (2019) evaluated the effectiveness of commercial household sanitizers used on fresh curly lettuce *(Lactuca sativa*). They observed that for 60% of the samples the population of thermotolerant Coliform remained above the acceptable levels for human consumption after sanitization. Baert et al. (2009) concluded that the organic matter content influences the effectiveness of sodium hypochlorite to reduce murine *norovirus* 1 (MNV-1), Listeria monocytogenes and *Escherichia coli* O157:H7 in chopped lettuce and in residual washing water, which could justify the lower reduction efficiency of the E. coli population verified in this study, since the fertigation methods used increased the concentration of organic matter. According to Maillard (2016), several factors can affect the biocidal effectiveness of the sanitizer, such as: concentration, contact time, organic of the food, temperature, pH, water hardness. However, considering the simulation of vegetable hygiene in a domestic environment, these parameters would hardly be evaluated by the consumer, who would follow the instructions printed on the label.

The occurrence of Escherichia coli in higher numbers than those recommended by the legislation can lead to the incidence of cases and/or foodborne outbreaks, which is a risk for public health. According to ANVISA, between January/2009 and June/2018, 6,903 foodborne outbreaks were recorded, which represented 122,187 patients, 16,817 hospitalized and 99 deaths. Approximately one third of these outbreaks (36.9%) occurred at home and 2.6% of the cases were associates with the consumption of vegetables. Additionally, vegetables can comprise other two categories of food: mixed foods (which have ingredients that belong to different groups in their composition) and multiple foods (preparations with two or more ingredients that were identified as responsible for the outbreak), which caused 25,5 % and 10.7% of outbreaks in this period, respectively. The microorganism *Escherichia coli* is the etiological agent of greatest concern, since it is responsible for 24% of outbreaks, followed by *Salmonella* spp. and *Staphylococcus aureus*, which represent 11.2% and 9.5% of outbreaks, respectively (Brasil, 2020).

5. Conclusion

Although the higher nitrogen dosages supplied has brought greater benefits to the plant from a commercial point of view (weight increase and higher number of leaves per plant), the use of fertigates are limited in terms of food safety. The treatments having the reference nitrogen dosage - A2 (90 kg ha-1) and 50% of the reference value - A1, were effective, showing a microbial population that could be reduced during the sanitization, and so are able to offer products within the microbiological standards established by Normative Instruction MS No. 60/2019. On the other hand, the use of greater amounts of fertigation (A3 and A4) resulted in an increased number of microorganisms, which could not be reduced to acceptable levels with the applied hygiene process with sodium hypochlorite following protocols commonly domestically used.

Thus, wastewater cannot be used indiscriminately. The results obtained indicate the limitation of the supply of 100% of the recommended dose of nitrogen to the plant, using this effluent as a source. The environmental and sustainable aspects should be evaluated in line with food safety, since the higher nitrogen contents studied led to the development of Escherichia coli, a microorganism related to the occurrence of numerous foodborne outbreaks, which can cause serious public health problems.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Macrothink Institute.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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