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Efficiency of common washing treatments in reducing microbial levels on lettuce in Mali

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Introduction

The consumption of raw salad is common in Francophone countries in West Africa including Mali (Tounkara *et al.*, 2019). Fast food in Bamako and its peri-urban areas has lettuce as one of its common ingredients (Karg & Drechsel, 2018). Most fresh lettuce used in Bamako and its peri-urban areas are cultivated along dams, drains and near open wells, where untreated irrigation water, manure and contaminated soils are commonly used (Traoré, 2020). In Bamako, these sources of irrigation water have been observed to have high faecal coliform contamination levels above the WHO recommended standards for unrestricted irrigation (Traoré, 2020). Thus, vegetables are normally washed in a basin of standing water that has been contaminated through previous use, which could increase contamination levels instead of decreasing them (Kumwenda, 2019). The disinfection methods for vegetables in Bamako vary widely and are applied ineffectively by vegetables consumers due to poor knowledge and inadequate information (Traoré, 2020). Some of the disinfectants sold in Mali have been repackaged from their original containers, with no labels, while others have foreign labels, are expired or are counterfeit. In addition, these disinfectants are also mostly exposed to ultraviolet radiation in the open market, which affects the quality and efficacy. Majority of Malians are not aware about the protocol prescribed by Mali Health Service (MHS, 2005) and their disinfection method has also been reported as ineffective for complete disinfection of lettuce (Traoré *et al.*, 2013). The efficiency of common vegetable disinfectants in Mali, in reducing pathogenic bacteria on irrigated lettuce from Bamako were thus evaluated in this study.

Material and Methods

The research was carried out in three lettuce farms with an average size of 0.25 hectare irrigated with untreated river water, in each of communities five and six within Bamako. Fifteen heads of lettuce, were collected at random per farm (Hayes, 1995), transported on ice to the laboratory where they were analysed for bacterial contamination. Five commonly used vegetable disinfectants in Mali: bleach (NaClO), potassium permanganate ($KMnO_4$), vinegar (CH_3COOH), common salt (NaCl) and tap water were used in this study based on three consecutive washing protocol recommended for vegetables in Mali (MHS, 2005). Three concentrations of the four chemical disinfectants were prepared as follows using sterile distilled water: NaClO and CH_3COOH at 0.00285, 0.00570 and 0.00855 ppm; $KMnO_4$ at 170, 340 and 510 ppm and NaCl at 500, 1000 and 1500 ppm. The three concentrations of NaClO, $KMnO_4$ and CH_3COOH were

chosen based on the two recommended vegetable disinfectants in Mali, while those of the common salt were based on estimates from the results of previous study by Traoré (2020). Hundred grams of each composite lettuce leaves from each farm were washed in two litres tap water for one minute, followed by immediate immersions in respective disinfection solutions at specific contact time depending on the concentration and finally rinsed in two litres tap water for one minute. The high, intermediate and low concentration of disinfection solutions were exposed for 5, 10 and 15 min, respectively. Disinfection with tap water was equally tested at 5, 10 and 15 min. All disinfection procedures were done in LaboREM-Biotech, FST, USTT-B at approximately 25±3°C. Faecal coliform were determined by the method of NFV 08 060 (2009). *Escherichia coli* was determined by the method of ISO 16649-2 (2019) and *Salmonella* spp. by the method of ISO 6579-1 (2017) and the biochemical confirmation was done by API 20E gallery. Bleach/vinegar combination disinfection was also tested at 15 min using the same protocol above. The entire experiment was repeated three times with fresh composite lettuce samples (from the same study area in Bamako) for the second and third repetitions. The mean faecal coliform populations on lettuce before disinfection for the three experiments was 4.5 log CFU/100 g and its corresponding *E. coli* populations were 2.8 CFU/100 g. *Salmonella* spp. were present in lettuce samples in all experiments before disinfection. Faecal coliform and *E. coli* populations data were log-transformed and subjected to ANOVA. Where statistical difference was found, means were separated using Turkey's HSD test ($P < 0.05$) in GenStat 11th Edition.

Results and Discussion

Efficiency of common disinfection methods on Salmonella spp. on irrigated lettuce

There was complete disinfection of *Salmonella* spp. on lettuce with all disinfectants at all contact times.

Efficiency of common disinfection methods on faecal coliform level on irrigated lettuce

At all contact times, the mean log reduction of faecal coliform populations on lettuce did not vary amongst disinfectants regardless of the concentration and ranged between 2.4–3.5 log CFU/100 g (Table 1). The log reductions of faecal coliform population in tap water at 5, 10 and 15 min ranged from 1.3–1.9 log CFU/100 g and did not differ significantly ($P < 0.05$). Bleach/vinegar combination (0.00143 ppm/0.00143 ppm) applied for 15 min reduced faecal coliform populations by 2.8 log CFU/100 g.

Efficiency of common disinfection methods on E. coli population on irrigated lettuce

E. coli populations after disinfection ranged between 2.0–2.1 log CFU/100 g (Table 2). Bleach/vinegar combination eliminated *E. coli* populations on lettuce samples at 15 min.

All disinfectants tested in this study reduced faecal coliform populations on lettuce, but could not completely eliminate them. This finding is in agreement with those of several other researchers where the use of similar disinfectants even at higher concentrations could not completely eliminate faecal coliform on lettuce and other vegetables (Pourzamani *et al.*, 2019). Disinfection with tap water at 15 min was as effective as the other sanitisers at all contact times. Clean water is well known to flash out some microorganisms from vegetables. Additionally, tap water contains chlorine with concentrations of 0.2–1.0 mg/l (WHO, 2003) which could also disinfect microorganisms. Traoré *et al.* (2013) using 2.6 mg/l of bleach for 15 min at almost a 1000-fold increase of the lowest concentration in this study, obtained complete disinfection of lettuce which did not affect its quality, and the chlorine residues on the produce was less than the maximum acceptable value (5 mg/l) in drinking water (WHO, 2017). Vinegar and sodium chloride also had at least 2 log reductions in faecal coliforms populations, which is in agreement with findings of Amoah *et al*. (2009). It can be inferred from this study that disinfection of lettuce before consumption could be a crucial point for health risk reduction in Mali.

Table 1. Faecal coliform populations and Log reduction on lettuce cultivated using untreated water from river in Bamako and washed with some vegetable disinfectants at different contact times.

Contact time (min)	Vegetable disinfectant (ppm)	Mean faecal coliforms (CFU/100 g)	Mean Log reduction of faecal coliforms (CFU/100 g)
	Unwashed	4.5	-
5	Tap water	3.2^{c}	1.3^{c}
	NaClO (0.00855)	2.0^{abc}	2.5^{abcd}
	$KMnO_4$ (510)	1.6^{abc}	2.9^{ab}
	Vinegar (0.00855)	2.1^{abc}	2.4^{abc}
	NaCl (1500)	2.1^{abc}	2.4^{abc}
10	Tap water	2.9^{bc}	1.6^{bc}
	NaClO (0.00570)	1.7^{abc}	2.8^{abc}
	$KMnO_4$ (340)	1.5^{ab}	3.0^{ab}
	Vinegar (0.00570)	1.8^{abc}	2.7^{abc}
	NaCl (1000)	1.7^{abc}	2.8^{abc}
15	Tap water	2.6^{abc}	1.9^{abc}
	NaClO (0.00285)	1.0^{a}	3.5^{a}
	$KMnO_4$ (170)	1.2^{a}	3.3^{a}
	Vinegar (0.00285)	1.3^{ab}	3.2^{a}
	NaCl (500)	1.1^{a}	3.4^{a}

Means within column followed with different letter are significantly different Tukey's HSD test $P < 0.05$).

Table 2. *E. coli* populations and reduction on lettuce cultivated using untreated water from river in Bamako and washed with some vegetable disinfectants at different contact times.

Contact time (min)	Vegetable disinfectant (ppm)	Mean *E. coli* populations (CFU/100 g)	Mean reduction of *E. coli* populations (CFU/100 g)
	Unwashed	2.8	-
5	Tap water	0.8	2.0
	NaClO (0.00855)	0.7	2.1
	$KMnO_4$ (510)	0.7	2.1
	Vinegar (0.00855)	0.7	2.1
	NaCl (1500)	0.7	2.1
10	Tap water	0.7	2.1
	NaClO (0.00570)	0.7	2.1
	$KMnO_4$ (340)	0.7	2.1
	Vinegar (0.00570)	0.8	2.0
	NaCl (1000)	0.8	2.0
15	Tap water	0.7	2.1
	NaClO (0.00285)	0.7	2.1
	$KMnO_4$ (170)	0.7	2.1
	Vinegar (0.00285)	0.7	2.1
	NaCl (500)	0.7	2.1

No significant difference ($P > 0.05$) was found amongst disinfectants at all contact times.

Conclusions and Outlook

Conclusions

All chemical disinfectants including tap water at 15 min reduced faecal coliform populations below the undesirable ICMSF (2018) level. The concentrations of all chemical disinfectants applied for 5, 10 and 15 min respectively, reduced faecal coliform populations on lettuce by 2.4–2.9, 2.7–3.0 and 2.8–3.5 log CFU/100 g. All chemical disinfectants and tap water, reduced *E. coli* populations on lettuce and completely eliminated *Salmonella* spp. on lettuce regardless of the

concentration and contact time. Bleach/vinegar combination at 15 min reduced faecal coliform populations on lettuce by 2.8 log CFU/100 g and completely eliminated *E. coli* and *Salmonella* spp.

Outlook

The disinfection methods tested in this study should be evaluated by other researchers and health authorities to determine their efficacies on lettuce and other raw eaten vegetables on a large scale. Sensory analysis following the disinfection methods should also be done.

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