Washing methods to avoid parasitic contamination of raw vegetables in Assiut, Egypt: A comparative efficacy study

Original Article

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ABSTRACT

Background: Investigating simple, low-cost household washing methods is critical to avoid parasitic infections before consumption of fresh vegetables.
Objective: To evaluate the effect of commonly feasible household washing methods, and identify the most effective one for eliminating diverse infective parasitic stages.
Material and Methods: A total of 202 samples of 6 different raw consumable vegetables were collected from local markets in Assiut, Egypt, and microscopically examined to confirm parasitic contamination. Each sample (~50 g) was subjected to four washing treatments: direct rinsing with distilled water (DW) and immediate removal (W1), soaking in diluted vinegar (4:1) for one hour (W2), soaking in DW for 1, and 24 h (W3, and W4, respectively). To provide quantitative and qualitative measures of decontamination efficacy, the wastewater of each wash was subjected to parasitological examination to assess parasite contamination frequency, and viability rate (%).
Results: Among all tested methods, the vinegar-based washing method (W2) demonstrated optimal efficacy. It exhibited 82% reduction in pathogenic protozoan contamination, the highest decontamination rate. Prolonged soaking (W4) showed moderate efficacy (68% contamination reduction), while brief rinsing (W1) was the least effective. For helminth contaminants, W1 and W2 showed comparable efficacy (55-60% reduction, respectively). Although W2 achieved maximal pathogen inactivation (only 15.8% viable stages remaining) a significant (P<0.01) result versus other methods. No method eliminated all pathogenic stages, demonstrating the need for combined preventive approaches.
Conclusion: Soaking in diluted vinegar not only eliminates infective parasitic stages more effectively, but also inactivates them, making it superior for ingestion of raw vegetables.

Keywords: Assiut; Egypt; household washing; parasitic contamination; raw vegetables; vinegar.

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INTRODUCTION

Raw vegetables are a fundamental component of a healthy human diet owing to their nutritional value. They are a great source of vitamins (A, B-complex, C and K) and their precursors (*e.g.*, beta-carotene), minerals (calcium, potassium, magnesium, and iron) and fibers[1]. Besides, they may reduce the risk of chronic diseases and cancer[2]. Dietary guidelines advocate a high intake of fresh vegetables[3]. There is now good evidence that there is a strong relationship between fresh vegetable intake and mental health, owing to their high content of antioxidants[4]. However, human infections caused by consuming contaminated raw fresh vegetables remain a significant public health concern, particularly in developing countries, where the issue remains underestimated[5,6]. Globally, over 40 million people are at risk of zoonotic parasitic infections[6]. Protozoa and helminths are the main parasites involved in vegetables. These organisms are characterized by their long survival through their environmental stages (cyst/oocyst, or ova/larva) that withstand harsh conditions[7].

A study conducted in Iran documented the role of raw vegetables in the transmission of helminth eggs, larvae (*e.g.*, *Fasciola* spp., *Taenia spp.*, *H. nana*, *A. lumbricoides*, *Toxocara* spp., *T. colubriformis*, and hookworms), protozoan cysts and oocysts (*e.g.*, *E. histolytica, G. lamblia*, and *Cryptosporidium* spp., and *T. gondii*)[8]. Vegetables are contaminated with soil-transmitted helminths through irrigation with contaminated water (wastewater, sewage); use of untreated manure as fertilizer; poor hygiene during harvesting, handling, and transportation[9]. Additionally, animal manure, used as fertilizer, harbors high concentrations of pathogenic microorganisms, with certain pathogens reaching levels of millions to billions per g of wet feces or millions per ml of urine[10].

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In Egypt, where agricultural practices, and food safety regulations face implementation challenges, Assiut and the surrounding regions of Upper Egypt exemplify this crisis. Raw vegetable consumption poses a significant public health risk in Egypt due to high rates of water and agriculture pollution, as well as poor hygienic handling. Several previous studies reported that up to 29–86% of fresh consumable vegetables in the Egyptian local markets harbored pathogenic protozoan cysts, and helminth eggs[5,11-13].

To ensure food safety and prevent parasitic contamination, several critical measures must be implemented. First, all raw vegetables and fruits should be thoroughly washed under running potable water, with consideration given to approved chemical disinfectants where appropriate[10]. Additionally, preventive measures should begin at the production stage, including proper fertilization practices, hygienic handling of organic fertilizers, and the use of safe irrigation water[14]. Finally, community education programs should be implemented to raise awareness about parasitic life cycles and proper food handling techniques[15]. These combined measures significantly reduce the risk of foodborne parasitic infections when consistently applied.

Vegetable washing employs physical, chemical, and biological approaches with varying efficacy against parasitic contaminants. Physical methods include mechanical removal (brushing, rinsing with tap/ distilled water or saline), energy-based treatments (UV irradiation, solar disinfection), and thermal processes[16,17]. They showed variable significant results in their capability to remove parasitic life cycle stages[8]. Chemical methods can significantly enhance the removal and inactivation of parasites, particularly in settings where mechanical washing alone is insufficient. Chemical-based washing methods including acetic acid (vinegar), chlorine (bleach), and sodium bicarbonate (baking soda) effectively reduce microbial load but they have many limitations, as they not all culturally acceptable in some regions, reduced efficacy in organic-rich water due to rapid degradation, may alter taste/texture if not rinsed well, they also have safety concerns requiring thorough rinsing to eliminate residues[17,18].

Proper washing should be routinely applied to avoid outbreaks of intestinal parasitic infections[19]. Worldwide, only a few studies have dealt with washing methods by comparing the effect of various washing procedures on eliminating parasitic contamination in vegetables[20,21]. This public health concern underscores the urgent need to explore affordable and practical household decontamination techniques that require neither specialized equipment nor high-cost access. The current study thoroughly assesses washing methods of raw vegetables for their effectiveness against parasitic contamination. On the other hand, previous studies confirmed the presence of parasitic contamination in Egypt's tap water[15,22], with notable prevalence in Assiut Governorate[23,24]. Consequently, DW was used in our study to confirm parasitic contaminants on vegetables rather than from washing using tap water[25].

MATERIAL AND METHODS

This descriptive analytical study was conducted at the Medical Parasitology Department, Faculty of Medicine, Assiut University, Assiut, Egypt, during the period from June 2022 to May 2025.

Study design: Different consumable raw vegetables were collected from local markets across Assiut Governorate over 24 months, and only parasite-positive samples were included in the study. Collection days were randomized to avoid market-specific biases. To identify the optimal washing method to avoid parasitic infections, each sample was processed using DW, and diluted vinegar. Parameters used for evaluation included parasite contamination frequency and viability rate.

Samples: Six different vegetables were collected from the local markets; including *Allium fistolosum* (green onion), *Raphanus sativus* (radish), *Petroselinum crispum* (parsley), *Eruca sativum* (watercress), *Lactuca sativum* (lettuce) and *Coriandrum sativum* (coriander). All collected samples were transported to the laboratory in individual sterile nylon bags to prevent cross-contamination. Upon arrival, samples underwent manual separation of leaves and roots, with strict exclusion of damaged parts. Each sample was divided equally into 5 portions.

Pre-washing examination: Following the methodology described by Ahmed *et al*.[5], the samples were processed and examined for parasitic contamination in the Parasitology Laboratory at Assiut University. Briefly: a 250 g sample of each vegetable was collected and divided into five groups (50 g each). The first group was immediately analyzed to confirm parasite contamination, while the remaining four groups were preserved for subsequent washes if the initial test proved positive.

For microscopic examination, the first 50 g sample was briefly soaked in 100 ml DW and then promptly removed. Resulting water was collected in individually labelled conical flasks, and sediment from each sample was centrifuged. Then, samples wash sediments underwent initial wet mount microscopy, and concentration techniques (formalin-ether sedimentation and sucrose flotation)[26], with Modified Ziehl-Neelsen staining applied for detection of acid-fast parasites, and lactophenol cotton blue staining for morphological identification, and parasite contamination quantification[27,28]. For samples testing

positive, acridine orange fluorescence microscopy was employed to determine viability rates based on nucleic acid staining patterns[29].

According to pre-washing examination, out of the collected samples, 202 were contaminated, distributed as follows: radish (n=37), watercress (n=37), parsley (n=38), green onion (n=34), coriander (n=29), and lettuce (n=27).

Washing methods: Two washing solutions were used; DW, and diluted vinegar. From each positive sample, ~50 g was processed in one of the following methods; 1) rinsing for 15-20 sec in 500 ml DW (W1); 2) soaking in 500 ml diluted vinegar with DW (1:4) for one hour (W2); 3) soaking in 500 ml DW for one hour (W3); and 4) soaking in 500 ml DW for 24 h (W4).

Parasitological examination: The wastewater from each washing treatment was collected separately in conical flasks to allow for sediment recovery. Collected samples were transferred to centrifuge tubes and subjected to centrifugation at 3000 rpm for 5 min. Each sediment was examined as previously described in pre-washing examination.

Parameters calculation: Mean (±SD) parasite contamination frequency = number of positive samples per wash/total number of positive samples[5] (n=202). Viability rate (%) was determined by intact cell walls, larval motility and vital dyes intake (acridine orange), and calculated according to the following formula: Viability rate (%) = (Number of viable parasitic stages/ Total number of parasitic stages observed) ×100[29].

Statistical analysis: The collected data were tabulated and statistically analyzed using SAS software (version 9.3; SAS Institute, 2011). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was conducted to assess significant differences among the washed groups. A $P<0.05$ was considered statistically significant.

Ethical consideration: The present study was approved by the Ethics Committee of the Faculty of Medicine, Assiut University (No. 17300166).

RESULTS

Efficacy of washing methods in removing parasites (Figure 1 and table 1): Vinegar for one hour (W2) was the most effective across all vegetables, yielding the highest parasitic contamination frequency (indicating better removal), followed by W4 (24-h). Radish showed a mean of 9.0±5.2 parasites/sample with W2 compared to only 2.33±1.7 parasitic contamination frequency with W1 (direct method). W1 was the least effective, consistently showing the lowest parasite contamination frequency (0.34±0.5 in lettuce). All vegetables exhibited significant differences ($P<0.05$) between washes, except for some pairwise comparisons.

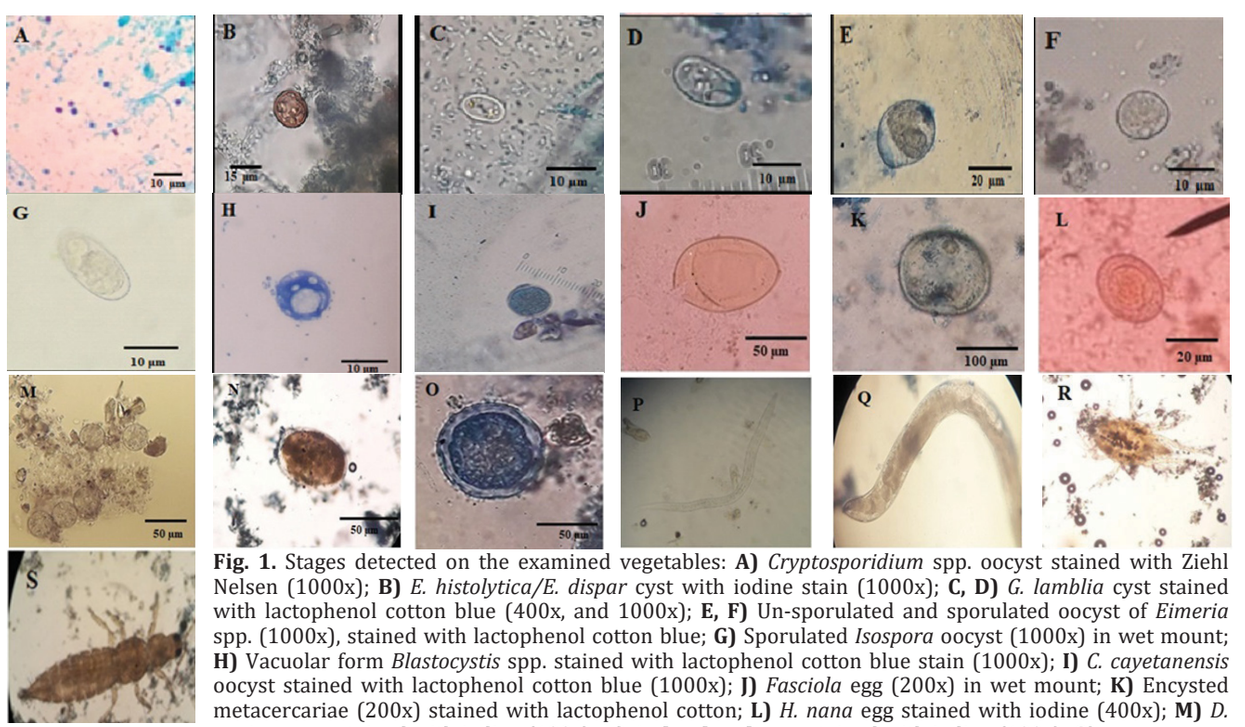

Fig. 1. Stages detected on the examined vegetables: **A)** *Cryptosporidium* spp. oocyst stained with Ziehl Nelsen (1000x); **B)** *E. histolytica/E. dispar* cyst with iodine stain (1000x); **C, D)** *G. lamblia* cyst stained with lactophenol cotton blue (400x, and 1000x); **E, F)** Un-sporulated and sporulated oocyst of *Eimeria* spp. (1000x), stained with lactophenol cotton blue; **G)** Sporulated *Isospora* oocyst (1000x) in wet mount; **H)** Vacuolar form *Blastocystis* spp. stained with lactophenol cotton blue stain (1000x); **I)** *C. cayetanensis* oocyst stained with lactophenol cotton blue (1000x); **J)** *Fasciola* egg (200x) in wet mount; **K)** Encysted metacercariae (200x) stained with lactophenol cotton; **L)** *H. nana* egg stained with iodine (400x); **M)** *D. caninum* egg stained with iodine (400x); **N) A. duodenale** egg stained with iodine (400x); **O)** *Toxocara* egg stained with lactophenol cotton blue (400x); **P, Q)** Free living nematode larvae on raw vegetables stained with iodine and lactophenol cotton blue (100x and 400x); **R)** Crustacean and sucking lice (100x).

Table 1. Means of total parasite contamination frequency in different vegetables according to the type of wash.

Vegetable	Parasite contamination frequency				Statistical analysis *P* value
	W1	W2	W3	W4	
Watercress	3.31 ± 4.4^{ac}	6.75 ± 5.4^{ad}	3.98 ± 3.4^{df}	5.30 ± 4.8^{cef}	= 0.004*
Radish	2.33 ± 1.7^{ac}	9.0 ± 5.2^{ad}	4.2 ± 3.0^{df}	7.2 ± 4.6^{cf}	<0.0001*
Green onions	2.14± 3.5^{ac}	6.5 ± 4.6^{ad}	3.0 ± 2.5^{df}	5.4 ± 4.5^{cef}	<0.0001*
Coriander	1.35 ±1.6^{a}	4.0 ± 4.3^{ad}	2.4 ± 2.6^{d}	3.1 ± 4.2^{c}	= 0.005*
Lettuce	0.34 ± 0.5^{ac}	1.5 ± 1.6^{ad}	0.5 ± 0.7^{d}	0.7 ± 0.9^{e}	<0.0001*
Parsley	2.8±2.6^{a}	8.5 ± 6.4^{ad}	4.0 ± 3.1^{d}	5.7 ± 4.9^{c}	<0.0001*

Significance between washing types using Student's t-tests; One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Superscript letters indicate statistically significant (P<0.05) differences between washing methods; **a:** Significantly different between W1 and W2; **b:** Significantly different between W1 and W3; **c:** Significantly different between W1 and W4; **d:** Significantly different between W2 and W3; **e:** Significantly different between W2 and W4; **f:** Significantly different between W3 and W4.

Impact of wash types on specific parasites (Table 2-7): Vinegar (W2 and W4) were significantly (P<0.001) more effective in removing Cryptosporidium spp. from in radish, green onions, coriander, and parsley. Similar significant (P=0.007) results were obtained for *E. histolytica/E. dispar* only in radish. While W2 was the most effective in eliminating arthropods (sucking lice, mites and Cyclops), W3 (one hour rinsing) was the least effective in radish. Variable abilities of washing solutions to eliminate nematode free-living larvae, *Toxocara* and *A. duodenale* eggs. For instance, W1 and 2 worked best for green onions (P=0.004), while W4 was superior for parsley (P=0.01).

Table 2. Mean (±SD) parasite contamination frequency in watercress calculated in washing solutions.

	W1	W2	W3	W4	*P* value
***G. lamblia* cyst**	4.0 ± 0.9	-----	6.0 ± 2.3	-----	$
***Cryptosporidium* oocyst**	2.1 ± 1.2	6.2 ± 4.2	3.7 ± 2.7	5.2 ± 3.6	*
***E. histolytica/E. dispar* cyst**	1.3 ± 0.5	2.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.4	*
***Eimeria* oocyst**	6.3 ± 4.4	4.0 ± 1.4	3.0 ± 1.7	4.0 ± 1.01	$
***Isospora* oocyst**	-----	2.0 ± 0.5	1.0 ± 0.3	8.0 ± 2.8	$
***Fasciola* egg**	1.0 ± 00	-----	-----	1.0 ± 0.1	$
***Fasciola* encysted metacercaria**	1.4 ± 0.9	1.4 ± 0.5	1.5 ± 0.7	1.3 ± 0.6	NS
Cestode eggs$^{@}$	-----	2.3 ± 1.5	1.0 ± 0.4	3.5 ± 2.1	NS
Nematode stages$^{\#}$	-----	1.3 ± 0.5	1.0 ± 0.3	3.5 ± 2.1	NS
Arthropods$^{\$}$	1.4 ± 0.5	2.6 ± 1.1	2.1 ± 1.6	2.7 ± 1.3	NS

^: Significance between washing solution using Student's t-tests; One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. **@:** Eggs of *D. caninum*, and *H. nana*; **#:** Free-living larvae, *Toxocara* and *A. duodenale* eggs; **$:** Sucking lice, mites and *Cyclops*; **NS:** Not significant; ***:** Significant (P<0.05); **$:** P value is not calculated due to small mean±SD.

Table 3. Mean (±SD) parasite contamination frequency in radish calculated in washing solutions.

	W1	W2	W3	W4	*P* value
***G. lamblia* cyst**	-----	-----	2.0 ± 0.0	1.7 ± 0.6	NS
***Cryptosporidium* oocyst**	2.0 ± 0.0	7.3 ± 3.9	3.18 ± 2.0	6.5 ± 3.3	*
***E. histolytica/E. dispar* cyst**	1.0 ± 0.0	2.2 ± 1.0	1.0 ± 0.0	2.0 ± 0.0	*
***Eimeria* oocyst**	1.5 ± 1.0	2.0 ± 0.8	2.7 ± 1.0	3.3 ± 2.0	NS
***Isospora* oocyst**	1.0 ± 0.0	2.3 ± 1.5	2.0 ± 0.0	1.3 ± 0.6	NS
***C. cayetanensis* oocyst**	-----	2.0 ± 0.0	2.0 ± 0.0	-----	$
***Fasciola* egg**	-----	1.7 ± 0.5	1.0 ± 0.0	1.0 ± 0.0	NS
***Fasciola* encysted metacercaria**	2.0 ± 1.4	2.0 ± 1.1	1.5 ± 1.0	1.0 ± 0.0	NS
Cestode eggs$^{@}$	-----	-----	-----	-----	$
Nematode stages$^{\#}$	1.0 ± 0.0	1.7 ± 0.5	1.5 ± 1.0	2.5 ± 1.7	NS
Arthropods$^{\$}$	2.8 ± 0.5	3.6 ± 0.6	1.0 ± 0.0	2.0 ± 0.0	*

^: Significance between washing solution using Student's t-tests; One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. **@:** Eggs of *D. caninum*, and *H. nana*; **#:** Free-living larvae, *Toxocara* and *A. duodenale* eggs; **$:** Sucking lice, mites and *Cyclops*; **NS:** Not significant; ***:** Significant (P<0.05); **$:** P value is not calculated due to small mean±SD.

Table 4. Mean (±SD) parasite contamination frequency in green onion calculated in washing solutions.

	W1	W2	W3	W4	*P* value
***G. lamblia* cyst**	-----	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	$
***Cryptosporidium* oocyst**	2.7 ± 2.0	5.6 ± 3.4	3.0 ± 2.0	5.1 ± 3.5	*
***Eimeria* oocyst**	1.5 ± 0.7	1.7 ± 1.3	1.0 ± 0.0	3.1 ± 1.8	*
***Isospora* oocyst**	1.0 ± 0.0	1.0 ± 0.0	-----	1.0 ± 0.0	$
***C. cayetanensis* oocyst**	5.0 ± 0.0	3.0 ± 2.0	2.0 ± 0.0	1.0 ± 0.0	NS
***Fasciola* egg**	-----	1.2 ± 0.4	3.0 ± 0.0	1.3 ± 0.5	*
***Fasciola* encysted metacercaria**	-----	1.0 ± 0.0	1.0 ± 0.0	-----	$
Cestode eggs$^{@}$	1.5 ± 0.7	1.5 ± 0.8	1.5 ± 0.5	1.3 ± 0.5	NS
Nematode stages$^{\#}$	2.0 ± 1.0	1.8 ± 0.1	1.3 ± 0.5	1.0 ± 0.0	*

^: Significance between washing solution using Student's t-tests; One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. **@:** Eggs of *D. caninum*, and *H. nana*; **#:** Free-living larvae, *Toxocara* and *A. duodenale* eggs; **NS:** Not significant; ***:** Significant (P<0.05); **$:** P value is not calculated due to small mean±SD.

Table 5. Mean (±SD) parasite contamination frequency in coriander calculated in washing solutions.

	W1	W2	W3	W4	*P* value
***Cryptosporidium* oocyst**	2.5 ± 1.3	5.5 ± 3.9	3.1 ± 2.4	5.1 ± 4.3	*
***Fasciola* egg**	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0	-----	$
Cestode eggs[@]	-----	2.0 ± 1.4	5.0 ± 0.0	-----	NS
Nematode stages[#]	1.0 ± 0.0	1.0 ± 0.0	-----	1.0 ± 0.0	$
Arthropods[$]	2.0 ± 1.0	3.0 ± 1.0	-----	1.0 ± 0.0	$

^: Significance between washing solution using Student's t-tests; One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. **@:** Eggs of *D. caninum*, and *H. nana*; **#:** Free-living larvae, *Toxocara* and *A. duodenale* eggs; **$:** Sucking lice, mites and *Cyclops*; **NS:** Not significant; ***:** Significant (P<0.05); **$:** P value is not calculated due to small mean±SD.

Table 6. Mean (±SD) parasite contamination frequency in lettuce calculated in washing solutions.

	W1	W2	W3	W4	*P* value
***G. lamblia* cyst**	1.1 ± 0.3	2.6 ± 1.4	1.2 ± 0.4	1.5 ± 0.8	*
Nematode stages[#]	1.0 ± 0.0	1.0 ± 0.0	-----	1.0 ± 0.0	$
Arthropods[$]	1.0 ± 0.0	1.0 ± 0.0	-----	1.0 ± 0.0	$

^: Significance between washing solution using Student's t-tests; One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. **#:** Free-living larvae, *Toxocara* and *A. duodenale* eggs; **$:** Sucking lice, mites and *Cyclops*; **NS:** Not significant; ***:** Significant (P<0.05); **$:** P value is not calculated due to small mean±SD.

Table 7. Mean (±SD) parasite contamination frequency in parsley calculated in washing solutions.

	W1	W2	W3	W4	*P* value
***Cryptosporidium* oocyst**	2.2 ± 1.2	6.2 ± 4.7	3.4 ± 2.6	5.4 ± 4.2	*
***E. histolytica/E. dispar* cyst**	1.0 ± 0.0	1.3 ± 0.5	1.0 ± 0.0	-----	NS
***Eimeria* oocyst**	2.3 ± 1.2	2.9 ± 2.6	1.5 ± 0.6	3.4 ± 3.3	NS
***Isospora* oocyst**	-----	3.5 ± 0.7	2.0 ± 1.4	1.0 ± 0.0	NS
***C. cayetanensis* oocyst**	3.0 ± 0.0	8.0 ± 4.2	2.3 ± 0.6	-----	NS
***Fasciola* egg**	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	-----	$
***Fasciola* encysted metacercaria**	1.1 ± 0.4	1.9 ± 1.0	1.5 ± 0.8	1.9 ± 0.9	NS
Cestode eggs[@]	1.0 ± 0.0	3.4 ± 2.6	1.0 ± 0.0	1.3 ± 0.5	NS
Nematode stages[#]	1.0 ± 0.0	1.5 ± 0.7	1.0 ± 0.0	4.0 ± 0.0	*
Arthropods[$]	2.1 ± 0.8	2.0 ± 0.9	1.4 ± 0.5	1.0 ± 0.0	NS

^: Significance between washing solution using Student's t-tests; One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. **@:** Eggs of *D. caninum*, and *H. nana*; **#:** Free-living larvae, *Toxocara* and *A. duodenale* eggs; **NS:** Not significant; ***:** Significant (P<0.05); **$:** P value is not calculated due to small mean±SD.

Parasite viability rate (%) reduction (Table 8): Vinegar (W2) drastically reduced viability rate to 15.8%, compared to 50–60.8% for other methods (P<0.0001). Although W3 and 4 were partially effective in eliminating the parasites from tested vegetables, they were ineffective at killing them, with viability rates similar to W1 (60%).

Table 8. Effectiveness of different wash types on the overall parasites' viability rate (%) regardless of the vegetable types.

Wash type	Viability rate (N= 202)		Statistical analysis	
	Non-reduced No. (%)	Reduced No. (%)	X^2	*P* value
W1	101 (50%)	101 (50%)	131.4	<0.0001*
W2	32 (15.8%)	170 (84.2%)		
W3	123 (60.8%)	79 (39.1%)		
W4	123 (60.8%)	79 (39.1%)		

***:** Significant (P<0.05).

DISCUSSION

Despite the nutritional benefits of raw vegetables[30], their consumption poses significant parasitic infection risks, particularly in regions where contaminated produce is linked to foodborne outbreaks[31]. This study evaluated practical household washing methods to address this public health challenge, providing evidence-based strategies to reduce parasite transmission in endemic areas.

According to US Department of Agriculture (USDA), no washing method completely removes or kills all microbes, however; thoroughly rinsing fresh product under running water is an effective way to reduce the number of microorganisms. Washing fruits and vegetables not only helps remove dirt, bacteria, and stubborn garden pests, but it also helps remove residual pesticides[32]. The FDA recommends 20-sec vinegar/

water washes for bacterial reduction[33,34]. Our study demonstrated that parasitological decontamination requires longer exposure to achieve significant efficacy (82% protozoan reduction, 84% viability loss), results that align with global evidence.

The relatively high efficacy of vinegar to remove parasites and reduce helminthic eggs and protozoa viability rates can be attributed to its ability to change washing water properties like pH making it inappropriate media for parasites survival[35,36]. As disinfectant, vinegar (20%) acts by infringement of nucleic acids and triggering proteins bonds, or by killing contaminating organic matter and metabolites produced from bacterial and other floral growth parasites. Besides, it may work by oxidation of the cell membrane phospholipids that leads to membrane dysfunction and cell death[37,38]. These vinegar properties align with Etewa *et al*.[11] who observed a decline in parasite viability after treatment with acetic acid (5%), and potassium permanganate (24 mg/l). Similarly, Elahi *et al*.[39] reported that washing vegetables with vinegar or germicide significantly (P<0.05) reduced parasitic contamination (particularly Free-living nematode larvae) compared to inadequately washed samples.

On the other hand, two studies[8,40] used different washing methods for more powerful elimination of parasites contaminating vegetables. However, these methods may leave an unpalatable taste on the vegetables and are not very safe as they contain detergents. Sadeghi and co-authors evaluated three washing methods: potable water with saline solution, a commercial detergent (1% sodium dodecyl sulfate plus 1% Tween 80), and physiological saline. Their results revealed that saline solution was most effective at removing helminth eggs, while the detergent proved optimal for eliminating protozoa[40]. Hajipour *et al*.[8] assessed the effectiveness of washing methods: 1% vinegar; 0.95% calcium hypochlorite (bleaching powder), 1% lemon juice; and potable water with dishwashing liquid. Their findings indicated bleaching powder was the most effective at eliminating parasitic organisms. In contrast to our findings, the investigators reported lower efficacy of vinegar, probably due to its weaker concentration compared to our 25% solution[8].

Direct washing and short time soaking in water for one hour revealed low parasitic removal level due to the tight attachment of some protozoan cysts and helminth eggs to vegetable surface, these findings agreed with Hajipour *et al*.[8] who reported that that dishwashing liquid combined with water removed the smallest number of parasites (40% still being contaminated) compared to other washing methods. Agreed also with Skowron *et al*.[41] who approved that water was significantly less efficient than any of the disinfectants in reducing the number of Listeria monocytogenes[41]. An earlier study performed in Iran found contamination frequency with nematode larvae in raw vegetables washed with water was significantly higher than those washed with vinegar and germicide solutions[39]. The superior efficacy of vinegar for vegetable decontamination stems from its unique combination of safety, cost-effectiveness, and multi-target antimicrobial action[42]. It has the advantages of avoiding toxic residues that require rinsing, its long shelf-life (stable efficacy for >2 years at pH <3), and cultural familiarity in local culture[43].

In conclusion, unlike many studies focusing on industrial sanitization, this work evaluates accessible, low-cost household methods, making findings directly applicable to daily life. By employing standardized laboratory techniques to quantify parasite reduction, the study ensures reliable and reproducible results, bridging a critical gap between controlled experiments and real-world implementation. Notably, our study demonstrates that vinegar not only removes parasites more effectively than conventional methods but also achieves pathogen inactivation, offering a natural, safe, and broadly effective solution. These data-driven insights advance the limited research on household-level interventions for parasite control in raw vegetables, granting actionable recommendations to inform public health guidelines and consumer practices. The findings underscore vinegar's dual role as both a physical remover and antimicrobial agent, positioning it as an optimal choice for household food safety.

Study limitations: Because of the limited resources, this study did not assess the efficacy of washing methods against *T. gondii*, a critical pathogen associated with raw vegetable consumption. This protozoon exhibits distinct environmental resilience and advanced diagnostic techniques compared to other parasites, and their inactivation may require tailored interventions[44]. Future studies should assess vinegar's efficacy against *T. gondii* using molecular diagnostics, as oocysts are highly resistant. Small sample sizes for some parasites may obscure statistical significance. Additional studies should evaluate the efficacy of vinegar at varying concentrations and exposure times to optimize decontamination protocols.

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