# Comparative sanitizers study of domestic aplication in vegetables contaminated with hookworms

## Estudo comparativo da ação sanitizantes de uso caseiros em hortaliças contaminadas com Ancilostomídeos

## Estudio comparativo de la acción higiénica de los cuidadores en vegetales contaminados con anquilostomas

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REVISA

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**RESUMO**

**Objetivo:** O presente trabalho tem como objetivo verificar a efetividade de sanitizantes de uso caseiros quanto a capacidade de paralisar e desinfetar larvas de Ancilostomídeos. **Método** Para obtenção das larvas de Ancilostomídeos foi utilizado o método de Hoffman, Pons e Janer (HPJ). Em seguida, foram preparadas lâminas com o sedimento e adicionou-se concentrações diferentes de saneantes empregados na rotina doméstica. Simultaneamente, foi avaliado, através de microscopia óptica, o tempo cronometrado que cada saneante em diferentes concentrações, necessitaria para paralisação completa do helminto. **Resultados:** O ácido acético e o hipoclorito não apresentaram efetividade em nenhuma das concentrações testadas. Já o vinagre de álcool puro na diluição de 40% paralisou 90% das larvas em um período de 14 minutos e 31 segundos. O vinagre composto de álcool, na concentração de 40% foi capaz de paralisar mais de 90% das larvas em um tempo médio de 9 minutos e 50 segundos. **Conclusão:** A utilização de 40% de sanitizantes de vinagre se mostraram eficazes na desinfecção de larvas de Ancilostomídeos. No entanto, este estudo alerta que a eliminação de larvas não é segura quando se leva em conta a concentração usada na rotina doméstica atual.
**Descritores:** Ancilostomídeos; Contaminação Alimentar; Sanitizantes Caseiros.

**ABSTRACT**

**Objective:** The present work aims to verify the effectiveness of homemade sanitizers for the ability to paralyze and disinfect hookworm larvae. **Method:** To obtain hookworm larvae, the method of Hoffman, Pons, and Janer (HPJ) was applied. The next slides were prepared with the sediment, and different sanitizing concentrations, routinely used in the domestic, were added. Simultaneously, we evaluated, by optical microscopy, the chronometer time that each sanitizer in different concentrations, would require for complete helminth paralysis**. Results:** Acetic acid and hypochlorite were not effective in any of the tested concentrations. Pure alcohol vinegar in the 40% dilution paralyzed 90% of the larvae in 14 minutes and 31 seconds. The vinegar composed of alcohol, in the 40% concentration, was able to paralyze more than 90% of the larvae in an 9,50 minutes average. **Conclusion**: The use of 40% of vinegar sanitizers proved to be useful in disinfecting hookworms larvae. However, this study cautions that larvae elimination is not safe when taking into account the concentration handled in the current domestic routine.
**Descriptors:** Ancylostomids; Homemade Sanitizers Parasites; Antiparasitic Agents.

**RESUMEN**

**Objetivo:** el presente trabajo tiene como objetivo verificar la efectividad de los desinfectantes caseros para la capacidad de paralizar y desinfectar larvas de anquilostomas. **Método:** para obtener larvas de anquilostomas, se utilizó el método de Hoffman, Pons y Janer (HPJ). Luego se prepararon los portaobjetos con el sedimento y se agregaron diferentes concentraciones de agentes desinfectantes utilizados en la rutina doméstica. Simultáneamente, se evaluó, usando microscopía óptica, el tiempo cronometrado que cada desinfectante en diferentes concentraciones necesitaría para completar la parálisis del helminto. **Resultados:** el ácido acético y el hipoclorito no fueron efectivos en ninguna de las concentraciones probadas. El vinagre puro de alcohol en la dilución al 40% paralizó el 90% de las larvas en un período de 14 minutos y 31 segundos. El vinagre compuesto de alcohol, en la concentración del 40%, fue capaz de paralizar más del 90% de las larvas en un tiempo promedio de 9 minutos y 50 segundos. **Conclusión:** el uso del 40% de desinfectantes de vinagre demostró ser eficaz para desinfectar larvas de anquilostomas. Sin embargo, este estudio advierte que la eliminación de las larvas no es segura cuando se tiene en cuenta la concentración utilizada en la rutina doméstica actual.
**Descriptores:** Anquilostomas; Contaminación de alimentos; Desinfectantes caseros

ORIGINAL

## Introduction

Vegetables are foods of great nutritional importance in the human diet, as they provide essential nutrients, such as mineral salts, vitamins, fibers and antioxidants, which directly contribute to the maintenance of the body's homeostasis.[1-3] They are classified in the vast majority of times as "in natura" foods, and are eaten raw, lettuce being the most consumed by the Brazilian population.[3-4]

Vegetables, because they are easily grown during all seasons of the year in Brazil, are widely used in Brazilian cuisine, being present in almost all diets, from portions of salad, sandwiches, buffet decorations to accompanying meats and juices.[4-6] Because they are consumed in their "in natura" form, raw or minimally processed, vegetables can be a vehicle for the transmission of important parasites, as they may be contaminated with parasitic evolutionary forms such as eggs, cysts, oocysts, as well as helminth larvae. These evolutionary forms when ingested can cause endoparasitosis in man.[7-9]

In general, contamination of vegetables occurs in several stages, ranging from the production, transport and storage process to commercialization in open markets, supermarkets.[8-11] The use of water contaminated with human fecal coliforms and / or other pathogenic microorganisms for irrigation is one of the main ways of contamination of vegetables, but it is not the unique.[6,8]

Other forms of contamination by parasites in vegetables occur through the indiscriminate use of organic fertilizers containing fecal material of human or animal origin, which is the most relevant route, since many parasites have a monoxenic cycle, whose man is the only host and reservoir.

Poor hygiene conditions can be observed in the entire production chain of transfer of vegetables, from handlers, which can contaminate vegetables during handling in the field; inadequate transport in unsuitable vehicles, favoring the proliferation of several microorganisms; storage in unhealthy places and without proper basic hygiene conditions, exposing vegetables to direct contact with rats and cockroaches; handling in commercial stalls by the population without proper hand asepsis; sharing knives and / or other household items to prepare more than one food. [6-8,12-14]

The main geo-helminths that can be transmitted to humans through the ingestion of contaminated food are: Ascaris lumbricoides, Trichuria trichiura and Hookworms. Among the species of hookworms pathogenic to man, we highlight Necator americanus and Ancylostoma duodenale, which the larvae are able to pass through the upper gastrointestinal tract without being digested and later install themselves in the lower gastrointestinal tract, causing severe pathophysiology, especially in children and immunodepressed patients.[15]

In oral infection caused by hookworms, the larvae, which are in stage L3, are ingested by the host and lose the cuticle when passing through the gastric juice of the stomach.[15,21] After about 3 days, these larvae migrate to the small intestine and change to L4 larva; and after two days or more, the larvae in stage L4 migrate to the lumen of the small intestine and attach themselves to the mucosa to carry out the hematophagy and copulation process.[15-17]

The presence of hookworms in the small intestine has a direct consequence of blood loss, being related to clinical conditions of severe anemia, hemorrhagic conditions, protein malnutrition, increased susceptibility to intestinal infections, mental and physical retardation and damage to maternal-fetal health in women pregnant.[15-19]

Because there are several studies demonstrating the importance of vegetables as a means of transmission of endoparasitoses, and the clinical seriousness that some of these parasites present, homemade methods are routinely applied in an attempt to eliminate parasites in vegetables. However, there is a lack of studies that assess the real effectiveness of these homemade methods, which may be contributing to the spread of worms present in the Brazilian diet, due to lack of information and studies on the subject.

## Method

The methodology used in this work consists of a set of adapted techniques, where, initially, hookworm larvae were isolated using the Hoffman method (HPJ). The isolated larvae were subsequently subjected to worm paralysis efficacy tests, testing different concentrations of certain commercial compounds, routinely used by the population, for disinfecting vegetables (Figure 1).

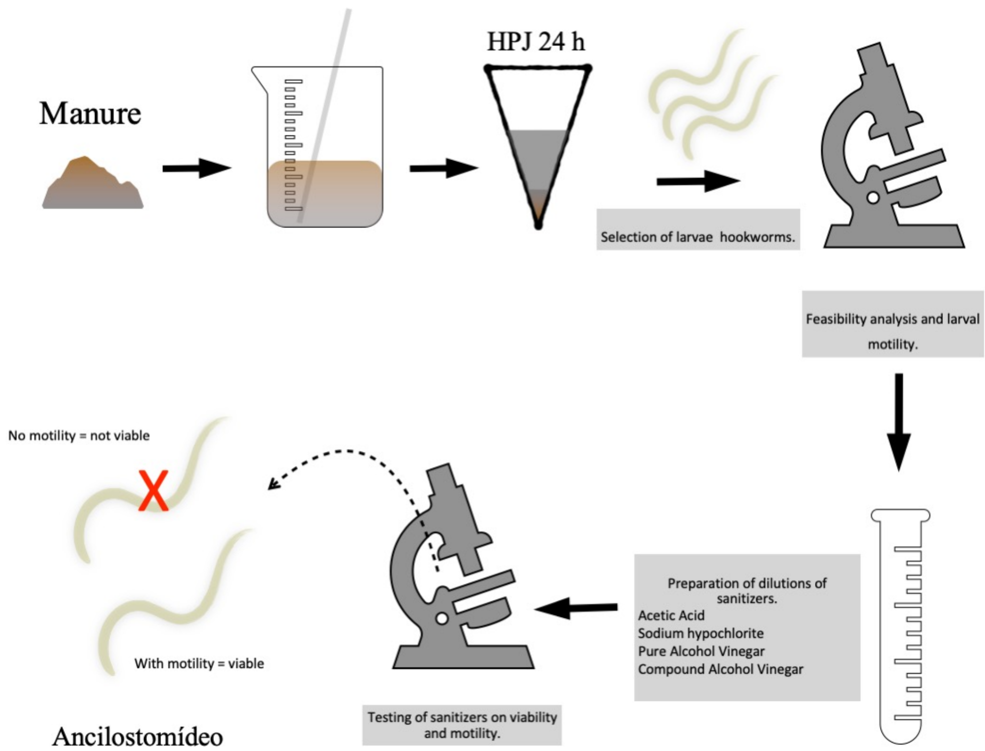


**Figure 1-** Schematic representative of the methodology applied in the study.

### *Helminth Isolation*

The larvae of Ancilostomídeos sp. were obtained through the HPJ method, from organic fertilizer, supposedly contaminated, 10-14,21 used in the cultivation of plantations around the Federal District. About 5 grams of the contaminated material was placed in a 250 ml conical cup and 50 ml of distilled water added, and then homogenized and strained in gauze. After the straining process, the calyx volume was filled with distilled water and left to stand for 24 hours for the spontaneous sedimentation process to occur. After 24 hours, slides were made with the content of the sediment and these were analyzed under microscopy in the 10x and 40x objective to verify the presence of helminth larvae.

*Research on the viability and motility of helminth larvae*

We prepared 51 slides, each with two drops of the sediment contained in the cup. Subsequently, microscopy of these slides was performed to check the concentration and presence of movement of the hookworm larvae. The slides that presented more than 90% of the larvae counted with movement were preserved, while the number of contact parasites with movement was less than 90% were discarded. As a test control, 3 slides were reviewed and analyzed without adding the substances to be tested, to ensure that the parasites would remain alive.

*Preparation of samples to be tested*

Based on ease of access in markets, the following compounds for home use in vegetable disinfection were selected: Acetic acid, Hypochlorite, Pure Alcohol Vinegar and Compound Alcohol Vinegar. Then, dilutions in distilled water of these substances were prepared in a graduated cylinder: Acetic acid [5%, 40%, 76.92% and 100%]; Hypochlorite [5%, 40% 76.92% and 100%]; Pure Alcohol Vinegar [5%, 40%, 76.92% and 100%]; and Vinegar composed of alcohol [5%, 40%, 76.92% and 100%].

*Testing the effectiveness of the samples*

Slides were prepared with a drop of the material contaminated with hookworm larvae and these slides were covered with coverslips and taken to microscopy. After the microscopy process, slides with parasitic loads with movement above 90% were reserved for the testing phase of previously prepared substances, while slides with parasitic loads with movement below 90% were discarded - the percentage values were obtained by counting the total number of parasites present on the slide and performing a simple 3 rule. Once the slides were selected, they were subjected to the test with the different solutions previously prepared. To perform this test, a drop of each solution was added on respective slides and a timer was started simultaneously, with a maximum time of 15 minutes being established to check if the hookworm larvae were paralyzed. The slides were taken for microscopy and were analyzed in the 40x objectives for the presence of movement of the hookworm larvae. For the accuracy and guarantee of reliable results, all samples were processed in triplicates and the average was obtained as a chronological result.

## Results

The hookworm larvae obtained through the HPJ method are shown in Figure 2. The identification of hookworms was based on phenotypic criteria through the observation of structures present in this genus of the nematode group, as described by Neves et al, (2016).[21]

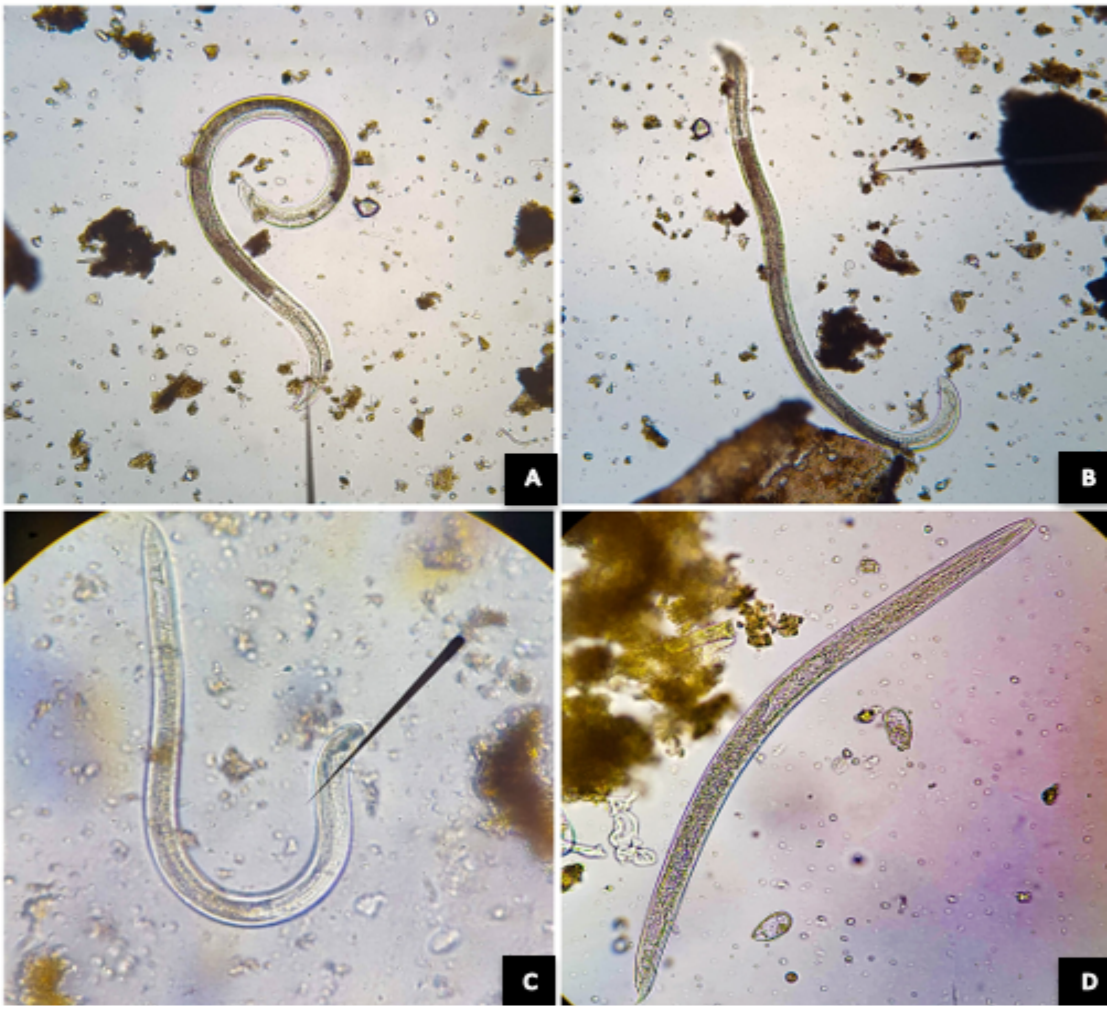


**Figure 2**- Hookworms isolated by the HPJ method used in this work.
**Legend:** A-D images show hookworm larvae. Hookworms were identified based on phenotypic criteria characteristic of this genus of nematodes, as reported by Neves (2016),[21] these being: cylindrical and robust body with tapered ends, spike at the tip of the tail and mouth capsule.

The values obtained as a result of effectiveness in terms of elimination capacity (paralysis of parasites) and effectiveness time of the tested agents and their respective dilutions are shown in Table 1.

**Table 1-** Kinetics of the effectiveness of triplicates regarding the paralysis of hookworm larvae when subjected to the tested substances and their dilutions. Brasília, Federal District, 2019.

| Tested substance | Efficiency time | Helminth | Movement |
|---|---|---|---|
| Acetic Acid 100% | 15:00 | Hookworms | Present |
| Acetic Acid 100% | 15:00 | Hookworms | Present |
| Acetic Acid 100% | 15:00 | Hookworms | Present |
| Acetic Acid 76,92% | 4:14 | Hookworms | Absent |
| Acetic Acid 76,92% | 15:00 | Hookworms | Present |
| Acetic Acid 76,92% | 15:00 | Hookworms | Present |
| Acetic Acid 40% | 15:00 | Hookworms | Present |
| Acetic Acid 40% | 15:00 | Hookworms | Present |
| Acetic Acid 40% | 15:00 | Hookworms | Present |
| Acetic Acid 5% | 15:00 | Hookworms | Present |
| Acetic Acid 5% | 15:00 | Hookworms | Present |
| Acetic Acid 5% | 15:00 | Hookworms | Present |
| Hypochlorite 100% | 03:19 | Hookworms | Absent |
| Hypochlorite 100% | 15:00 | Hookworms | Present |
| Hypochlorite 100% | 15:00 | Hookworms | Present |
| Hypochlorite 76,92% | 15:00 | Hookworms | Present |
| Hypochlorite 76,92% | 15:00 | Hookworms | Present |
| Hypochlorite 76,92% | 15:00 | Hookworms | Present |
| Hypochlorite 40% | 15:00 | Hookworms | Present |
| Hypochlorite 40% | 15:00 | Hookworms | Present |
| Hypochlorite 40% | 15:00 | Hookworms | Present |
| Hypochlorite 5% | 15:00 | Hookworms | Present |
| Hypochlorite 5% | 15:00 | Hookworms | Present |
| Hypochlorite 5% | 15:00 | Hookworms | Present |
| Pure alcohol vinegar 100% | 7:41 | Hookworms | Absent |
| Pure alcohol vinegar 100% | 13:02 | Hookworms | Absent |
| Pure alcohol vinegar 100% | 07:16 | Hookworms | Absent |
| Pure alcohol vinegar 76,92% | 14:00 | Hookworms | Absent |
| Pure alcohol vinegar 76,92% | 10:36 | Hookworms | Absent |
| Pure alcohol vinegar 76,92% | 14:50 | Hookworms | Absent |
| Pure alcohol vinegar 40% | 14:39 | Hookworms | Absent |
| Pure alcohol vinegar 40% | 13:54 | Hookworms | Absent |
| Pure alcohol vinegar 40% | 15:00 | Hookworms | Present |
| Pure alcohol vinegar 5% | 15:00 | Hookworms | Present |
| Pure alcohol vinegar 5% | 15:00 | Hookworms | Present |
| Pure alcohol vinegar 5% | 15:00 | Hookworms | Present |
| Alcohol-based vinegar 100% | 08:07 | Hookworms | Absent |
| Alcohol-based vinegar 100% | 06:06 | Hookworms | Absent |
| Alcohol-based vinegar 100% | 12:56 | Hookworms | Absent |
| Alcohol-based vinegar 76,92% | 07:00 | Hookworms | Present |
| Alcohol-based vinegar 76,92 | 6:56 | Hookworms | Present |
| Alcohol-based vinegar 76,92% | 6:34 | Hookworms | Present |
| Alcohol-based vinegar 40% | 9:00 | Hookworms | Absent |
| Alcohol-based vinegar 40% | 9:50 | Hookworms | Absent |
| Alcohol-based vinegar 40% | 10:40 | Hookworms | Absent |
| Alcohol-based vinegar 5% | 15:00 | Hookworms | Present |
| Alcohol-based vinegar 5% | 14:00 | Hookworms | Absent |
| Alcohol-based vinegar 5% | 15:00 | Hookworms | Present |

Based on the raw data obtained, it is possible to exclude samples that were not effective when the helminths stopped in the interval from 00:00 to 15:00 minutes, as these were inefficient at the maximum concentration of 100% when taking into account the stipulated period. The samples that showed the ability to paralyze the worms in only one of the three slides tested were excluded and identified as negative, since only those substances that could paralyze about 90% of the parasites in two or more slides were considered positive.

In order to determine among the tested sanitizers the most efficient in relation to the ability to inhibit the movement of worms, the average time of action of each sanitizer was evaluated, within a period of fifteen minutes, and the minimum inhibitory concentration that had an effect (Table 2).

**Table 2-** Average time of effectiveness of the tested samples regarding the ability of paralyzing larvae of hookworms. Brasília, Federal District, 2019.

| Tested substance | Average Time (minutes) | Helminth | Ability to paralyze hookworm larvae |
|---|---|---|---|
| Acetic acid 100% | 15:00 | Hookworms | Not effective |
| Acetic acid 76,92% | 15:00 | Hookworms | Not effective |
| Acetic acid 40% | 15:00 | Hookworms | Not effective |
| Acetic acid 5% | 15:00 | Hookworms | Not effective |
| Hypochlorite 100% | 15:00 | Hookworms | Not effective |
| Hypochlorite 76,92% | 15:00 | Hookworms | Not effective |
| Hypochlorite 40% | 15:00 | Hookworms | Not effective |
| Hypochlorite 5% | 15:00 | Hookworms | Not effective |
| Pure alcohol vinegar 100% | 9:16 | Hookworms | Effective |
| Pure alcohol vinegar 76,92% | 13:35 | Hookworms | Effective |
| Pure alcohol vinegar 40% | 14:31 | Hookworms | Effective |
| Pure alcohol vinegar 5% | 15:00 | Hookworms | Not effective |
| Alcohol-based vinegar 100% | 4:29 | Hookworms | Effective |
| Alcohol-based vinegar 76,92% | 6:50 | Hookworms | Effective |
| Alcohol-based vinegar 40% | 9:50 | Hookworms | Effective |
| Alcohol-based vinegar 5% | 15:00 | Hookworms | Not effective |

Acetic acid was not effective at its maximum concentration in any of the three slides evaluated. However, in one of the triplicates at a concentration of 76.92%, 90% of the parasites present were paralyzed. The other dilutions did not show significant results and were discarded, as the stoppage rate was less than 90%.

Hypochlorite and its respective dilutions, as well as acetic acid, have not shown to be effective in paralyzing the larvae.

On the other hand, pure alcohol vinegar showed significant results when compared to acetic acid and hypochlorite. At 100% concentration, the rate of loss of movement of hookworm larvae reached 90%, in the period of 9:16 min. The concentration of 76.92%, the level of loss of movement of hookworms (> 90% of the total hookworms) was reached in 13:35 min. The 40% concentration also showed effectiveness, reaching the peak of stoppage in 14:30 min, while the 5% concentration did not show effectiveness within the established time interval.

The vinegar composed of alcohol was the one that showed the best efficiency in relation to its ability to paralyze the larvae. The concentration of 100% was able to paralyze more than 90% of the larvae, with an average time of

4:29 seconds. The concentrations of 76.92% and 40% also showed good results, being able to paralyze more than 90% of the worms in an average time of 6:50 and 9:50 respectively. The 5% concentration had no effect. Figure 3 shows the general scheme of the effect of tested home use sanitizers and with effect against hookworm larvae.

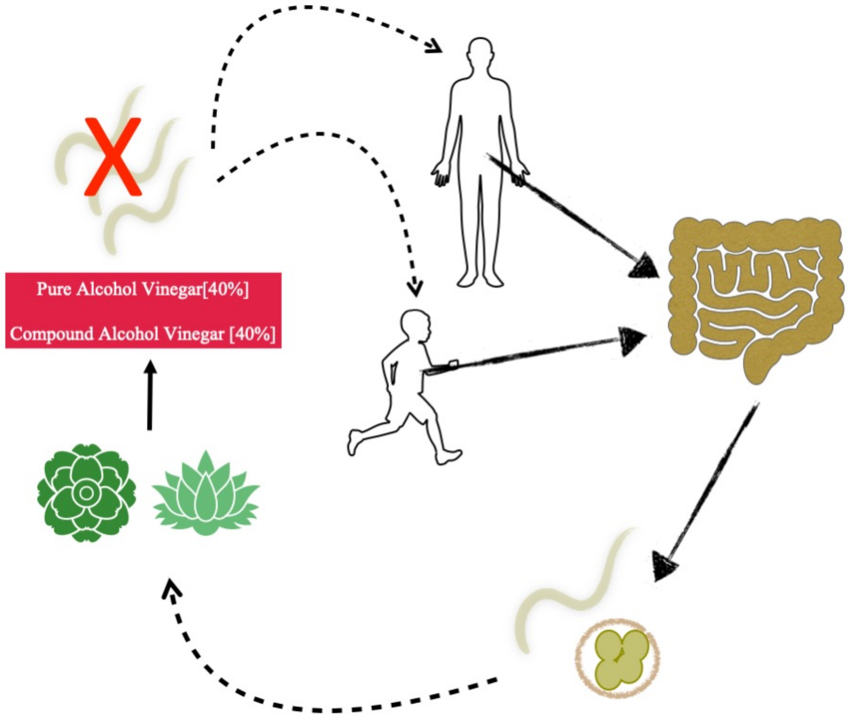


**Figure 3-** General scheme of the effect of tested home use sanitizers and with effect against hookworm larvae.
**Legend:** Pure and compound alcohol vinegar [40%] proved to be able to paralyze hookworm larvae.

## Discussion

The present work demonstrated that the hypochlorite has no significant activity in any of the tested concentrations, in the determined period of time, as to the ability to paralyze the helminth larvae present in the samples. These data corroborate a study carried out at the University of Brasília (2017), testing various commercial and homemade sanitizers, based on hypochlorite or chlorine salts as an active ingredient, demonstrating that none of the sanitizers was able to eliminate infective larvae of Strongyloides venezuelensis, in the concentrations recommended by manufacturers.[22] This same study also showed that there was elimination of S. venezuelensis larvae when sanitizers were used without dilution in vegetables. However, it is worth mentioning that the use of these sanitizers at a concentration of 100% is impracticable, since, in addition to being economically impracticable, they can alter the flavor of vegetables and are considered carcinogenic agents.[22]

Acetic acid did not show good results in most of the tested samples. Interestingly, it is worth mentioning that in one of the slides tested, the concentration of 76.92% was able to eliminate more than 90% of the hookworm larvae, in 4:14 min. This fact was not taken into account for the analysis because in the other two slides he was not able to reach the limit stipulated as ideal. What is known about the sanitary action of acetic acid is that, in addition to changing the pH of the medium in question, it can act on lipopolysaccharides and carrier proteins, present on the surfaces of Gram-negative bacteria, as reported by Utyama (2003) in a study on the use of vinegar and acetic acid as possible antimicrobial agents.[23] This fact may explain the ineffectiveness of acetic acid in relation to causing loss of hookworm movement, since taxonomically, nematodes belong to the Eukarya domain, present different proteins and different lipopolysaccharides on the surface of their cells when compared to bacteria.

Another important point to be considered about the ineffectiveness of acetic acid is that this fact may be related to some proteins produced and / or secreted by hookworms, as reported by Costa (2012) in his doctoral thesis, where he showed that ASPs (Ancylostoma Secreted Protein), proteins of the SCP / Tpx / Ag5 / PR-1 / Sc7 family (SCP / TAPS), act to protect hookworms from possible environmental stresses, such as changes in pH and osmolarity.[23] Costas (2012) also demonstrated that when hookworm larvae are in the infective phase (L3), they increase the secretion of ASPs and produce a protective cuticle, which is able to resist extremely low pH.

The study by Souza et al[24], reports that acetic acid was able to decrease the total load of fecal coliforms and other bacteria in vegetables when they were submerged in a solution of acetic acid in a concentration of 1% for at least 15 minutes. This work also suggests that this fact is linked not only to a change in pH, but also to the chemical nature of acetic acid, assuming that it can destabilize proteins responsible for ensuring the osmotic balance of bacterial cells. Although this study demonstrates a sanitizing effect against bacteria, our data test the effect against helminths, which are phylogenetically very distant from bacteria, and closer to animals, the same cannot be observed.

A study carried out in Natal - Rio Grande do Norte, with disinfectants used to disinfect vegetables, demonstrated that one of the best ways to improve methods for sanitizing vegetables, vegetables and fruits is to reduce contact with contaminating agents, that is, to ensure the minimum basic hygiene conditions from the cultivation of these vegetables to the processes of transportation, marketing and preparation for human consumption.[25]

Another study on sanitizers used in vegetables carried out by Barbari (2001), showed that the bleach solution with 200 ppm of active chlorine was able to reduce the initial microbial load of heterotrophic bacteria, thermotolerant coliforms and Escherichia coli after 15 minutes of immersion. However, it was not effective in reducing the load of filamentous fungi and yeasts.[26] In other studies using methodology similar to the previous study, no significant decreases in the numbers of microorganisms were observed in lettuces treated with hypochlorite.[25-27]

Fernandes (2017) and Santos (2015) demonstrated a better acetic acid activity for the elimination of E. coli when compared to hypochlorite, however, the use of high concentrations of acetic acid can interfere both in the appearance and flavor of vegetables.[28-29]

An experiment carried out with the use of hypochlorite in minimally processed cassava, demonstrated that it was not effective in reducing the load of total coliforms, fungi and lactic and / or helminth bacteria. However, this study also demonstrated that hypochlorite together with citric acid caused a significant decrease in the total microbial load, suggesting that changes in pH can significantly increase the effectiveness of the hypochlorite to eliminate bacteria and fungi.[30]

Regarding the efficacy of the vinegars tested, it is suggested that such efficacy may be related to the presence of alcoholic groups in their composition, as these groups can penetrate the interior of cells and interact with important metabolic pathways, as reported by Brito et al[31], in an experiment testing the anthelmintic action of Morinda citrifolia (noni) on Heterakis gallinarum.

Another hypothesis raised about the action of vinegars composed of alcohol and vinegar of pure alcohol is that they can act not only causing metabolic stress, but also interacting with the plasma membrane and causing severe damage and / or lysis to cells. This effect was suggested by Utyama (2003) in his work to assess the antimicrobial activity and cytotoxicity of two types of vinegars and acetic acid.[31]

In general, it can be said that, although sanitizers are used by the vast majority of the population to eliminate possible parasitic agents and taking into account the sanitary conditions of production (soil preparation, cultivation, harvesting, transportation, marketing and preparation ) in our country, this is a matter of great concern, since endoparasitosis caused by nematelminths is very prevalent in a developing country.[1-5] Another point of concern is respect for the lack of work being evaluated, which are actually used to eliminate helminth larvae present in vegetables and legumes, and such a gap represents a serious health problem, particularly for immunocompromised individuals.

## Conclusion

Based on the results obtained about home sanitization methods, it is suggested that most of the sanitizers evaluated in this study, are not effective against contamination of vegetables by helminths. In addition, concentrations routinely used in the disinfection of vegetables have proved ineffective in making larvae of hookworms, a globally dispersed geo-helminth and an important infectious agent in humans, unfeasible.

Findings suggest that both the vinegar composed of alcohol and the vinegar of alcohol in the concentration of 40%, were efficient in paralyzing hookworm larvae. However, it is noteworthy that although the antiparasitic activity of these sanitizers has shown good results, there is a scarcity of studies that better elucidate the action kinetics to a safe concentration for domestic use. In addition, it is suggested that studies be carried out in an attempt to elucidate the mechanisms of action, thus being able to lead to an accessible and safe methodology for the population.

Finally, the production chain of this type of food consumed "in natura" must pay close attention to the risk of contamination by geo-helminths, and good hygiene practices must be strictly followed.

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