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# PROTOZOA CONTAMINATED RAW VEGETABLES AND FRUITS IN MINIA CITY (EGYPT): CONTROL BY THREE SAFE DISINFECTANTS

By

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**Abstract**

Intestinal protozoan infection is a common zoonotic parasitic disease worldwide. Contaminated fresh vegetables and fruits are important sources of parasitic infections. This study identified the parasites infesting raw green vegetables and fruits and effect of some washing solutions to eliminate them.

A total of 398/1000 samples of green vegetables and fruits collected from October 2020 to September 2021 were infested with intestinal protozoa. Contaminated parasites were diagnosed microscopic by stained smears and flotation of each materials sample separately. Lemon juice 10%, sodium salt solution 1.5% and vinegar 0.5% were used to control parasitic stages as com-pared to tap water & potassium permanganate solution 150ppm as standard ones.

The commonest parasites were *Entamoeba histolytica* (19.2%) followed by *Cryptosporidium parvum* (18%). The most contaminated vegetables were watercress (64/113) followed by lettuce (56/99), with a significant seasonal variation in all parasites prevalence and incidence on plants. Vinegar 5%, lemon 10% & salt 1.5% gave highly significant lethal effect on all parasites as compared to water (P= 0.0001). Besides, potassium permanganate, lemon juice gave a significant lethal effect on *C. parvum*, but the sodium salt solution was only significant on *E. histolytica*, *G. lamblia* and *Blastocystis* hominis.

**Key words:** Minia city, Raw plants, Disinfectants, Potassium permanganate, Water

## Introduction

Generally speaking, fruits and vegetables include a diverse group of plant foods that vary greatly in content of energy and nutrients. Also, fruits and vegetables supply dietary fiber, and fiber intake is linked to lower incidence of cardiovascular disease and obesity (Slavin and Lloyd, 2012). WHO (2004) recommended eating at least 400grams of fruits and vegetables per day for micronutrient supplementation as well as prevention of chronic diseases. Darmon *et al.* (2005) reported that the Dietary Guidelines for Americans 2005 recommended that consumers give priority to nutrient-dense foods, which contain substantial amounts of key nutrients related to the dietary energy calculated/ weight (nutrient adequacy score), /calorie (nutrient density score), or /unit cost (nutrient-to-price ratio). Nevertheless, Beuchat (2002) in USA reported that outbreaks of human infections associated with consumption of raw fruits & vegetables during the past decade. He added that a general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables was attributed to their inaccessibility to locations within other structures and tissues that may harbor the pathogens.

Wadamori *et al.* (2017) in New Zealand reported that microbiological foodborne outbreaks associated with consumption of fresh products were increasing as *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and others.

Moreover, Mahvi and Kia (2006) in Iran reported contaminated plants were due to using insufficiently treated wastewater and animal waste fertilizers. El Shazly *et al.* (2007) in Egypt detected protozoa pollution in water used for irrigation. Abdalla *et al.* (2013) in Saudi Arabia reported that raw vegetables and fruits play an important role in transmitting zoonotic parasites. Therefore, Lee *et al.* (2009) reported that the US/FDA approved chemical compounds for food surface disinf-

ection include chlorine, chlorine dioxide, iodophors and quaternary ammonium compounds. However, chlorine concentration in tap water didn't eliminate parasites such as *Giardia* spp. and *Cryptosporidium* spp. on fresh vegetables and fruits (Singh *et al,* 2002).

This study aimed to estimate the prevalence of intestinal protozoa parasites in raw vegetables and fruits and to detect the effect of three safe disinfectants as washing solutions.

## Materials and Methods

A cross-sectional study was conducted from October 2020 to September 2021 to detect the parasites in some fresh green vegetables and fruits collected from shopping markets in Minia City. A total of 1000 samples were collected from in separate moisture labeled nylon bags, and immediately transferred to Parasitology Department to be put clean labeled glass backers. Sodium salt, sugar cane vinegar and lemon were used as disinfectant washing solutions for vegetables and fruits to test their effect in parasites viability as compared to water (negative control) and potassium permanganate (positive control).

Sample preparation: Each sample (250gm) was soaked for 30 minutes in sterile tap water, followed by vigorous shaking with a mechanical shaker for 20 minutes. Samples were removed and the washing solution was left to precipitate and tested directly (wet mount, or stained smears).

Concentration procedure separate parasites increasing the chances of detecting them even in small numbers, which was divided into flotation techniques and sedimentation techniques. Flotation techniques by zinc sulfate or Sheather's sugar solutions rise parasites to the top and debris to the bottom. But, the disadvantages of flotation techniques were that walls of eggs and cysts often collapsed hindering the identification. The sedimentation techniques proved easier to perform and less prone to technical errors, used at CDC (2016) is the formalin-ethyl acetate technique, a diphasic sedimentation technique that avoids the problems of flammability of ether, and which can be used with the specimens preserved in formalin, MIF or SAF. Positive washing solutions were divided into 4 equal aliquots to evaluate the disinfectants' efficacy.

Sample examination: Wet mount and sediment were examined by low and high powe rs of a light microscopy (Garcia, 1993). The modified Ziehl-Neelsen (MZN) stained was indicated for some protozoa as *Cryptosporidium* spp., *Isospora belli*, *Cyclospora cayetanensis* and others (El Shazly *et al,* 2006).

Disinfectants: Three disinfectant solutions were used. Fresh lemon juice solution was diluted to 10% with sterile distilled water. Sodium salt crystals were dissolved in sterile distilled water to have 1.5% solution. Sugar cane vinegar solution was diluted in sterile distilled water to have 0.5% solution. Potassium permanganate powder was dissolved in sterile distilled water to have 150 ppm.

Disinfection of vegetables and fruits: Different disinfectant solutions were added separately to each one for 20 min., and then were centrifuged at 1500 rpm for 5 minutes. Sediment and supernatant were examined to detect their effects on the detected parasites.

Statistical analysis: Data were analyzed by using Statistical Package for Social Science (SPSS) software version 25 (SPSS Inc, Chicago, Illinois, USA). Qualitative data was presented as a frequency distribution. Pearson's Chi-square test was used, $P \leq 0.05$ was considered significant to test for concordance between the used disinfectants and each of tap water and K. permanganate, Related-Samples McNemar's Change Test were used in this research (non-parametric test).

## Results

In this study, of 1000 samples of different green vegetables and fruits 398 were contaminated with at least one type of intestinal parasite with an average rate of (0.40).

The contaminated vegetables were watercress (64/113), lettuce (56/99) and cucumber (49/91), followed by coriander (46/96), parsley (46/104) and finally dill (41/109). Fresh fruits, banana was highly contaminated ones

(31/92) followed by apple (28/100), orange (24/104) and then apricot (13/91).

The summer was highly contaminated season (49.2%) followed by spring (41.3%), winter (36%) and autumn (25.5%). In summer, watercress contaminated rate was (23.12%), and then lettuce (20%), coriander (12.5%), parsley (9.38%) and dill (6.25%) with P = 0.025, 0.035, 0.016, 0.452 & 0.188, respectively. In spring, parsley (15.32%) was contaminated followed by coriander (14.52%), cucumber and dill (both 12.9%). Fruits (apples, bananas and apricots didn't show seasonal significant differences, but orange was significantly contaminated in winter.

Protozoa parasites detected were *Entamoeba histolytica* (19.2%) followed by *Cryptosporidium parvum* (18%), *Giardia lamblia* (6.1%), *Blastocystis hominis* (4.2%), *Isospora belli* (4.1%), *Cyclospora caytenensis* (2.4%), and *Dientamoeba fragilis* (0.3%). In summer *E. histolytica* (32.6%), *C. parvum* (24.6%) and *G. lamblia* (11.7%) were the commonest ones. But, in spring, *C. parvum* (26%) and *E. histolytica* (19.7%) were the commonest. In winter, *C. parvum* (7.4%), *B. hominis* (3.4%) and *E. histolytica*. (2.3%) were the commonest. In autumn, *E. histolytica* (11.5%) and *C. parvum* (4.5%) were the commonest.

Tap water had no lethal effect on the detected protozoa, but vinegar, lemon and salt showed a highly significant lethal effect (P 0.0001). Vinegar, lemon and salt solutions showed high significant effect than K. permanganate, which differed with different detected parasite.

Vinegar 0.5%, lemon 10% & salt solution 1.5% as compared to K. permanganate, *E. histolytica* showed significant only with salt solution (P= 0.0001). Also, the three disinfectants showed lethal effect on *C. parvum*, but more significant only with 10% lemon (P= 0.002). Salt solution gave a significant lethal effect on *G. lamblia* and *B. hominis* than others compared to K permanganate (P = 0.009, 0.006 respectively). *I. belli*, without significant difference disinfectants.

Details were given in tables (1, 2, 3 & 4) and figures (1 & 2).

Table1: Rate of contamination in green vegetables and fruits.

| Type of plant | Positive N= 398 | Negative N= 602 | Parasites detected One | Two | Three | Z test | P value |
|---|---|---|---|---|---|---|---|
| Watercress (113) | 64 | 49 | 44 | 14 | 6 | 3.88 | 0.0001 |
| Parsley (104) | 46 | 58 | 34 | 7 | 5 | 0.97 | 0.327 |
| Dill (109) | 41 | 68 | 25 | 10 | 6 | -0.49 | 0.624 |
| Lettuce (99) | 56 | 43 | 46 | 8 | 2 | 3.59 | 0.0003 |
| Coriander (96) | 46 | 50 | 38 | 5 | 3 | 1.71 | 0.087 |
| Cucumber (91) | 49 | 42 | 40 | 6 | 3 | 2.87 | 0.004 |
| Oranges (105) | 24 | 81 | 16 | 6 | 2 | -3.75 | 0.0002 |
| Apples (100) | 28 | 72 | 16 | 8 | 4 | 2.54 | 0.011 |
| Banana (92) | 31 | 61 | 20 | 7 | 4 | -1.25 | 0.208 |
| Apricot (91) | 13 | 78 | 9 | 2 | 2 | -5.21 | <0.0001 |

*Chi-square test was used

Table 2: Number and percentage of samples contaminated with parasites in different seasons

| Seasons Positive | Summer (160/325) 49.2% | | Spring (124/300) 41.3% | | Winter (63/175) 36% | | Autumn (51/200) 25.5% | | $X^2$ test | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | +ve | % | +ve | % | +ve | % | +ve | % | | |
| Watercress | 37/160 | 23.21 | 12/300 | 9.68 | 7/175 | 11.11 | 8/200 | 15.69 | 9.29 | 0.025 |
| Parsley | 15/160 | 9.38 | 19/300 | 15.32 | 7/175 | 11.11 | 5/200 | 9.8 | 2.63 | 0.452 |
| Dill | 10/160 | 6.25 | 16/300 | 12.9 | 8/175 | 12.7 | 7/200 | 13.72 | 4.79 | 0.188 |
| Lettuce | 32/160 | 20 | 12/300 | 9.68 | 5/175 | 7.94 | 7/200 | 13.72 | 8.60 | 0.035 |
| Coriander | 20/160 | 12.5 | 18/300 | 14.52 | 0 | 0 | 8/200 | 15.69 | 10.28 | 0.016 |
| Cucumber | 22/160 | 13.75 | 16/300 | 12.9 | 5/175 | 7.94 | 6/200 | 11.76 | 1.48 | 0.687 |
| Apple | 9/160 | 5.62 | 8/300 | 6.45 | 6/175 | 9.52 | 5/200 | 9.8 | 1.74 | 0.627 |
| Banana | 11/160 | 6.88 | 14/300 | 11.29 | 6/175 | 9.52 | 0 | 0 | 6.87 | 0.076 |
| Orange | - | - | - | - | 19/175 | 30.16 | 5/200 | 9.8 | 7.02 | 0.008 |
| Apricot | 4 | 2.5 | 9/300 | 7.26 | - | - | - | - | 3.62 | 0.057 |

Table 3: Prevalence rate of parasitic infection in examined fruits and vegetables in different season

| Parasite | Prevalence | Summer (325) | | Spring (300) | | Winter (175) | | Autumn (200) | | X-test | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1000 (%) | +ve | % | +ve | % | +ve | % | +ve | % | | |
| *E. histolytica* | 192 (19.2) | 106 | 32.6 | 59 | 19.7 | 4 | 2.3 | 23 | 11.5 | 77.66 | < 0.0001 |
| *C. parvum* | 180 (18) | 80 | 24.6 | 78 | 26 | 13 | 7.4 | 9 | 4.5 | 60.59 | < 0.0001 |
| *G. lamblia* | 61 (6.1) | 38 | 11.7 | 16 | 5.3 | 3 | 1.7 | 4 | 2 | 29.80 | < 0.0001 |
| *B. hominis* | 42 (4.2) | 16 | 4.9 | 16 | 5.3 | 6 | 3.4 | 4 | 2 | 4.04 | 0.257 |
| *I. belli* | 41 (4.1) | 20 | 6.2 | 16 | 5.3 | 0 | 00 | 5 | 2.5 | 13.43 | 0.004 |
| *C. caytanensis* | 24 (2.4) | 10 | 3.1 | 11 | 3.7 | 0 | 00 | 3 | 1.5 | 6.81 | 0.078 |
| *D. fragilis* | 3 (0.3) | 1 | 0.3 | 2 | 0.66 | 0 | 00 | 0 | 00 | 0.42 | 0.516 |
| Total | 543 | 271 | 83.4 | 198 | 65.96 | 26 | 14.8 | 48 | 24 | 306.5 | < 0.0001 |

Table 4: Effect of natural disinfectants compared to tape water and potassium permanganate in distorting parasites

| Disinfectant / Parasite | Water and K. permanganate N (%) | Vinegar 0.5% | | Lemon 10% | | Salt solution 1.5% | |
|---|---|---|---|---|---|---|---|
| | | distorted parasite | P-value | N (%) | P-value | N (%) | P-value |
| *E. histolytica* | 0/192<br>48/192 | 58 (30.2%) | 0.0001<br>0.3 | 61<br>(31.8%) | 0.0001<br>0.1 | 86<br>(44.8%) | 0.0001<br>0.0001 |
| *C. parvum* | 0/180 (0%)<br>36/180(20%) | 40 (22.2%) | 0.0001<br>0.6 | 63<br>(35 %) | 0.001<br>0.002 | 38<br>(21.1%) | 0.0001<br>0.8 |
| *G. lamblia* | 0/61 (0%)<br>16/61 (26.2%) | 18 (29.5%) | 0.0001<br>0.8 | 24<br>(39.3%) | 0.0001<br>0.1 | 31<br>(50.8%) | 0.0001<br>0.009 |
| *B. hominis* | 0/42 (0%)<br>9/42(21.4%) | 11 (26.2%) | 0.001<br>0.7 | 17<br>(40.5%) | 0.0001<br>0.09 | 22<br>(52.4%) | 0.0001<br>0.006 |
| *I. belli* | 0/41(0%)<br>9/41 (22%) | 11 (26.8%) | 0.001<br>0.7 | 16<br>(39.02%) | 0.001<br>0.1 | 10<br>(24.4%) | 0.002<br>0.7 |

## Discussion

Dorny *et al.* (2009) in Belgium reported that parasitic food-borne diseases are generally under-recognized, but they are becoming more common. Globalization of the food supply, increased international travel, increase of population of highly susceptible persons, change in culinary habits, but improved diagnostic tools and communication were factors associated with the increased diagnosis of food-borne parasitic diseases worldwide. Generally, consumption of raw green vegetables and/or raw fruits without good washing is one of the main sources of soil transmitted parasites (El Shazly *et al,* 1994).

In the present study, there was a high rate of contaminated parasites (Protozoa, 40%) on green vegetables and fruits with intestinal. This agreed with Said (2012) in Alexandria reported (31.7%), Eraky *et al.* (2014) in Qalyubia reported (29.6%), and Alhabbal (2015) in Syria reported (31.38%), Bekele and Shumbej (2019) in Ethiopia reported (42.6%). Lower contaminated rates were (14.6% & 8.4%) reported by Rahmati *et al.* (2017) and Matini *et al.* (2017) in South and West Iran respectively. This difference may be due to difference farmers' hygienic levels and sellers, fertilizers and irrigation water.

In the present study, watercress (64/113) was the highly contaminated green plants followed by lettuce (56/99), cucumber (49/91), coriander (46/96), parsley (46/104) and lastly dill (41/109). This more or less agreed with Hussein *et al.* (2021) in Baha Region, Saudi Arabia reported that watercress and lettuce were the most contaminated leafy vegetables followed by parsley, dill and coriander. Also, the present study the contaminated raw fruits in a descending order were apples (28/100), then oranges (24/105) and lastly apricots (13/91). This more or less agreed with Alhabbal (2015) in Syria and Taherimoghaddam *et al.* (2018) in Iran, they reported that parsley was the highly contaminated fruits**.** These slight differences raw vegetables and fruits may be due to climatic and ecological variations as well as the morphological features of the different vegetables & fruits examined. Apart from the cold weather in Syria, the extent of the crisis has affected all aspects of Syrians' life (Alhaffar and Janos, 2021). In Saudi Arabia, despite difficulties of scarce rain, meager subterranean water, limited local manpower & widely dispersed cultivatable lands, but fluctuations in maximum & minimum temperature, water availability and humidity of plant growth di-

fferent stages for few major crops of country which include wheat, date, and vegetables (Bin Alam *et al,* 2010). Egyptian agricultural products depends mainly on water coming from River Nile branches, and groundwater although contributes with little amounts, but generally, agricultural sector is considered the largest water consumer about 80% of the total water budgets (Mahmoud, 2017). Watercress and lettuce have rough surfaces so that the infective stage of the parasite can easily stick to the surface of these vegetables. However, vegetables and fruits with smooth surfaces had a lower contamination rate due to smooth surfaces reduce the chance of parasitic attachment (Damen *et al,* 2007).

In the present study, the summer had the highest contaminated rates on raw vegetable and fruit samples (49.2%), followed by spring (41.3%), winter (36%) and then autumn (25.5%). This also agreed with Eslami *et al* (2003) who reported a higher rate of parasitic contamination in vegetables during warm seasons compared to those in cold ones.

In the present study, watercress was highly contaminated parasite (23.12%) in summer, followed by lettuce (20%) and coriander (12.5%), but parsley (9.38%), dill (6.25%), apple (5.62%) and apricot (2.5%) were the least contaminated ones. This more or less agreed with Eraky *et al,* (2014), who reported that lettuce (45.5%) was highly contaminated followed by watercress (41.3%). Al-Binali *et al.* (2006) and Al-Megrin (2010) in Saudi Arabia reported that the contaminated plants were lettuce (27.8% & 17%), followed by watercress (22.8% & 17%) respectively. Also, Mohamed *et al.* (2016) in Sudan reported that lettuce was highly parasitic contaminated fresh vegetable (36.4%), they added that fresh lettuce was 36.4% (4/11), but cayenne pepper, and cucumber were not contaminated. No doubt, the differences in parasitic prevalence rate may be due to different climatic and ecological environmental factors (Saleh *et al,* 2014).

In the present study, *E. histolytica* was the most detected parasite (19.2%), followed by *C. parvum* (18%), and *G. lamblia* (6.1%), but *B. hominis* (4.2%), *I. billi* (4.1%) and *C. caytenens is* (2.4%) were the least frequently detected ones. This agreed with Hussein *et al.* (2021) in Egypt who reported that *C. parvum* (29.3%) and *G. lamblia* (21.3%) were the commonest protozoan parasites, but *I. belli* was the least detected parasite (1.8%). Gabre and Shakir (2016) in Tabuk, Saudi Arabia reported the *E. histolytica* commonly contaminated vegetables than *G. lamblia*.

Also, in the present study, *E. histolytica* (32.6%), *C. parvum* (24.6%) and *G. lamblia* (11.7%) were more prevalent in summer (P=0.0001. This agreed with Jaran (2016) in Jordan who found that the high parasites (*G. lamblia* & *E. histolytica*) were in summer (62%) compared to winter (16%), with were the most detected parasites. Khalifa *et al.* (2014) in Minia Governorate reported a significant high rate of protozoa contamination in summer (66.7%). Siwila *et al.* (2020) in Zambia reviewed the results of 17/54 African countries, and reported that *Cryptosporidium* prevalence ranged from 6 to 100% in surface water, 4 to 100% in tap water and up to 100% in wastewater and sludge. Besides, *Cryptosporidium* was in five countries with prevalence of 0.8-75%. *Giardia* was in 47 countries with prevalence ranged from 2.4% in surface water; 1% to over 70% in tap water; 28-100% in wastewater and 2% - 99% in fresh products, but *Cyclospora cayetanensis* was lower. *E. histolytica* was 78% in surface water; 100% in wastewater and up to 99% in fresh produce.

In the present study, tap water had no lethal effect, but the vinegar destroyed the parasites as potassium permanganate did. This agreed with Costa *et al.* (2009) who reported that vinegar (4% v/v acetic acid) killed *G. duodenalis* cysts. Also, Etewa *et al.* (2017) in Sharkia reported that acetic acid (5%) had a significant killed *E. histolytica & G. lamblia* (cysts) and *C. parvum* (oocyst) by nucleic acids and protein bonds precipitation by changing the pH of parasite ecology. The 10% lemon solution proved to be an effecti-

ve disinfectant in more distorting all five detected parasites in comparison with potassium permanganate and vinegar, and more obvious effect was on *C. parvum*. Bushkin *et al.* (2013) in USA reported that the lemon contains lemon oil, which contains several essential oils known for their anti-parasitic efficacy that damage the wall of both cysts and oocysts. Xavier-Junior *et al.* (2017) in Brazil reported that the lemon oils combine with the protein of the cell and precipitate it, as good solvents of fatty substances due to their ability to decompose the membranes of the parasites living cells and as a result, the internal components come out and destroyed. This agreed with Maaroufi *et al.* (2021) in Tunisia who reported that the lemon juice proved effective as anti-parasitic agent.

Apart from parasites, the lemon grass leaf extract's mean concentration which inhibited the growth of *Staphylococcus aureus* was at 15.59%, which is significantly lower than the mean concentrations of both the calamansi peel extract and the positive control, Gentamicin, which were 76.43% and 25% respectively (Jafari *et al,* 2012). Moreover, the lemongrass leaf extract's mean concentration which inhibited the growth of *Escherichia coli* was at 42.94%, which is significantly lower than the mean concentrations of both the calamansi peel extract and the positive control, Ceftriaxone, which were 97.65% and 100% respectively (Falah *et al,* 2015).

## Conclusion

No doubt, using natural safe disinfectants as lemon juice, sodium salt & vinegar solutions to wash raw vegetables and fruits are more appropriate practice for consumers at home. These materials are household ingredients easy to get, affordable and safe.

The results showed that contamination levels of raw vegetables and fruits with seven zoonotic protozoa. However, the safe use of disinfectants; such as lemon (10%), potassium permanganate (150ppm), sodium salt solution (1.5%), and vinegar (10%) effectively reduced parasitic infective stages.

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**Explanation of figures**

Fig.1: Parasites: A- Acid-fast Kinyoun stain showed *Isospora belli* oocysts, and *Cryptosporidium parvum* oocysts. B- Direct iodine showed *E. histolytica*. C- Direct saline showed *Blastocystis hominis* cysts. D-Acid-fast Kinyoun stain showed *Cyclospora cayetanensis* oocysts.

Fig. 2: Parasitic distortion. A- Giemsa stained smear showed *E. histolytica/dispar* trophozoite with highly vacuolar cytoplasm and protruding pseudopodia. B- Direct iodine stained smear showed *E. histolytica/dispar* trophozoite in salt solution. C- Direct iodine showed *E. histolytica/dispar* cysts. D- Direct iodine showed *E. histolytica/dispar* cysts. E- Direct iodine showed *E. histolytica/dispar* cyst in salt solution. F- Acid-fast Kinyoun stained showed *C. parvum* oocysts. G- Acid-fast Kinyoun stain showed *C. parvum* oocysts distorted cyst by lemon solution.