Parasitic Contamination of Commonly Consumed Fresh Vegetables and Fruits in Some Rural Areas of Sharkyia Governorate, Egypt

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Background and study aim: The healthy diet must basically contain fresh fruits and vegetables. Contaminated vegetables and fruits consumption is a way of getting people infected with intestinal parasites. This study aimed to detect the parasitic contamination in some common fresh vegetables and fruits in Sharkyia Governorate, Egypt and the effect of potassium permanganate (24mg/L) and acetic acid 5% upon these parasites.

Patients and Methods: The study examined 420 samples: lettuce, watercress, parsley, cucumber, carrots and strawberry. Their collection was done from some rural areas of Sharkyia governorate's markets. Samples were washed and the solution resulted from washing was filtered and centrifuged to concentrate the parasitic stages. Sediments and supernatants were microscopically examined using iodine and modified Ziehl–Neelsen stained smears. Then the positive samples were soaked separately in acetic acid 5% and potassium permanganate 24ml/L for 15 and 30 minutes. Then they were tested by 0.2% trypan blue stain to detect the viability of parasites.

Results: The parasites were determined in 164/420 (39%) samples. *Giardia lamblia* cysts were the most prevalent parasite (12.6%) followed by *Cryptosporidium spp.*oocysts (7.6%), *Entamoeba spp.* cysts (6.2%), *Blastocystis hominis* cysts (3.8%), *Hymenolepis nana* eggs (2.8%), *Ascaris lumbricoides* eggs (1.9%), various helminths larvae (1.6%), *Enterobius vermicularis* eggs (1.4%) and *Balantidium coli* cysts (0.9%). The highest contaminated one was watercress (55.7%) followed by lettuce (45.7%), parsley (44.2%), cucumber (31.4%), strawberry (30%) and carrots (27.1%). There was a reduction in the viability of the parasites after exposure to acetic acid 5% and potassium permanganate 24mg/L but the statistical difference between the percentages was insignificant.

Conclusion: The results of the study emphasized a possible role of contaminated raw vegetables and fruits in the spread of parasitic diseases in Sharkyia governorate, Egypt. Acetic acid 5% and potassium permanganate 24ml/L are considered effective disinfectants to reduce parasitic contamination of fresh vegetables and fruits.

INTRODUCTION

Maintenance of a healthy human diet can be achieved by vegetables and fruits as they contain many vitamins, carbohydrates and minerals **[1]**. Also, the risk of certain diseases as chronic diseases and cardiovascular diseases can be reduced by them **[2]**. In less developed countries, there are certain factors which contribute to the contamination of vegetables and fruits during the planting process including the usage of insufficiently treated wastewater for irrigation, soil contamination by the animal wastes and increased application of improperly composted manures to the soil. The bad hygienic practice by food handlers during the preparation of fresh vegetables and fruits can lead to high prevalence of contamination **[3]**. Certain new habits contribute to the increase in the occurrence of food borne illness linked to consuming fresh vegetables and fruits. These include the increase in consumption of raw or improperly cooked vegetables and fruits in fast meals eaten in restaurants, canteens and from street sellers of food **[4].**

In the last years, the breaking off the barriers in between the countries leads to the widespread of parasitic infections all over the world. This is made by the export of contaminated vegetables from developing countries to developed ones **[5]**. Consumers are advised to wash fresh vegetables and fruits with running tap water before consuming them because the tap water contains a chlorine oxide which can kill the microorganisms which found on the surface of fruits and vegetables **[6].** This chlorination method is considered a traditional disinfection method but it isn't particularly effective in reducing helminths and some protozoan numbers to low levels **[7].** Nowadays, certain types of disinfectants are used worldwide such as acetic acid, potassium permanganate, Ozone, Ultraviolet irradiation and several chemicals **[8].**

In less developed countries including Egypt, there is a lack in the diagnosis of outbreaks caused by contaminated vegetables and fruits and no adequate previous surveys have been conducted to record the incidence of parasitic contamination in vegetables and fruits **[9].** So, this work aimed to investigate the prevalence of helminths and protozoa contamination of some selected types of raw vegetables and fruits commonly consumed and sold in Sharkyia governorate rural markets with an offer of reducing these contaminations by using common disinfectants like acetic acid 5% and potassium permanganate 24mg/l. So, this reduction may decrease the hazards upon the health.

PATIENTS AND METHODS

Study type:
A cross sectional study was performed during the period from January to December 2016. The practical work was done at the laboratories of Medical Parasitology Department, Faculty of Medicine, Zagazig University, Egypt. The study included (420) vegetables and fruits samples (70 samples from each type). Five types of vegetables and one type of fruits; (lettuce, watercress, parsley, cucumber, carrots and strawberry).

Sample collection:
Samples were collected from all available sales outlets (street vendors, commercial groceries and supermarkets) in some rural areas of Sharkyia governorate including (Faqous, Banayuos, Kafr El Hamam, Kafr Sakr, Abo Hammad, El Qenayat, Abbasa). Each sample was put with its root in a separate nylon bag and labeled.

Procedure for sample preparation and washing:
About two hundred grams was weighted from each sample, and then about one liter of physiological saline was used to immerse each vegetable and fruit's sample in aseparate sink. The samples were left soaked overnight for sedimentation to take place.The top layer was discarded and the remaining solution was sieved to remove debris after that, they were transferred to the tubes for centrifugation to be centrifuged at 2000 rpm for twenty minutes. The supernatant was transported to a test tube and the examination of the sediment was done **[10]**.

Detection of parasites:
The supernatant was examined by Zinc sulphate flotation method to detect light weight eggs and cysts of protozoa **[11].** Then, the sediment was examined microscopically after mixing it well by the following methods: simple smear and iodine stained smear for detection of parasitic eggs, cysts and larvae. In the unstained smear, the sediment's drop was placed on a clean slide and covered by a cover slip, then, examined under a light microscope using x10 and x40 objectives. In iodine smear, a drop of Lugol's iodine was added to the sample before being covered by the cover slip **[12].** To detect the oocysts of coccidian protozoa, we used Modified Ziehl–Neelsen stain smears. Then they were examined microscopically by oil immersion lens (x1000) **[13].**

Disinfection of vegetables:
The used disinfectants were acetic acid 5% and potassium permanganate 24mg/l. The positive samples for each parasite were collected separately in one container with 15 ml of saline. Each parasite suspension was divided in two tubes after mixing it well. Then, it was centrifuged at 1500 r.p.m for 5 minutes. Then, the following was done for each parasite: 5ml of 5% of acetic acid was added to the sediment and the supernatant of the 1st tube. 5ml of 24 mg/l potassium permanganate was added to the sediment and the supernatant of 2nd tube. After 15 minutes from exposure to these disinfectants. About 5 ml of the suspension from the two tubes were taken and centrifuged again. Then, they were exposed again to these disinfectants for 30 minutes **[14]**. These procedures were conducted at the room temperature (25°c).

Viability assay:
Preparation of slides smears from the solutions exposed to the disinfectants was done and stained

by 0.2% trypan blue and examined microscopically (x100, x400, and x1000). Viable parasites appeared clear with light blue color and showed dye exclusion activity, while dead parasitic stages appeared dark blue in color with some changes of their external structure **[15].**

Statistical Analysis:
The results were analyzed using chi-square tests of the SPSS software version 17. The significance's threshold is fixed at 5% level (P-value). P-values >0.05 were considered insignificant, while P-values <0.05 were considered significant and <0.001 highly significant.

RESULTS

As shown in tables and figures.

Table (1): Prevalence rate (percentages) of parasitic contamination of the examined raw vegetables and fruits

	Lettuce (70)		**Water cress (70)**		**Parsley (70)**		**Cucumber (70)**		**Carrots (70)**		**Straw berry (70)**		**Total examined (420)**		**X^2**	**P**
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**		
Positive	32	45.7	39	55.7	31	44.2	22	31.4	19	27.1	21	30	164	39	18.57	0.002
Negative	38	54.3	31	44.2	39	55.7	48	68.6*	51	72.8*	49	70*	256	60.9		
X^2	1.03		1.03		1.83		19.3		29.26		22.4					
P-value	0.31		0.31		0.1>		<0.001**		<0.001**		<0.001**					

*Significant P value (P < 0.05)

Table (2): The distribution of each parasitic contamination of examined raw vegetables and fruits

Parasite	**Total No. recovered (out of 420)**	**% of +ve (164)**	**Lettuce (70)**		**Water cress (70)**		**Parsley (70)**		**Cucumber (70)**		**Carrots (70)**		**Straw berry (70)**	
			+ve	**%**	**+ve**	**%**	**+ve**	**%**	**+ve**	**%**	**+ve**	**%**	**+ve**	**%**
Enterobius egg	6	3.6	1	1.4	3	4.2	2	2.8	0	0	0	0	0	0
H. nana egg	12	7.3	3	4.2	5	7.1	4	5.7	0	0	0	0	0	0
Ascaris egg	8	4.96	1	1.4	4	5.7	3	4.2	0	0	0	0	0	0
Helminths larvae	7	4.2	4	5.7	2	2.8	1	1.4	0	0	0	0	0	0
E. histolytica cyst	26	15.9	2	2.8	4	5.7	3	4.2	6	8.5	5	7.1	0	8.5
Cryptosporoidium spp. oocyst	32	19.5	8	11.4	7	10	6	8.5	5	7.1	4	5.7	2	2.8
B. hominis cyst	16	9.7	1	1.4	3	4.2	2	2.8	3	4.2	3	4.2	4	5.7
G. lamblia cyst	53	32.3	12	17.1	11	15.7	10	14.2	8	11.4	7	10	5	7.1
B. coli cyst	4	2.4	0	0	0	0	0	0	0	0	0	0	4	5.7
P-value		<0.001**		0.11		0.24		0.56		0.38		0.5		

No: total examined samples. %: percentage from total examined for each type of vegetables and fruits.

Table (3): Percentage reduction in viability of some parasites after exposure to acetic acid (5%) and potassium permanganate (KMno4) 24mg/L for 15 and 30 minutes**.**

Parasite	K permanganate Loss of viability		P1	Acetic acid Loss of viability		P1	P2	P3
	15 min (%)	30 min (%)		15 min (%)	30 min (%)			
Enterobius egg	16%	18%	0.68	20%	23%	0.6	0.4	0.38
H. nana egg	10%	12%	0.65	12%	10%	0.65	0.6	0.6
Ascaris egg	9%	12%	0.47	15%	18%	0.56	0.19	0.23
Helminths larvae	3%	6%	0.49	9%	12%	0.48	0.07	0.13
E. histolytica cyst	21%	25%	0.5	23%	27%	0.51	0.7	0.7
Cryptosporoidium spp. oocyst	19%	21%	0.72	25%	28%	0.6	0.3	0.24
B. hominis cyst	22%	25%	0.61	30%	32%	0.7	0.19	0.27
G.lamblia cyst	22%	26%	0.5	28%	31%	0.64	0.3	0.4
B. coli cyst	21%	25%	0.5	25%	28%	0.63	0.5	0.63

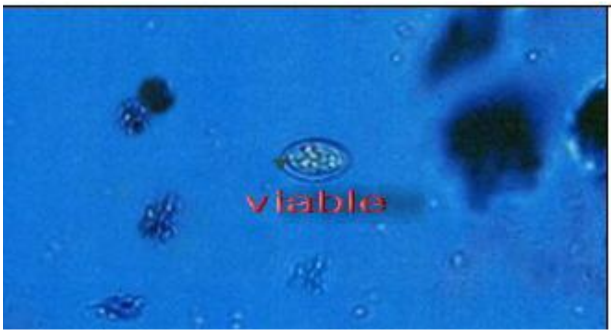

Figure (1): Viable *G. lamblia* cyst as detected by trypan blue 0.2% stain and appeared clear with light blue colour (X40).

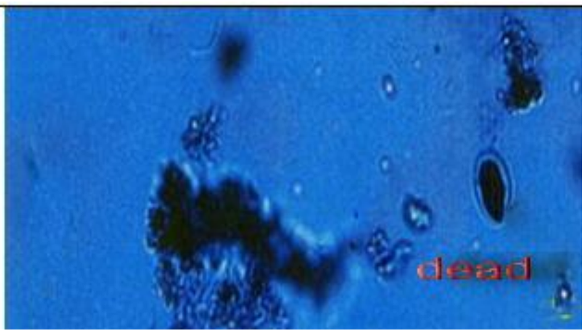

Figure (2): Non viable *G. lamblia* cysts as detected by trypan blue 0.2% stain and appeared dark blue and their structure could not be detected (X40).

The morphological changes of *Hymenolepis nana* egg due to exposure to potassium permanganate 24mg/L.

Figure (3): Dipping in the egg shell of *H. nana* egg after exposure to potassium permanganate 24mg/L for 15 minutes (X40).

Figure (4): Marked damage in the egg shell of *H. nana* egg after exposure to potassium permanganate 24mg/L for 30 minutes (X40).

DISCUSSION

Vegetables and fruits are one of the most important sources of nourishment. But, they may be contaminated by intestinal parasites. They affect about 3.5 billion people **[16].** In less developed countries, there is a facilitation of transmission of parasitic infections to vegetables and fruits during cultivation by sewage contaminated irrigation water and untreated organic fertilizers carrying fecal helminths eggs and larvae. Also, the post-harvest handling, washing, transfer and storage contribute in their contamination **[17].** Consumption of the raw vegetables especially salads and fruits is almost daily in Egypt. Thus, it has been found that, there are high incidences of intestinal parasitic infections in raw vegetables consumed communities **[18].** Sharkyia governorate is mainly an agriculture community with large areas of agricultural fields where people mainly depend upon fresh raw vegetables in their food. Therefore, this study highlighted the most common types of vegetables and fruits which may transmit parasitic infection.

The current study showed a considerable high level of parasitic contamination of examined vegetables and fruits (39%) (Table 1). This result was nearly in agreement with Said **[19]** who reported that the rate of the parasitic contamination of vegetables was 31.7% in Alexandria, Egypt. In Nigeria and Ghana, Parasitic contamination of the vegetables was 36% **[20,21].** Another study recorded slightly lower levels of parasitic contamination as in Benha city, Egypt which reported a prevalence rate (29.6%) **[22].** Also, Abe et al. **[23]** showed that 37.5% of the studied samples of fruits and vegetables in Lafia's markets, Nigeria had some geohelminths contamination. Another study recorded slightly lower levels of parasitic contamination as in Ardabil city, Iran where a prevalence of 29% was recorded upon the contamination of garden vegetables with intestinal parasites **[24].** Also, Hassan et al. **[25]** reported 19.4% contamination in Alexandria, Egypt. Another study in Riyad, Saudi Arabia had a much lower level of vegetable contamination of parasitic infection (16.2%) **[26]**. But, the contamination of green vegetables was reported in a higher level in retail markets in Tripoli, Libya (58%) **[10]**. In Nigeria, 55% of the examined samples of vegetables were positive for different species of parasites **[27].** It was found that, the rate of parasitic contamination of collected samples of fruits and vegetables from local markets in Arab Minch town, Southern Ethiopia was 54.4% **[28].** Also, Nyarango **[29]** revealed (75.9%) contamination of the examined vegetables in Kenya. These findings 'differences are mainly due to the difference of the used techniques of investigations, the soil's type, type of water used for irrigation, climatic conditions, geographical locations and sanitary habits **[30].**

Table (1) showed the prevalence rate of parasitic contamination of the examined vegetables and fruits, where watercress samples were found to have the highest parasitic contamination (55.7%) followed by lettuce samples (45.7%), parsley (44.2%), cucumber (31.4%) and strawberry (30%). While carrots were found to be the least contaminated ones (27%). This variance of contamination is due to the variance in the shape and the surface area of the used vegetables and fruits. Lettuce, parsley and watercress have rough surfaces so that parasitic eggs, cysts and oocysts can attach easily to the surface of these vegetables. The pits on the surface of the strawberry are considered the chief factor leading to its contamination by protozoan stages. On the other hand, vegetables with smooth surface had less contamination because its smooth surface reduces the rate of parasitic attachment **[21].** These results were partially in accordance with a study in Khartoum state, Sudan, as lettuce recorded the highest level of contamination in fresh vegetable samples (36.4%) but cucumber wasn't contaminated **[31].** Similar results were reported in a study performed in Riyad, Saudi Arabia, as lettuce recorded with the highest rate of contamination (27.8%) followed by watercress (22.8%) **[26].** In Tripoli, Libya, watercress and lettuce samples were found to be contaminated more than other samples recording 100% and 96% respectively **[10]**. In Benha city, it was found that; lettuce had the highest parasitic contamination (45.5%), followed by watercress (41.3%) **[22].** Also, there was a high rate of lettuce contamination (40%) in Nigeria **[21].** In contrast, lettuce and watercress reported with lower rate of contamination (17%) than green onions (28%) in a study performed in Saudi Arabia 2006 **[32].**

Table (2) in the present study showed that *Giardia lamblia* cysts were the highest prevalent parasitic stage detected in the samples of raw vegetables and fruits (12.6%) followed by *Cryptosporidium spp.* oocysts (7.6%), *Entamoeba spp.* cysts (6.2%), *Blastocystis hominis* cysts (3.8%), *Hymenolepis nana* eggs (2.8%), *Ascaris lumbricoides* eggs (1.9%), different helminths larvae (1.6%), *Enterobius vermicularis* eggs (1.4%)

and finally *Balantidium coli* cysts were the least detected (0.9%).

In the present work, *G. lamblia* was the most prevalent parasite contaminating fruits and vegetables (12.6%), strawberry had a percentage (7.1%); lettuce had the highest percentage (17.1%). These findings are particularly like previous reports in Egypt, including the studies conducted in Alexandria, by Hassan et al. **[25]** who reported a prevalence rate of 8.8%. The prevalence of *G.lamblia* cysts was 23% of the examined samples of salad vegetables in Amman and Baqa'a in Jordan **[33].** Another study in Libya reported that *G.lamblia* cysts were found in 10% of the total examined vegetable samples **[10].** *G.lamblia* parasite was the third parasite that was detected in 1.6% of the samples in a study performed by Matini et al. **[30]**. On the other hand, *Giardia* cysts were detected in a higher rate in green vegetables in Riyad, Saudi Arabia **[26].** This high prevalence of *G. lamblia* in our study may be attributed to the long periods of survival *of Giardia* under cool and moist conditions and due to its resistance to the used chlorine in drinking water **[34].**

Cryptosporidium was the second prevalent parasite causing contamination of the examined samples (7.6%). *Cryptosporidium* was found in 4% of samples of fresh vegetables in Norway **[35].** This widespread of *Cryptosporidium* is mainly associated with the used water for irrigation and the use of the human excreta as manure. These findings agree with the study performed in Alexandria which detected a high rate of contamination of irrigation water in El Mahmoudeya canal by *Cryptosporidium* oocysts **[36]**.

The third prevalent parasite detected in this study was *Entamoeba spp*. cysts (6.2%). These results agree partially with a study in Shahrekord, Iran as cysts of *Entamoeba spp.* were detected in 9.2% of vegetable samples **[18].** In Sudan, it was found that *E. histolytica* was the most predominant parasite in fresh vegetables samples (42.9%) **[31].** *E. coli* was the most abundant parasite (2.6%) found in the survey done by Matini et al. **[30].** Much higher rates of the prevalence of *E. coli* cysts (11.2%) were recorded by Hassan *et al.* (2012) from a study in Alexandria, Egypt **[25]**. In Gaza, Palestine, the prevalence rate of vegetable samples contamination was 37.5% for *E. histolytica* **[37].** On the other hand, less rate of the prevalence of *E. histolytica* (0.6%) was recorded in Philippines **[38].** Detection of *E. histolytica* indicates the possibility of the contamination of the vegetables and fruits by human feces since the organism only lives in the human intestine **[18].**

H. nana eggs were detected in 2.8% of the examined samples. This level was like that recorded by Said (2012) as the prevalence of *H. nana* was 2.6% of vegetables samples in Alexandria, Egypt **[19].** *H. nana* eggs were 2.4% of vegetable samples in a previous study in Libya **[10],** 5% in another study in Zahedan, Iran **[39].** On contrast, other studies showed higher findings as in Riyad, Saudi Arabia in which *H. nana* eggs were found to contaminate 14.5% of vegetables samples **[26].** In Arba Minch town, Thousern Ethiopia, *H. nana* eggs were detected in 15.56% of examined vegetables samples **[28].** Lower rate was detected in Qazvin, Iran, as *H. nana* eggs were detected in 0.5% only from the parasite contaminated green vegetables **[40].** The observed difference might be due to difference in the climatic conditions and geographical location **[41].**

In the present study, *A. lumbricoides* eggs were detected in (1.9%) of the examined samples. Similar rate of contamination of vegetables samples with *A. lumbricoides* eggs was recorded (1.8%) of total examined samples in Turkey **[42].** Also, the rates of contamination with *Ascaris* eggs in vegetables were detected in Iran, as it was found to be 2.5% in Jiruft **[43],** 2.3% in Kazvin **[40].** Our results disagree with other studies that showed higher findings as in Alexandria governorate where *Ascaris* eggs were detected in 20.3% of the examined samples **[19].** The occurrence of *A. lumbricoides* (56.31%) among the examined fruits and vegetables was recorded in a study performed in rural areas of Zamfara States, Nigeria **[44].** A higher rate of contamination (68%) of fresh vegetables used in making the salad in Tripoli, Libya was recorded **[10].** A very high percentage of *A. lumbricoides* (89.33%) was recorded by Abe et al. **[23]**. The presence of *Ascaris* eggs in the vegetables is attributed to the usage of untreated night soil. This parasite's ubiquitous distribution could be attributed to the resistant nature of the eggs that enables them to survive under unfavorable conditions being unaffected by desiccation for several weeks.

In this study, helminths larvae were detected in 1.6% of the examined samples**.** These findings disagree with the higher rates of helminths larvae which were recorded in imported vegetable 6% and in native vegetable 7% in Tabriz, Iran **[45]**. On the other hand, Ezaptour et al. **[46]** reported that helminths larvae were detected in (40%) of total vegetable samples in a study performed in Khorramabad, Iran. The increase in the prevalence of helminths larvae in some regions is due to subsequent silting of local rivers causing deposition of sandy loam top soils and increased soil moisture that might promote the emergence of these larvae **[47].**

In this study, *E. vermicularis* eggs were detected in 1.4% of vegetables samples. Nearly similar results were reported in Nigeria as the contamination rate of vegetables by *Enterobius spp*. was 0.8% **[48].** In Turkey, *Enterobius spp.* rate among the examined vegetables was 0.9% **[42].** On the other hand, higher levels of *E. vermicularis* (4.5%) were detected in a study conducted on vegetables in Philippines **[38].** Al-Binali et al. **[32]** recorded a rate (6.3%) of *E. vermicularis* eggs of vegetables samples in South Western Saudi Arabia. In Iran, *E. vermicularis* eggs were detected in 5.1% of the examined vegetables samples in Khorramabad **[46],** 8.1% in Zahedan **[39].** The predominance of *E. vermicularis* is associated with the socio-economic and environmental conditions and the bad hygienic practice of the vegetables handlers **[42].**

Table (2) showed the distribution of each parasitic contamination in various examined raw vegetables and fruits, where *G. lamblia* cysts were the most prevalent on lettuce samples (17.1%) followed by watercress (15.7%), parsley (14.2%), cucumber (11.4%), carrots (10%) and finally strawberry (7.1%). These results agree with a study conducted in Amman, Gordan **[33]** where lettuce samples were being the most contaminated (63%). In contrast, it was found in Libya that only 4% of lettuce was contaminated by *G. lamblia* cysts **[10].**

The results showed that *Cryptosporidium spp.* were most prevalent upon lettuce samples (11.4%) followed by watercress (10%), parsley (8.5%), cucumber (7.1%), carrots (5.7%) and finally strawberry (2.8%). These results disagree with a study performed by Said **[19]** who recorded that *Cryptosporidium spp.* oocysts were found in 2.5% of lettuce samples.

The results showed that *E. histolytica cysts* were most prevalent upon cucumber and strawberry samples (8.5%) followed by carrots (7.1%), watercress (5.7%), parsley (4.2%) and finally lettuce (2.8%).

As regards *H. nana* eggs, watercress samples were the most contaminated (7.1%), followed by parsley (5.7%) and lettuce (4.2%). But cucumber, carrots and strawberry samples were not contaminated**.** In our study, *A. lumbricoides* eggs were higher in watercress (5.7%) followed by parsley (4.2%) and finally lettuce (1.4%)**.** These results agree with a study performed in Tripoli, Libya which recorded that *Ascaris* eggs were detected in a high percentage in watercress [**10].**

This study showed that watercress was the highest contaminated vegetables by *E. vermicularis* eggs (4.2%), followed by parsley (2.8%) and then lettuce (1.4%). As regards *B. coli* cysts, strawberry is the only one of the examined samples which was contaminated by it (5.7%). This result may be due to the close contact of the mammillated fruits with soil.

Comparing these results with a previous study in Alexandria, Egypt, records were found as follows: watercress (46.7%), lettuce (45%) and parsley (36.7%) while leek (16.7%) and green onion (13.3%) **[19].** another study in Tripoli, Libya, revealed that watercress had the highest rate of contamination (100%) followed by lettuce (96%) while tomato had the least rate of contamination **[10].** In Benha, Egypt, lettuce (45.5%), watercress (41.3%), parsley (34.3%), green onion (16.5%) and leek (10.2%) **[22].** Also, in Riyad, Saudi Arabia, the highest rate of contamination was detected in 27.8% of lettuce, followed by watercress (22.8%) and parsley (17.4%) **[32].** these differences in the percentage among different kinds of vegetables are attributed to the nature of the building of the vegetables and fruits. As the smooth surface reduce the parasitic attachment **[49].**

Table (3) showed the comparison between the effects of two common disinfectants for vegetables as acetic acid (5%) and potassium permanganate (24mg/L) upon the viability of the parasites after exposure for 15 and 30 minutes. As regards *E. histolytica* cysts, the percentage of loss of viability was 21% after 15 minutes exposure to potassium permanganate, and then it reached 25% after 30 minutes exposure. Upon exposure to acetic acid 5%, the reduction in viability reached 23% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 27%.

Table (3) showed that the percentage of loss of viability of *G. lamblia* changed from 22% after 15 minutes to 26% after 30 minutes when exposed to potassium permanganate. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 28% to 31% respectively. This difference was statistically insignificant. A study on the giardiacidal effect of lemon juice and vinegar on *G. lamblia* cysts was done by Sadjjadi et al. **[50]** revealed that the giardiacidal activity at 4°C after 3 hours for lemon juice and vinegar was 18.9% and 28.4% and at 24°C 28.3% and 40.6% respectively.

As regards *Cryptosporidium spp.* oocysts, the percentage of loss of viability was 19% after 15 minutes exposure to potassium permanganate, and then it reached 21% after 30 minutes exposure. Upon exposure to acetic acid (5%), the reduction in viability reached 25% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 28%. But in the last protozoal one (*B. coli)*, the percentage of loss of viability was 21% when exposed to potassium permanganate for 15 minutes and became 25% when extended to 30 minutes of exposure. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 25% to 28% respectively.

The explanation of the effect of the used disinfectants upon the different protozoa stages in this study may be that the acidic disinfectants act by breaking the nucleic acids and precipitating proteins bonds. Also, it may work by changing the pH of the environment making it unsuitable to these parasites **[51].** Abuladze *et al.* (2009) explained in his study how the exposure of the protozoa to potassium permanganate to the oxidation of the cell membrane phospholipids which led to membrane dysfunction and cell death **[8].**

According to this study, the percentage of loss of viability in *E. vermicularis* eggs changed from 16% after 15 minutes to 18% after 30 minutes when exposed to potassium permanganate. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 20% to 23% respectively.

H. nana eggs, the percentage of loss of viability was 10% after 15 minutes exposure to potassium permanganate, then it reached 12% after 30 minutes exposure. Upon exposure to acetic acid (5%), the reduction in viability reached 12% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 10%.

About the helminths larvae, the percentage of loss of viability was doubled from 3% after 15 minutes to 6% after 30 minutes when exposed to potassium permanganate. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 9% to 12% respectively.

In the case of *Ascaris* eggs, the percentage of loss of viability was 9% after 15 minutes exposure to potassium permanganate, and then it reached 12% after 30 minutes exposure. Upon exposure to acetic acid (5%), the reduction in viability reached 20% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 23%.

Considering these results, it was found that the effect of both potassium permanganate and acetic acid upon different helminths stages was less than upon protozoa stages. This may be attributed to the nature of the structure of helminths eggs **[52].** In general, *H.nana* eggs are very resistant to chemicals such as acetic acid and kept viable for several months and this is due to having an onchosphere covered by three basic layers which form the egg shell making an obstacle to the chemicals to reach the infective stage **[53].** *E. vermicularis* eggs have five membranes: one inner, lipoid layer, three middle layers called membrane Lucida and an outer, albuminous membrane which coats the egg. This membrane makes the eggs sticky leading to increase in the resistance of the eggs to disinfection **[54].**

Our study reported that, the percentage of the loss of viability of most of the examined parasites by using acetic acid (5%) is more than that by using potassium permanganate (24mg/L), however this difference was found to be insignificant. This effect appeared under the light microscope different magnifications by some morphological changes in the form of deformity, damage of the egg shell and shrinkage of the cyst wall. After exposure of raw vegetables and fruits in this study (lettuce, parsley, watercress, cucumber, carrots and strawberry) to both acetic acid (5%) and potassium permanganate (24 mg/L) for 15 and 30 minutes, they showed nearly normal taste, smell, consistency without color changes or softening in the vegetable leaves.

CONCLUSION

The results obtained from this research showed high contamination levels of raw vegetables and fruits with different helminths and protozoa stages. Thus, the consumption of raw vegetables and fruits may be one of the sources of parasitic infections among the public. The usage of certain types of disinfectants as potassium permanganate (24mg/L) and acetic acid (5%) is one of the effective ways of reducing fruits and vegetables borne parasitic infections. This study calls for the need of strict hygienic measures in the areas where the vegetables and fruits are cultivated, sold, prepared and consumed. This is properly achieved by treatment of soil, manure and water used for cultivation of vegetables and fruits and disinfecting them before consumption. In addition, the surveys must be done in different governorates in Egypt to evaluate the levels of parasitic contamination in raw vegetables and fruits in both rural and urban areas.

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