ZANCO Journal of Pure and Applied Sciences

TYPE Original Research
DOI http://dx.doi.org/10.21271/ZJPAS.36.4.2

OPEN ACCESS

*Corresponding author

Chreska Nooraldin Ahmed
chreska.ahmed@su.edu.krd

RECEIVED :10/10/2023
ACCEPTED :03/07/2024
PUBLISHED :31/08/2024

KEYWORDS:

Parasites, Fruits and vegetables, Erbil markets,
Iraq.

Detection of Parasites in Locally Sourced Fresh Fruits and Vegetables Using Various Diagnostic Techniques

Chreska N. Ahmed[1*], Qaraman M.K. Koyee [1], Lana O. Kanabe[2], Abbas M. Faraj[3], Zohair I.F. Rahemo[4]

1Department of Biology, College of Science, Salahaddin University-Erbil, Erbil, Kurdistan Region, Iraq
2General Education Directorate, Ministry of Education, Erbil, Kurdistan Region, Iraq
3College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq
4Department of Biology, College of Science, University of Mosul, Mosul, Iraq

ABSTRACT

A prospective study was conducted from September 2022 to March 2023 to examine the occurrence and contamination rates of different parasitic stages on fresh edible fruits and vegetables in local markets of Erbil city. Eight different types of fruits and vegetables were selected from eight markets, and 144 samples were analyzed for parasites using direct sedimentation, indirect floatation ($ZnSO_4$), Kinyoun, and trichrome staining methods. The highest total frequencies of parasites were detected using the direct sedimentation method (18.75%), followed by the indirect floatation method (6.25%), Kinyoun technique (5.55%), and modified trichrome staining (4.16%). *A. lumbricoides* (Ova) was detected with 4.16% using direct sedimentation, while no positive detections were observed with other techniques. Kinyoun technique identified *Cryptosporidium* spp. oocysts. For *E. histolytica* 1.3% were positive using the direct sedimentation method, while the other three methods showed no positive detections. Similar results were observed for *G. lamblia* where only the modified trichrome staining technique detected 0.69% with trophozoites and 1.3% with cysts. Additionally, the direct sedimentation method revealed the presence of nematodes at 2.77%, but no positive detections were made with other methods. Concerning Taeniid eggs, the indirect floatation identified 0.69% with ova, while other methods showed no positive detections. Lastly, the direct sedimentation method yielded 6.25% with unidentified flagellate protozoa, but no positive detections were found using the indirect floatation method, Kinyoun technique, or modified trichrome staining technique. The study concluded that Kinyoun and trichrome techniques are the gold standard for detecting different parasite stages.

1.Introduction

Fresh fruits and vegetables can be a source of gastrointestinal parasite transmission. Contaminations could occur in the field during manuring or fertilizing, harvesting, transportation, processing, distribution, and marketing, as well as in the house. Furthermore, during postharvest handling, such as at places of preparation by street vendors, food service facilities, and, in most cases, irrigation water, in addition to dust dispersal during wind blow. Intestinal parasites in certain districts infect millions of populations worldwide. The vegetables and fruit contamination by parasites have been established with different groups of parasites (Protozoa, Nematodes, Cestodes, and Trematodes) (Fallah et al., 2012; Ola-Fadunsin et al., 2022).

Minerals such as iron, vitamin C, vitamin B_{12}, nicotinic acid, and vitamin B_2 are abundant in vegetables and fruits. Consumption of raw or inadequately prepared vegetables is recognized as a risk factor for parasite infections in humans (Yandev et al., 2019). Various foodborne pathogens may be present in raw fruits and vegetables cultivated close to the soil. Food supply globalization, pathogen introduction into new geographical areas through import, use of wastewater and manure as fertilizers in agricultural fields as crop production, irrigation, various agronomic practices, level of hygiene of food handlers, and other factors all contribute to an increase in diseases related to raw vegetables and fruits. The constant use of untreated wastewater and manure as fertilizer in the yielding of vegetables and fruits is a significant contributing factor in contamination that causes numerous food-borne infection outbreaks (Berhanu et al., 2022).

Water sources (rain and irrigation during wet and dry seasons respectively) are highly contaminated with human and animal faces, posing a significant risk to producers and consumers of fruit and vegetable goods. Fruit markets are frequently contaminated with eggs of human intestinal nematodes, and human and animal faces are widely utilized as fertilizers and reused wastewater (Alemu et al., 2020; Mogaji et al., 2021). This practice is growing in prominence in Iraq due to the mineral fertilizers cost and the high demand for basic vegetables and fruits as a nutrient diet due to poor socio-economic conditions. Consumption of vegetables and fruits traditionally in Iraq either raw or cooked, in addition to their usage as a great vitamin and mineral source. Vegetables are eaten extensively in various regions of Iraq, but still, Iraqi populations do not know how to wash them carefully. A few studies have been done on parasites associated with fruits and vegetables in Iraq (Al-Mukhtar and Al-Dabbagh, 1991).

Epidemiological studies reveal that food cross-contamination during preparation significantly contributes to the prevalence of foodborne infections (Gilling et al., 2001; El Safadi et al., 2023). As far as we know, there has been little research on parasite contamination of vegetables and fruits in our region. Thus, this study aims to assess the parasitic contamination rate of fruits and vegetables and associated factors in selected local markets of Erbil city where they are commonly sold in open markets and streets, and this will enable proffering solutions to the consumers in Erbil city, as well as it was done to know the possible role of them in the transmission of human parasitic diseases.

2. Material and Methods

2.1 Study Area and Subjects:

The study was carried out from the beginning of September 2022 to the end of March 2023. Samples of fruits and vegetables were taken from Erbil's main marketplaces. The fruits and vegetables used in the study were apples, oranges, bananas, celery, lettuce, cress, rocca, and chard. The fruits and vegetables were picked from the major markets in Erbil city (Kasnazan, Zeelan, Shawes, Rapareen, Kwran, 100 Metry Street, Saidawa, and Manitikawa).

2.2 Sample Collection:

A total of 144 samples of fruits and vegetables were picked up to obtain a quantitative estimation of parasitic contamination of these fruits (Apple, Orange, and Banana) and vegetables (Celery, Chard, Cress, Lettuce, and Rocca), which they classified scientifically according to Quattrocchi (2017) as shown in

Table 1.
Table 1: Types of vegetables and fruits used in the present study

No.	Type of sample	Common name	Scientific name	No. of examined samples	Examined parts	Weight of each sample (gm)
1.	Vegetable	Celery	*Apium graveolens*	22	Leaves	200 gm
2.	Vegetable	Chard	*Beta vulgaris*	17	Leaves	200 gm
3.	Vegetable	Cress	*Lepidium sativum*	25	Leaves	200 gm
4.	Vegetable	Lettuce	*Lactuca sativa*	19	Leaves	200 gm
5.	Vegetable	Rocca	*Eruca sativa*	20	Leaves	200 gm
6.	Fruit	Apple	*Pyrus malus*	17	Fruit	200 gm
7.	Fruit	Banana	*Musa spp.*	12	Fruit	200 gm
8.	Fruit	Orange	*Citrus sinensis*	12	Fruit	200 gm
	Total			144		

2.3 Detection of parasites

2.3.1 Direct Sedimentation Method:

Fruit and vegetable samples were separately washed in distilled water and/or normal physiologic saline within a plastic jar for the presence of parasite eggs, larvae, trophozoites, or cysts. The suspension was mixed and centrifuged at 5000 rpm for about 5 minutes and the supernatant was removed into the sterilized container. The sediment was remixed and a drop of it was added to the clean slide, a clean cover slip was placed carefully to avoid air bubbles and over-flooding. The prepared slides were examined under a microscope for detecting parasite cysts, eggs, or larval stages using 10X and 40X objective lenses (Nyarango et al., 2008; Beyene, 2019).

2.3.2 Indirect Floatation Method:

Similarly in the sedimentation method, samples of each fruit and vegetable were prepared by washing them in distilled water and/or normal physiologic saline in a plastic jar for the presence of eggs, larvae, cysts, or trophozoites of parasites. The mixed suspension was centrifuged at 5000 rpm for about 5 minutes and the supernatant was removed into the disinfectant container. The sediment separated and re-suspended in 33% Zinc-Sulphate solution (floatation fluid) and re-centrifuged then the floatation fluid was added to fill to the rim of the tube and a cover slip was picked up and put on a clean slide and examined under a light microscope using 10X and 40X objective lenses (Nyarango et al., 2008; Beyene, 2019).

2.3.3 Kinyoun Technique

In this instance, 3 ml of each sample of sterile, distilled water that was recovered during the washing procedure was centrifuged at 2500 rpm (700 g) for 10 min. The silt was spread on a slide and allowed to dry before the smear was fixed with methanol. Carbol-fuchsin staining was applied to each smear for two minutes. The smears were first decolored with sulfuric acid (10%), and then malachite green was used to recolor them. For microscopic sporozoan observation with the 100X objective, the colored smears were dried and immersion oil was applied (Garcia et al., 2018). The Kinyoun method has demonstrated effective performance in identifying *Cryptosporidium* spp. and *Cyclospora* spp. Oocysts (Alemu et al., 2020).

2.3.4 Trichrome Staining Method

As part of this procedure, following washing, 3 ml of each sample of sterile distilled water was centrifuged for 10 min at 2500 rpm (700g). A thin smear of the sediment was applied, air dried, and then fixed for 3 min with methanol. The slides were stained with trichrome stain for detection of protozoan parasites. Slides were then examined microscopically with an oil immersion lens (100X). The microscopic examination was done 3 times on each slide to ensure accuracy. Depending on their morphology, parasitic stages were identified according to El-Sayed et al. (2023).

2.4 Statistical Analysis

Both Graph-Pad Prizm (version 9) and SPSS (version 23) were used to examine the data. To describe market characteristics and the level of contamination of fruits and vegetables, descriptive statistics like frequency and percentage were determined. Significant associations were those with p-values <0.05 at 95% confidence intervals.

3. Results

The results are summarized in Table (2), which presents a comparison of the rate of parasite contamination in fruits obtained from different markets (Kwran, Saedawa, and 100 metry) in Erbil City using 4 diagnosing techniques: Modified trichrome staining, Kinyoun technique, indirect floatation method ($ZnSO_4$) and direct sedimentation. The contamination rates are expressed as percentages and represent the presence of parasitic contaminants in the examined fruits. in which only one kind of fruit sample (apple) was positive for parasites by direct sedimentation method from both replications in the same market (100 metry market) which was the ova of Ascaris lumbricoides with a percentage of 14.63%. In contrast, there were no positive cases for the other fruit samples and markets (Kwran and Saedawa) for all methods (0.0%).

Table 2: Comparison of Parasite Contamination Rates in Fruits from Different Markets Using Various Detection Techniques

Market	Fruit Type (examined No.)	Direct Sedimentation Method Rate of contamination (%)	Indirect Floatation Method ($ZnSO_4$) Rate of contamination%	Kinyoun Technique	Modified Trichrome Staining Technique	
					Before Cent.	After Cent.
Kwran Market	Apple (6)	0.0	0.0	0.0	0.0	0.0
	Banana (4)	0.0	0.0	0.0	0.0	0.0
	Orange (4)	0.0	0.0	0.0	0.0	0.0
Saedawa Market	Apple (5)	0.0	0.0	0.0	0.0	0.0
	Banana (4)	0.0	0.0	0.0	0.0	0.0
	Orange (4)	0.0	0.0	0.0	0.0	0.0
100 Metry Market	Apple (6)	*A. lumbricoides* (16.7%)	0.0	0.0	0.0	0.0
	Banana (4)	0.0	0.0	0.0	0.0	0.0
	Orange (4)	0.0	0.0	0.0	0.0	0.0
Total	41	14.63 %	0.0 %	0.0	0.0	0.0

In the present study, it was investigated the frequencies of isolation of various parasites from a total of 144 samples collected from different places in Erbil City (Kasnazan, Shawes, Zeelan, Mantikawa, and Raparin markets), using four different detection methods. The results revealed varying detection rates for different parasites with each method (Table 3). The parasite profile of each fruit and vegetable sample evaluated in different marketplaces have been compared using four distinct approaches, namely sedimentation, flotation using zinc-sulphate ($ZnSO_4$), trichrome staining, and Kinyoun techniques represented in Table 4. Among the parasites examined, ova of *A. lumbricoides* was detected in 6 samples (4.16%) using the direct sedimentation method, while no positive detections were observed with the indirect floatation method ($ZnSO_4$), Kinyoun technique, or modified trichrome staining technique. *Cryptosporidium* spp. (Oocyst) was not detected in any of the samples through the direct sedimentation method or indirect floatation Method ($ZnSO_4$).

However, the Kinyoun technique identified *Cryptosporidium* spp. oocysts in 8 samples (5.55%), and the modified trichrome staining technique detected them in 4 samples (2.77%).

Table 3: Number of Vegetable Samples contaminated with Intestinal Parasites in Each Market by using different techniques.

Market	Vegetable Type	Direct Sedimentation Method	Indirect Floatation Method ($ZnSO_4$)	Kinyoun Technique (%)	Modified Trichrome Staining Technique	
					Before Cent.	After Cent.
Kasnazan Market	Celery (3)	Flagellated Protozoa (33.4%)	Larvae of Nematodes (66.7%) Leg of insects (33.3%)	*Cryptosporidium* spp. (33.4%)	0.0	*Cryptosporidium* spp. (66.7%) *Alternaria* sp. (33.3%)
	Chard (3)	0.0	Leg of insect (33.3%)	0.0	0.0	0.0
	Cress (3)	0.0	**0.0**	*Cryptosporidium* spp. (33.4%)	*Cryptosporidium* spp. (66.7%)	0.0
	Lettuce (4)	Ova of *A. lumbricoides* (50%)	**0.0**	0.0	0.0	0.0
	Rocca (4)	0.0	0.0	0.0	0.0	0.0
Shawes Market	Celery (3)	0.0	0.0	0.0	0.0	0.0
	Chart (3)	Ova of *A. lumbricoides* (100%)	0.0	0.0	0.0	0.0
	Cress (3)	Flagellated Protozoa (33.4%)	0.0	0.0	0.0	0.0
	Lettuce (4)	Larvae of Nematodes (50%)	Larvae of Nematodes (50%)	0.0	0.0	0.0
	Rocca (4)	0.0	0.0	0.0	0.0	0.0
Mantikawa Markets	Celery (10)	Female of Nematodes (20%) flagellated protozoa (40%)	Larvae of Nematodes (25%)	*Cryptosporidium spp.* (20%)	0.0	*Alternaria* sp. (30%) *Cryptosporidium* spp. (10%)
	Chart (4)	Flagellated Protozoa (25%)	0.0	0.0	0.0	0.0
	Cress (10)	*Alternaria* spp. (30%)	Egg of *Taeniid spp.* (10%)	*Cryptosporidium* spp. (20%)	*Cryptosporidium* spp. (10%) And cyst of *E. histolytica* (10%)	Trophozoite of *Giardia* spp. (10%)
	Lettuce (4)	Male of Nematodes (25%) Flagellated Protozoa (25%)	Larvae of Nematodes (25%)	0.0	0.0	0.0
	Rocca (4)	0.0	0.0	0.0	0.0	0.0
Zeelan Market	Celery (3)	0.0	0.0			
	Chard (3)	Larvae of Nematodes (66.7%)	0.0	0.0	0.0	0.0
	Cress (4)	0.0	0.0	*Cryptosporidium* spp. (25%)	0.0	*Alternaria* spp. (50%)
	Lettuce (3)	0.0	0.0	0.0	0.0	0.0
	Rocca (4)	0.0	0.0	0.0	0.0	0.0
Raparin Market	Celery (3)	Adult Nematodes (33.4%)	Leg of insects (33.4%)	0.0	0.0	0.0
	Chart (4)	Flagellated Protozoa (25%)	0.0	0.0	0.0	0.0
	Cress (5)	0.0	**0.0**	*Cryptosporidium* spp. (20%)	0.0	0.0
	Lettuce (4)	Cysts of *E. histolytica* (50%) *G. lamblia* (50%)	Cysts of *G. lamblia* (50%)	0.0	0.0	0.0
	Rocca (4)	0.0	0.0	0.0	0.0	0.0
Total	103	26 (25.24%)				

For *E. histolytica* (Cyst), 2 samples (1.3%) were positive using the direct sedimentation method, while the other three methods showed no positive detections. Similar results were observed for *G. lamblia* (trophozoite and cyst), where only the modified trichrome staining technique detected one sample (0.69%) with trophozoites and 2 samples (1.3%) with cysts, respectively. Additionally, the direct sedimentation method revealed the presence of nematodes (adult stage)

Table 4: Frequency and prevalence of isolated parasites in fruit and vegetable samples with four different methods

Parasites	Frequency of isolation from 144 samples			
	Direct Sedimentation Method +No.* (%)	Indirect Floatation Method ($ZnSO_4$) +No. (%)	Kinyoun Technique +No. (%)	Modified Trichrome staining Technique +No. (%)
***A. lumbricoides* (Ova)**	6 (4.16%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
***Cryptosporidium spp*. (Oocyst)**	0 (0.0%)	0 (0.0%)	8 (5.55%)	4 (2.77%)
***E. histolytica* (Cyst)**	2 (1.3%)	0 (0.0%)	0 (0.0%)	1 (0.69%)
***G. lamblia* (Trophozoite)**	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.69%)
***G. lamblia* (Cyst)**	2 (1.3%)	2 (1.3%)	0 (0.0%)	0 (0.0%)
Nematodes (Adult stage)	4 (2.77%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Nematodes (Larva Stage)	4 (2.77%)	6 (4.16%)	0 (0.0%)	0 (0.0%)
Taeniid eggs (Ova)	0 (0.0%)	1 (0.69%)	0 (0.0%)	0 (0.0%)
Unidentified Flagellate protozoa	9 (6.25%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	27 (18.75%)	9 (6.25%)	8 (5.55%)	6 (4.16%)

in 4 samples (2.77%). For *E. histolytica* (cyst), 2 samples (1.3%) were positive using the direct sedimentation method, while the other three methods showed no positive detections. Similar results were observed for *G. lamblia* (trophozoite and cyst), where only the modified trichrome staining technique detected one sample (0.69%) with trophozoites and 2 samples (1.3%) with cysts, respectively. Additionally, the direct sedimentation method revealed the presence of nematodes (adult stage) in 4 samples (2.77%), but no positive detections were made with the other methods. Nematodes in the larval stage were found in 4 samples (2.77%) using the direct sedimentation method and 6 samples (4.16%) using the indirect floatation method ($ZnSO_4$), while no positive detections were recorded with the Kinyoun technique or modified trichrome staining technique. Regarding Taeniid eggs, the indirect floatation method ($ZnSO_4$) identified one sample (0.69%) with ova, while the other methods showed no positive detections. Lastly, the direct sedimentation method yielded 9 samples (6.25%) with unidentified flagellate protozoa, but no positive detections were found using the indirect floatation method ($ZnSO_4$), Kinyoun technique, or modified trichrome staining technique.

Overall, the highest total frequencies of parasites were detected using the direct sedimentation method (27 samples, 18.75%), followed by the indirect floatation method ($ZnSO_4$) (9 samples, 6.25%), the Kinyoun technique (8 samples, 5.55%), and the modified trichrome staining technique (6 samples, 4.16%). These findings highlight the importance of the choice of detection method in accurately identifying and quantifying different parasites in clinical samples. The different detection rates of various methods highlight the importance of choosing the right technique for each parasitic infection.

Regarding statistical analysis (Table 5), the current study's results were compared to the frequencies of parasites detected using various methods. The significance of the differences was assessed by applying the chi-square test.

Table 5: Statistical analysis (p-values) depending on Chi-Square results for different techniques used in diagnosing fruit and vegetable parasites

Techniques	Direct Sedimentation	Indirect Floatation ($ZnSO_4$)	Kinyoun Technique
Indirect Floatation ($ZnSO_4$)	Chi-Square: 2.11 p-value: 0.1462 NS		
Kinyoun Technique	Chi-Square: 19.16 p-value < 0.0001 Sig ***	Chi-Square: 14.64 p-value: 0.0001 Sig ***	
Modified Trichrome Staining Technique	Chi-Square: 13.02 p-value: 0.0015 Sig ***	Chi-Square: 4.11 p-value: 0.1273 NS	Chi-Square: 2.56 p-value: 0.1096 NS

The comparison conducted between direct sedimentation and indirect floatation ($ZnSO_4$) did not show any significant difference in the frequencies of parasites ($p = 0.1462$). The frequencies of parasites detected through direct sedimentation and the Kinyoun technique displayed a significant difference ($p = 0.0001$), implying that the method adopted impacts detection rates. Likewise, there existed a notable variation between direct sedimentation and modified trichrome staining technique ($p = 0.0015$).

The occurrence of parasites identified by indirect floatation ($ZnSO_4$) and modified trichrome staining technique, on the other hand, did not vary significantly ($p = 0.1273$). There was no significant difference between the Kinyoun technique and the modified trichrome staining technique ($p = 0.1096$).

The results suggest that the choice of detection method can influence the frequency of parasites detected in the samples. For some parasites, certain methods may yield higher detection rates, for instance, using the Kinyoun method for detecting *Cryptosporidium* spp. Is more effective than other staining techniques, while for others, no detection was observed using the same method. These findings may result differently in terms of the accuracy and effectiveness of each detection technique in diagnosing parasitic infections.

Overall, the highest total frequencies of parasites were detected using the direct sedimentation method (27 samples, 18.75%), followed by the indirect floatation method ($ZnSO_4$) (9 samples, 6.25%), the Kinyoun technique (8 samples, 5.55%), and the modified trichrome staining technique (6 samples, 4.16%). These findings highlight the importance of the choice of detection method in accurately identifying and quantifying different parasites in clinical samples. The different detection rates of various methods highlight the importance of choosing the right technique for each parasitic infection.

The results suggest that the choice of detection method can influence the frequency of parasites detected in the samples. For some parasites, certain methods may yield higher detection rates, for instance, using the Kinyoun method for detecting *Cryptosporidium* spp. Is more effective than other staining techniques, while for others, no detection was observed using the same method. These findings may result differently in terms of the accuracy and effectiveness of each detection technique in diagnosing parasitic infections.

The current study has revealed the investigation of different types of both fruit and green vegetable plants grown for human consumption regularly in Erbil province. It seems that various parasitic organisms are being detected in different vegetable types across different markets. The prevalence of these parasites varies, with some vegetable types and markets showing higher percentages of specific parasites. The usage of two different staining techniques (Modified Trichrome and Kinyoun staining methods) might also contribute to differences in detection rates.

This data suggests a potential risk of parasitic contamination in these vegetables, which could have implications for public health. Further analysis and investigation would be needed to understand the exact sources of contamination and the potential health risks associated with consuming these vegetables. Increasing demands for vegetables, fruits, and ethnic foods, some of them imported in regions without modern sanitary facilities and inspection systems may introduce stages of parasites (Lengai et al., 2022).

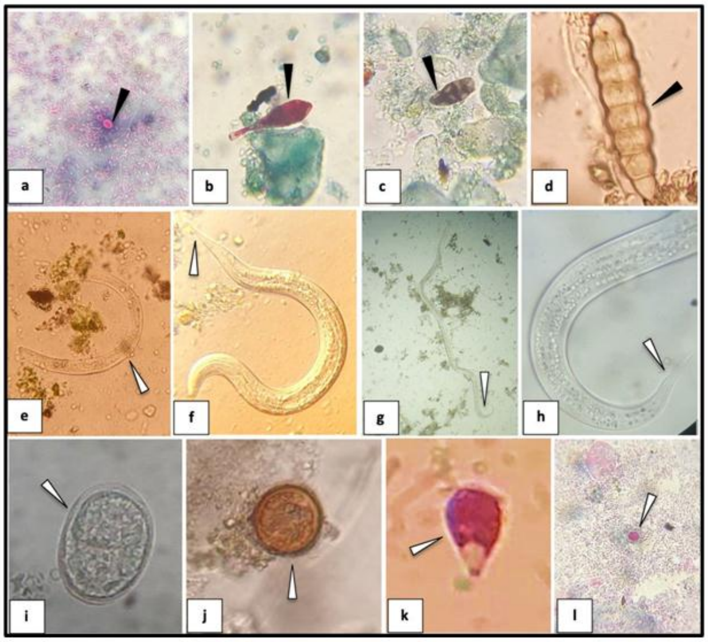

Figure 1: Photomicrographs representing certain pseudo-parasites (black arrow) and parasites (white arrow) identified from examined fruit and vegetable samples based on different diagnostic methods (Direct sedimentation, Trichrome Staining and Kinyoun Techniques)

a. Unicellular yeast with bud using Trichrome Staining (1000X).
b. Multicellular fungal spore (*Alternaria* spp.) using Kinyoun Technique (1000X).
c. Multicellular fungal spore (*Alternaria* spp.) using Trichrome Staining (1000X).
d. Multicellular fungal spore (*Alternaria* spp.) using Direct Sedimentation (1000X).
e. Female straight posterior end nematode using Direct Sedimentation (100X).
f. Female straight posterior end nematode using Direct Sedimentation (400X).
g. Male curvature posterior end nematode using Direct Sedimentation (100X).
h. Male curvature posterior end nematode using Direct Sedimentation (400X).
i. Thin-shelled Nematode egg isolated from $ZnSO_4$ floatation technique (400X).
j. Taeniid egg isolated from Direct Sedimentation (400X).
k. Trophozoite of *Giardia lamblia* using Trichrome Staining (1000X).
Oocyst of *Cryptosporidium* spp. using Kinyoun Technique (1000X).

4. Discussion

Based on the presented data, it was found that the total occurrence rate of parasites among examined fruits and vegetables was 18.75% with direct sedimentation method; this rate was lower than those reported by Klapec and Borecka (2012) in Poland which was 34.7% among conventional farms, whereas approximately was similar (18.9%) for organic farms using both sedimentation and floatation methods. The type of vegetables examined in this study by flotation method also exerted an effect on the results because the rate of contamination of leafy vegetables reported with the ova and cysts of parasites was considerably higher, in comparison to underground vegetables using the same method (Abougrain et al., 2010; Abubakr et al., 2020). The higher rate of vegetable contamination (88.3%) was reported by Al-Mozan and Dakhil (2019) from Thi-Qar province using direct sedimentation on different types of vegetables including celery, garden grass, and lettuce, this is in agreement with the present study. On the other hand, a lower contamination rate (10.2%) than the current study was reported by Abdullah (2021) in Duhok province by direct sedimentation method. Using the same method by Mirzaei et al. (2021) in Soran City reported 48.44% which was higher than the present study.

As previously reported the percentage of fruit contamination was 14.63% and vegetable contamination was 25.24%, this was relatively in agreement with those reported by Mahmood et al. (2011)in Mosul with a percentage of 17% for fruit contamination, while, lower than the same study (52%) for leafy vegetables using direct sedimentation and floatation methods. The investigations of the present study showed that 1.3% of the samples were contaminated with cysts of *Entamoeba histolytica*, which was reported on the lettuce samples. This is approximately similar to those reported by Mahmood et al. (2011) in Mosul which was 2%, but it was higher than Al-Mukhtar and Al-Dabbagh (1991) in the same city with percentages of 9% and 18% respectively on lettuce samples. This may be due to the use of chemical fertilizers more than the manure of animals or humans in different regions.

From the reported results of the present study, the lower occurrence rate with cysts of *G. lamblia* and ova of *Ascaris lumbricoides* was 1.3% and 4.16% respectively lower than the rates of the study done by Al-Megrin (2010) in Saudi Arabia (31.6% and 26.36% respectively). On the other hand, higher than the rates reported by Garedaghi et al. (2011) in Iran and Omowaye and Audu (2012) in Nigeria (1% and 0.78% for *A. lumbricoides* respectively). Overall, the rate variations depend on the geospatial distribution of the parasites in different regions. The occurrence of parasitic stages on lettuce was 52.63% which was higher than those reported by Al-Megrin (2010) in Saudi Arabia (27.8%). The results of *Giardia lamblia* (cysts and trophozoites) in the current study represented 2.08%, which was in disagreement with other studies, for instances Al-Mukhtar and Al-Dabbagh (1991) reported *G. lamblia* with a percentage of 16.7% on celery and 15.3% on lettuce by direct sedimentation method. Mahmood et al. (2011) reported 13% and 9% with both floatation and sedimentation methods respectively. Al-Mozan and Dakhil (2019) from two regions of Thi-Qar province reported the highest contamination rate with this protozoan (71.1%) using the direct sedimentation method. Abdullah (2021) in Duhok province and Mirzaei et al. (2021) in Soran City reported 4.2% and 10.6% contamination rates respectively for the previously mentioned flagellated protozoa using the same methods. They described that *G. lamblia* and *E. histolytica,* among the most prevalent intestinal parasites in the world, are transmitted directly (without the requirement for a secondary host).

These variations may be due to the place of cultivation of vegetables and the types of manure used, possibly the lettuce of Erbil is imported from Iran or Syria, while lettuce in Mosul markets is usually brought from Iraqi lands (Samara-mid of Iraq). This might be because the lettuce utilized in the current investigation was heavily infected with parasite stages. This observation is confirmed by the fact that similar solutions rarely use wastewater. Also, some studies indicated that the agricultural use of wastewater was the main cause of the occurrence of intestinal parasites (Salamandane et al., 2021).

Results of this study showed that only one kind of fruit sample (apple) was positive for parasites by direct sedimentation method from both replications in the same market (100metry market) with the ova of *A. lumbricoides* (16.7%), whereas there was no positive contamination rate for the other fruit samples (orange and banana) and markets (Kwran and Saedawa) for direct sedimentation, indirect floatation, the Kinyoun and trichrome staining techniques (0.0%), this was disagreement with the results of Elom et al. (2012) in Nigeria in which they reported 32% and 24% occurrence rates for orange and banana respectively by direct sedimentation method. Furthermore, the prevalence of *A. lumbricoides* and other unidentified Nematodes (larvae and adults) for the present study were 4.16% and 9.7% respectively by direct sedimentation method. The former was lower than the study done by Elom et al. (2012) in Nigeria, as they recorded 54.50% and the latter was higher (6.90%) than the same study. Al-Mozan and Dakhil (2019) from two regions of Thi-Qar province reported higher contamination rates (15.6% with *A. lumbricoides* and 35.15% with different species and stages of nematodes). Mirzaei et al. (2021) in Soran City reported 12% and 18.66% contamination rates respectively for *A. lumbricoides* and other species and stages of nematodes using the direct sedimentation method. Conversely, Tchounga et al. (2017) reported different percentages of contamination for *A. lumbricoides* and Nematodes, which were 8.5% and 3.5% respectively, as authors mentioned that this high incidence of geohelminths on fruits and vegetables is attributable to the fact that the outdoor markets used for this study were characterized by the presence of local garbage dumping sites, inadequate drainage, incorrect disposal of faces from sellers' children, and poor hygiene behavior. Variations in observed parasites might be attributed to changes in the research area's geographical location. Despite variations in individual parasites, the eggs of *A. lumbricoides* and hookworm were prevalent in all fruits and studies. This is possibly due to the parasites' ability to endure various unfavorable environmental conditions, which may indicate water pollution resulting from indiscriminate defecation and contamination of water and farmland, as observed by Adejumoke and Morenikeji (2015). Other reasons include contamination of fruits and vegetables due to a variety of sources, including the air, during storage, use, or handling (Mostafidi et al., 2020). In addition, the selection of diagnostic technique is considered as a standard reason for exhibiting the parasite contamination rates, for instance, using both trichrome staining and the Kinyoun techniques separately in identifying different species of protozoan parasites considered gold techniques. These two techniques above were measured as the first attempt to diagnose fruit and vegetable parasites in Iraq. The high parasite contamination rate and spread in this investigation were exacerbated by the deranged way of transportation of these consumable items. Additionally, the local practice of utilizing organic manure as fertilizer, such as cow and poultry droppings, contributed significantly to most of the helminthic infections in the studied locations. In this investigation, the direct sedimentation method had the highest recovery rate (18.75%), whereas the modified trichrome technique had the lowest (4.16%). Because most geohelminth ova are heavy, they settle towards the bottom of the tubes and can be overlooked using the flotation approach; this result was in disagreement with the result reported by Elom et al. (2012) in Nigeria with a contamination rate of 86.10%, as well as, those reported by Mahmood et al. (2011) in which they reported 5.09% for the sedimentation method.

Conclusions

At the end of this study, it was concluded that the contamination of both vegetables and fruits with different parasitic stags is still there in Erbil City markets. The most effective method for detecting various stages of the parasites is demonstrated by the direct sedimentation method. Different parasitic stages were reported including *G. lamblia*, *E. histolytica*, *Cryptosporidium* spp., *A. lumbricoides*, various nematodes, and taeniid eggs. The use of Trichrome staining and Kinyoun techniques in diagnosing different stages and species of parasites is considered a gold standard method in detecting fruit and vegetable parasite species besides direct sedimentation and floatation techniques. The findings underscore the importance of employing both broad-spectrum methods like direct sedimentation and more targeted techniques like the Kinyoun (for *Cryptosporidium* spp.) and trichrome staining (for *G. lamblia*) methods. This dual approach ensures a comprehensive analysis, allowing researchers to detect a wide variety of parasites and their various stages, enhancing the understanding of contamination profiles in fresh produce. The study highlights the necessity of continuous monitoring and employing a range of detection methods to ensure food safety and prevent health risks associated with parasite contamination in fruits and vegetables.

Acknowledgment: The authors would like to express their sincere gratitude to the Biology Department, College of Science, Salahaddin University-Erbil for valuable guidance and support throughout the research process in shaping our research and helping us to overcome challenges.

Financial support: No financial support.

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article.

References

Abdullah, A. M. (2021). Contamination of fresh vegetables with protozoan parasites, in Duhok City, Kurdistan Region of Iraq. *Medical Journal of Babylon,* 18 (4), 416-420. https://doi.org/10.4103/MJBL.MJBL_69_21

Abougrain, A. K., Nahaisi, M. H., Madi, N. S., Saied, M. M. & Ghenghesh, K. S. (2010). Parasitological contamination in salad vegetables in Tripoli-Libya. *Food control,* 21 (5), 760-762. https://doi.org/10.1016/j.foodcont.2009.11.005

Abubakr, B. M., Wabi, A. A., Gagman, H. A. & Mohammed, U. A. (2020). Prevalence of Helmenths Parasites in Vegetables Sold in Jama'are Metropolis, Bauchi State, Nigeria. *Bima Journal of Science and Technology,* 3 (2), 40-47. https://journals.gjbeacademia.com/index.php/bimajst/article/view/142/160

Adejumoke, A. & Morenikeji, O. (2015). Prevalence of intestinal parasites in vegetables sold in major markets in Ibadan city, south-west Nigeria. *Global journal of pure and applied sciences,* 21 (1), 7-12. https://doi.org/10.4314/gjpas.v21i1.2

Al-Megrin, W. A. (2010). Prevalence of intestinal parasites in leafy vegetables in Riyadh, Saudi Arabia. *International Journal of Tropical Medicine,* 5 (2), 20-23.

Al-Mozan, H. D. K. & Dakhil, K. M. (2019). Prevalence of parasites in fresh vegetables from two regions of Thi-Qar Province, Iraq. *Journal of Pure and Applied Microbiology,* 13 (2), 1103-1110. https://dx.doi.org/10.22207/JPAM.13.2.49

Al-Mukhtar, A. M. & Al-Dabbagh, N. Y. (1991). Occurrence of parasites of public health significance on lettuce and celery sold in local markets in Mosul-Iraq. *Journal of Community Medicine,* 4 (2), 167-175. https://pesquisa.bvsalud.org/portal/resource/pt/emr-20271

Alemu, G., Nega, M. & Alemu, M. (2020). Parasitic contamination of fruits and vegetables collected from local markets of Bahir Dar City, northwest Ethiopia. *Research and Reports in Tropical Medicine*, 17-25. https://www.tandfonline.com/doi/full/10.2147/RRTM.S244737

Berhanu, L., Abebe, M., Gizeyatu, A., Berihun, G., Teshome, D. & Walle, Z. (2022). Evaluation of the Effect of Wastewater Irrigation on the Microbiological Quality of Vegetables in Northeast Ethiopia: Implication for Food-Borne Infection and Intoxications. *Environmental Health Insights,* 16, 1-7. https://doi.org/10.1177/11786302221127856

Beyene, M. (2019). *Prevalence and Intensity of Intestinal Parasitic Infections of School Children in Ahmed Gurey Primary School, Jigjiga Town, Somali Regional State, Ethiopia.* M.Sc., Haramaya university. 65 pp.

El-Sayed, N. M., Gawdat, S. S., El-Kholy, H. S. & Elmosalamy, A. (2023). Parasitic Contamination in Five Leafy Vegetables Collected from Open Marketplaces in Giza, Egypt. *Journal of food quality and hazards control,* 10, 13-20. https://doi.org/10.18502/jfqhc.10.1.11984

El Safadi, D., Osman, M., Hanna, A., Hajar, I., Kassem, I. I., Khalife, S., Dabboussi, F. & Hamze, M. (2023). Parasitic Contamination of Fresh Leafy Green Vegetables Sold in Northern Lebanon. *Pathogens,* 12 (8), 1014. https://doi.org/10.3390/pathogens12081014

Elom, M. O., Eze, U. A., Nworie, A. & Akpotomi, I. O. (2012). Prevalence of geohelminths on edible fruits and vegetables cultivated in rural villages of Ebonyi State, South East Nigeria. *American Journal of Food and Nutrition,* 2 (3), 58-64. https://doi.org/doi:10.5251/ajfn.2012.2.3.58.64

Fallah, A. A., Pirali-Kheirabadi, K., Shirvani, F. & Saei-Dehkordi, S. S. (2012). Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: influence of season and washing procedure. *Food control,* 25 (2), 617-620. https://doi.org/10.1016/j.foodcont.2011.12.004

Garcia, L. S., Arrowood, M., Kokoskin, E., Paltridge, G. P., Pillai, D. R., Procop, G. W., Ryan, N., Shimizu, R. Y. & Visvesvara, G. (2018). Practical guidance for clinical microbiology laboratories: laboratory diagnosis of parasites from the gastrointestinal tract. *Clinical Microbiology Reviews,* 31 (1), 1-81. https://doi.org/10.1128/cmr.00025-17

Garedaghi, Y., Farhang, H. H. & Pooryagoobi, S. (2011). Parasitic contamination of fresh vegetables consumed in Tabriz, Iran. *Research Journal of Biological Sciences* 6(10), 518-522. http://docsdrive.com/pdfs/medwelljournals/rjbsci/2011/518-522.pdf

Gilling, S. J., Taylor, E. A., Kane, K. & Taylor, J. Z. (2001). Successful hazard analysis critical control point implementation in the United Kingdom: understanding the barriers through the use of a behavioral adherence model. *Journal of Food Protection,* 64 (5), 710-715. https://doi.org/10.4315/0362-028x-64.5.710

Klapec, T. & Borecka, A. (2012). Contamination of vegetables, fruits and soil with geohelmints eggs on organic farms in Poland. *Annals of Agricultural and Environmental Medicine,* 19 (3), 421-425. https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-0bf5fc66-8d1d-46b8-ba25-5d55149e285b

Lengai, G. M., Fulano, A. M. & Muthomi, J. W. (2022). Improving access to export market for fresh vegetables through reduction of phytosanitary and pesticide residue constraints. *Sustainability,* 14 (13), 8183. https://doi.org/10.3390/su14138183

Mahmood, M., Rahimo, Z. & Abdul-Faraj, M. (2011). Intestinal parasitic pollution of vegetables and fruits in Mosul. The second scientific conference of environment and pollution control research center table . The University of Mosul. Mosul Iraq. 159-173. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Intestinal+parasitic+pollution+of+vegetables+and+fruits+in+Mosul.&btnG=.

Mirzaei, Y., Mohammadi, C., Ahmad, S. F., Hamad, P. M. & Samiei, A. (2021). Prevalence of intestinal parasites in raw vegetables consumed in Soran city, Kurdistan region, Iraq. 67 (2), 275-279. https://doi.org/10.17420/ap6702.339

Mogaji, H. O., Ishola, A. Y., Adekunle, O. N., Adekoya, M. A., Oluwafemi, F. S., Ajayi, O. A., Odoh, I. M. & Ekpo, U. F. (2021). Parasitic Contamination on Raw Vegetables Sold in Selected Open Markets in Ekiti, Southwest Nigeria. *Pan African Journal of Life Sciences,* 5 (3), 342-348. https://doi.org/10.36108/pajols/1202.50.0360

Mostafidi, M., Sanjabi, M. R., Shirkhan, F. & Zahedi, M. T. (2020). A review of recent trends in the development of the microbial safety of fruits and vegetables. *Trends in Food Science & Technology,* 103, 321-332. https://doi.org./10.1016/j.tifs.2020.07.009

Nyarango, R. M., Aloo, P. A., Kabiru, E. W. & Nyanchongi, B. O. (2008). The risk of pathogenic intestinal parasite infections in Kisii Municipality, Kenya. *BMC Public Health,* 8 (237), 1-6. https://doi.org/10.1186/1471-2458-8-237

Ola-Fadunsin, S. D., Adebanjo, A. O., Abdullah, D. A., Hussain, K., Sanda, I. M., Rabiu, M., Ganiyu, I. A., Elelu, N., Aiyedun, J. O. & Oludairo, O. O. (2022). Epidemiology and public health implications of parasitic contamination of fruits, vegetables, and water in Kwara Central, Nigeria. *Annals of parasitology,* 68 (2), 339-352. https://doi.org/10.17420/ap6802.440.

Omowaye, O. & Audu, P. (2012). Parasites contamination and distribution on fruits and vegetables in Kogi, Nigeria. *Cibtech Journal of Bio-Protocols,* 1 (1), 44-47. https://scholar.google.com/scholar?hl=en&as_sdt=0%

2C5&q=Parasites+contamination+and+distribution+on+fruits+and+vegetables+in+Kogi%2C+Nigeria.&btnG=

Quattrocchi, U. (2017). *CRC world dictionary of plant names: common names, scientific names, eponyms, synonyms, and etymology*, Routledge, 728. https://doi.org/10.1201/9781315140599.

Salamandane, C., Lobo, M. L., Afonso, S., Miambo, R. & Matos, O. (2021). Occurrence of intestinal parasites of public health significance in fresh horticultural products sold in Maputo markets and supermarkets, Mozambique. *Microorganisms,* 9 (9)**,** 1806. https://doi.org/10.3390/microorganisms9091806

Tchounga, K. S., Ajugwo, A. O., Nsa, M., Oshoma, C. E., Dunga, K. E. & Ikenazo, H. (2017). Prevalence of Intestinal Parasites in Vegetables Sold in Some Local Markets in Port-Harcourt, Rivers-State, Nigeria. *Archives of Microbiology & Immunology,* 1 (1)**,** 41-49. https://www.fortunejournals.com/abstract/prevalence-of-intestinal-parasites-in-vegetables-sold-in-some-local-markets-in-portharcourt-riversstate-nigeria-34.html

Yandev, D., Abam, M., Olasan, J., Ishwua, M. & Osar, J. (2019). Assessment of Enteric Helminthes Ova on Ready to Eat Selected Vegetables (Garden Egg, Cucumber and Carrot) in Ushongo LGA of Benue State. *International Journal of Science,* 8 (7)**,** 28-33. https://doi.org/10.18483/ijSci.2104.