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Research Article

The Level of Pesticide Residues in Cucumber Fruits Collected from Central Vegetable Markets in Khartoum State

Mona Mahmoud Ahmed Aldawi[1], Azhari Omer Abdelbagi[2], Abd Elaziz Sulieman Ahmed Ishag[2]* and Ahmed Mohammed Ali Hammad[2]

[1]*Sudanese Metrology and Standards Organization, Sudan*

[2]*Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan*

***Corresponding Author:** Abd Elaziz Sulieman Ahmed Ishag, Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.

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Abstract

Cucumber fruit samples were collected from three central vegetable markets in Khartoum State (Bahari, Omdurman, and Khartoum) for residue analyses. Care was given to collect samples from both greenhouses and open fields sources. Collected samples were, analyzed by GLC-FID and results were expressed in mg/kg fruits. Pesticides residues were detected in all samples from greenhouses specially dimethoate and heptachlor, while diazinon, chlorpyrifos, endosulfan (beta and alpha isomers) were not detected. Omethoate was detected in 67% of the samples. The highest level detected corresponds to omethoate (2.45 mg/kg), followed by dimethoate (2.24 mg/kg). The highest frequency of violation (> MRLs) corresponds to dimethoate and heptachlor (100%), followed by omethoate (66.6%). The highest residue load per Kg fruit was found in fruits collected from Khartoum North (5.79 mg/kg), followed by Omdurman (1.97 mg/kg) while the least load was found in fruits collected from Khartoum (1.44 mg/kg). Washing of fruits seemed to decrease the residue load by 8 - 76%. The reduction corresponds to heptachlor 27 - 83%, and dimethoate 0 - 53%. Pesticides residues were detected in all samples from open fields, specially dimethoate, and heptachlor, while diazinon, chlorpyrifos, and endosulfan beta were not detected. Omethoate was detected in 67% of the samples while endosulfan alpha was detected in 33% of the samples. The highest level detected corresponds to dimethoate (1.34 mg/kg) followed by heptachlor (1.24 mg/kg). Whereas the highest frequency of violation (> MRLs) corresponds to dimethoate and heptachlor (100%), followed by omethoate (66.6%). The highest residue load per Kg fruit was found in fruits collected from Khartoum North (3.08 mg/kg) followed by Khartoum (2.33 mg/kg) and the least load was found in fruits collected from Omdurman (1.68 mg/kg). Washing of fruits seemed to decrease the pesticide residue load by 16 - 21%. The reduction corresponds to heptachlor 15 - 54% and dimethoate 0 - 22%.

Keywords: *Cucumber; Khartoum State; Greenhouses; Pesticides Residue*

Introduction

Fruits and vegetables are the most valuable and nutritious food commodities, which can substantially improve the social welfare and health status of the rural and urban population. For hundreds of years, conventional types of vegetables such as okra, jews mallow and cucurbits were consumed by many Sudanese people. By the turn of the twentieth century, new kinds and varieties of vegetables were introduced [1]. Sudan is nominated as a major producer of vegetable crops for local consumption, and to play a significant role in alleviating the world food shortage, expected to occur in the near future [2,3]. Administration of Strategic Planning in Agriculture Ministry of Province Khartoum reported in 2013 - 2014 the area cultivated with vegetables was estimated at 400,394 Fadden with the production of

about 3,803.75 tons; as we find the cultivated area with cucumber was estimated at 2,235 394 Fadden with production of about 11,987 tons. Furthermore, the international airport of Khartoum encourages the development of specialized vegetable export in Khartoum State.

Agricultural and horticultural crops and their products are exposed to great loss as a result of an attack by various pests, Plant Protection Team of the Food and Agriculture Organization estimated the loss in agricultural crops before and after harvest by 30 - 50% as a result of an attack by insects, diseases, and various weeds.

The use of pesticides represents the major method of pest control in many countries especially when the natural way fails. However, the proper use of pesticides to reduce crop losses and alleviate pest problems, should be based on a full understanding of the nature of the chemical, the proper diagnosis of the pest to be controlled, the proper time to perform the control operation, strict follows up of instructions and guidance to avoid dangerous damage to humans, animals and the environment. Several reports in the media and local newspaper claimed that farmers of open fields and greenhouses in Sudan use a high rate of pesticides and do not follow the recommended dose and safety instructions. Dabrawski [4] mentioned that farmers have a tendency to overuse pesticides they may apply any pesticides to reduce losses and increase their yield which may lead to increased risks to human health and the environment.

The hypotheses of the current study is that; cucumber fruits cultivated in open fields and greenhouses contain pesticides residue exceeding the maximum residue limits allowed in cucumber fruits and those grown in greenhouses contain even higher levels than those from the open fields. Workers and farmers of open fields and greenhouses do not follow the recommended dose and application methods and they are unaware of safety and precautionary measures that should be followed. Based on the above hypotheses this study was initiated to cast light on the level of pesticides residues in cucumber fruits from open fields and greenhouses in Khartoum state. The limited available information on this subject offer than strengthen our goals.

Materials and Methods

Chemicals and Reagents

All solvents used were of analytical HPLC or GLC grades, Solvents used were; Acetone (99.8%), Dichloromethane (99.8%), and N-hexane (99.9%). Other reagents used are; Anhydrous sodium sulfate (99.0%), Sodium chloride (99.5%), Celite and C18 Column.

Sample collection methods

Greenhouses sampling

The FAO/WHO Food standards method [5] was used. Representative samples (5 - 6 Kg) containing at least 25 piece of cucumber fruits were collected from Bahari Almarkzi Market (Possible sources; East Nile, Silet and Eid Babiker), Almalaga Market, Omdurman (Possible sources; Karari, El gekheas and West Omdurman), Almarkzi Market, Khartoum (Possible sources; Soba, Mayo, Gabal Awlia).

These markets represent the main suppliers of cucumber to Khartoum State from greenhouses. Samples were collected in paper bags, labeled and taken to the laboratory for immediate preparation; samples were temporarily stored at -4°C for short period before analysis.

Open fields sampling

The FAO/ WHO Food standards method [5], P.384 was followed. Representative samples (5 - 6 Kg) containing at least 25 piece of cucumber fruits were collected from Bahari Almarkzi Market (Possible sources; Algili, Al kabashi and Alrizergab), Almalaga Market, Omdurman (Possible sources; Karari, Abohorira and Algalaa), Almarkzi Market, Khartoum (Possible sources; Al horka, Aldwam and Gabal Awlia). Nine samples were collected from these markets which represent the main suppliers of cucumber to Khartoum state from open fields. Samples were collected in paper bags, labeled and taken to the laboratory for immediate preparation. Samples were temporarily stored at 4°C for short before analysis.

Method of analysis

The method of pesticides residues analysis used in this study described by Specht., *et al.* [6] was followed. Below is a brief description of the method.

Samples preparation and extraction

Cucumber surfaces were washed fourth times with small portions of distilled water (10 ml) for each. The washings process took about 30 second. All washings (40 ml) were collected and combined in 100 ml flask using Pasteur pipette. Washings were stored in the freezer at -20°C for dislodgeable residue analysis.

The sample was divided randomly into five equal portions, each was sliced into small pieces by the knife, thoroughly mixed and forty grams were taken from each sample and blended with five ml distilled water and 100 ml acetone on the high-speed blender for two minutes. Ten grams of celite was added, shaken gently, blended again for ten seconds, filtered through a fast rate filter paper in buncher funnel with aid of low water jet pump suction into Erlenmeyer flask (500 ml). The filtrates were collected in an Erlenmeyer flask (500 ml) for partitioning.

Partitioning

Extracts from each sample were transformed into a 500 ml separatory funnel. Fifty ml of dichloromethane and 10 ml of saturated NaCl solution were added, the content was carefully shaken for 2 minutes and left to stand for 10 minutes to allow separation of layers. The organic layer was collected and the aqueous layer was re-extracted with 50 ml of dichloromethane. The combined extracts of dichloromethane were filtered through cotton wool and mixed with 25g of anhydrous sodium sulfate and collected in 500 ml round-bottom flask. Extracts were again re-filtered through cotton wool with a 3 cm layer of anhydrous sodium sulfate in a separatory funnel. The solvent was removed to dryness by rotary evaporator operating under vacuum at a temperature of 40°C. Dried extracts were reconstituted in 10 ml hexane and kept in a volumetric flask (50 ml) at -20°C for clean-up.

Clean-up

Clean-up was done on using C18 column. The column was first rinsed with few ml of n-hexane. Extracts from each sample were added to the column and allowed to elute with solvent mixture of ten ml acetone: n-hexane (1:19). The combined eluate (eluate + washing) were transferred to rotary evaporator for complete removal of solvent and the content was reconstituted (resolved) in ten ml n-hexane and transferred into ten ml volumetric flask and stored at -20°C for residues analysis.

Analytical standards preparation

Analytical standards (99% pure) of diazinon, omethoate, dimethoate, heptachlor, chlorpyrifos, endosulfan-alpha, endosulfan-beta, were obtained from the pesticide Residues Analytical Laboratory, Agricultural Research Corporation (ARC), Wad Medani, Sudan. Stock solutions of 2 mg/ml of these pesticides were made by dissolving 20 mg from each of the analytical standards in 10 ml of n-hexane. Lower concentrations (0.01 - 0.04 ppm) were made by serial dilution.

Gas Chromatography (GC) Analysis

Residues analysis was done on used GC (Shimadzu - Japan, 2010) equipped with a flame ionization detector (FID) and a DB-5 capillary column of 30 ml length and 0.25 mm inner diameter (ID).

Nitrogen was used as a carrier and make-up gas at a flow rate of 6.8 ml/min and 30.0 ml/min, respectively. The split mode of injection was followed. The air flow rate was 400.0 ml/min. The operating temperature condition of the injection port and the detector were 280°C and 300°C respectively. The column temperature was programmed from an initial temperature of 50.0°C held for 5 minutes, raised at 5°C/min to 75.0°C, held for 10 minutes then raised at 10.0°C/min to 160.0°C, held for 5 minutes, raised again at 5°C/min to 185.0°C and held for 3 minutes and finally raised at 3°C/min to 240.0°C at which it was held for 10.0 minutes, the total run time was 46.83 minutes.

One µl of the pesticides standards was injected into the GC using a syringe of 5 cm long needle. Different concentrations of each standard solution were injected three times, the peak area of each standards solution was plotted against their respective concentrations and used for the construction of the standard curves (Figure 1). Small variations in response were corrected by obtaining fresh chromatograms of the standard mixture every nine injections (Figure 2).

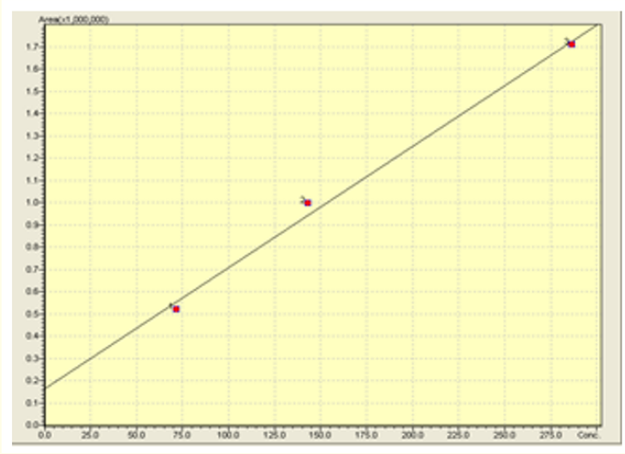

Figure 1: *Chromatogram of the standard pesticides (mixtures of diazinon, omethoate, dimethoate, chlorpyrifos, heptachlor, endosulfan alpha and beta) analyzed by GC-FID.*

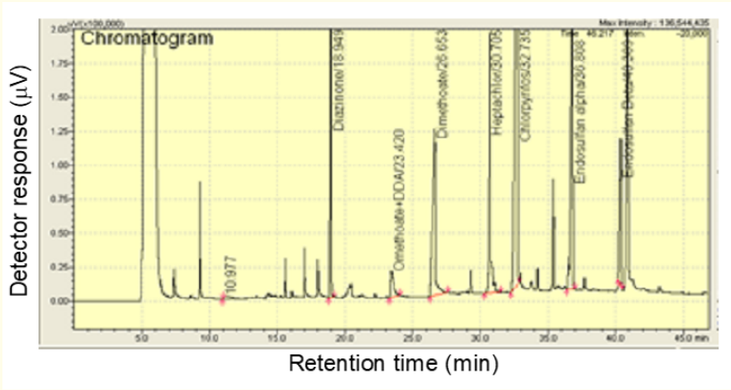

Figure 2: *Chromatogram of the standard pesticides (mixtures of diazinon, omethoate, dimethoate, chlorpyrifos, heptachlor, endosulfan alpha and beta) analyzed by GC-FID.*

Triplicate injections (2 µl each) of each sample were analyzed by the same GC and under the above-mentioned conditions. Analyzed pesticides were identified by comparing their retention times to those of the authentic standards and concentrations were determined from the standard curve and expressed in mg/kg fruit.

Statistical analysis

Analysis of variance (ANOVA) was performed according to Gomez and Gomez [7]. Mean were separated by using Duncan Multiple Range Test (DMRT).

Results and Discussion

Level of pesticide residue in greenhouses cucumber

The results (Table 1) indicated that pesticides residues were detected in 100% of greenhouses samples collected from central vegetable markets. Dimethoate and heptachlor were detected in all of the samples analyzed, while diazinon, chlorpyrifos, endosulfan beta, and endosulfan alpha were not detected. Omethoate was detected in some samples at a frequency of detection ranging from ND to 66.6%. The highest average level detected corresponds to omethoate (2.45 mg/kg) followed by dimethoate (2.24 mg/kg). Which the least average level detected correspond to heptachlor (1.1 mg/kg).

Pesticides	Parameters	Location					
		Khartoum North		Khartoum		Omdurman	
		Unwashed fruits	Washing water	Unwashed fruits	Washing water	Unwashed fruits	Washing water
Diazinon	Average	ND	ND	ND	ND	ND	ND
	Range	ND	ND	ND	ND	ND	ND
	Median	ND	ND	ND	ND	ND	ND
	Sample tested +ve%	ND	ND	ND	ND	ND	ND
	Violative sample %	ND	ND	ND	ND	ND	ND
	SE ±	0.0	0.0	0.0	0.0	0.0	0.0
Omethoate	Average	2.45	ND	ND	ND	ND	ND
	Range	ND- 4.09	ND	ND	ND	ND	ND
	Median	3.39	ND	ND	ND	ND	ND
	Sample tested +ve%	66.6	ND	ND	ND	ND	ND
	Violative sample %	66.6	ND	ND	ND	ND	ND
	SE ±	0.62	0.0	0.0	0.0	0.0	0.0
Dimethoate	Average	2.24	ND	0.86	0.46	0.73	ND
	Range	1.15 - 3.59	ND	0.59 - 1.01	ND - 1.49	ND - 1.45	ND
	Median	2.03	ND	0.79	1.28	1.0	ND
	Sample tested +ve%	100	ND	100	33.3	66.6	ND
	Violative sample %	100	ND	100	33.3	66.6	ND
	SE ±	0.34	0.0	0.05	0.23	0.2	0.0
Chlorpyrifos	Average	ND	ND	ND	ND	ND	ND
	Range	ND	ND	ND	ND	ND	ND
	Median	ND	ND	ND	ND	ND	ND
	Sample tested +ve%	ND	ND	ND	ND	ND	ND
	Violative sample %	ND	ND	ND	ND	ND	ND
	SE ±	0.0	0.0	0.0	0.0	0.0	0.0
Ops Total		4.69	ND	0.86	0.46	0.73	ND
Endosulfan alpha	Average	ND	ND	ND	ND	ND	ND
	Range	ND	ND	ND	ND	ND	ND
	Median	ND	ND	ND	ND	ND	ND
	Sample tested +ve%	ND	ND	ND	ND	ND	ND
	Violative sample%	ND	ND	ND	ND	ND	ND
	SE ±	0.0	0.0	0.0	0.0	0.0	0.0
Endosulfan beta	Average	ND	ND	ND	ND	ND	ND
	Range	ND	ND	ND	ND	ND	ND
	Median	ND	ND	ND	ND	ND	ND
	Sample tested +ve%	ND	ND	ND	ND	ND	ND
	Violative sample %	ND	ND	ND	ND	ND	ND
	SE ±	0.0	0.0	0.0	0.0	0.0	0.0
Total Endosulfan		ND	ND	ND	ND	ND	ND
Heptachlor	Average	1.1	0.51	0.63	0.58	1.24	0.33
	Range	0.56 - 1.63	0.45 - 0.56	0.39 - 0.79	0.4 - 0.98	1.02 - 1.74	ND- 0.59
	Median	1.2	0.53	0.56	0.55	1.08	0.45
	Sample teste +ve%	100	100	100	100	100	66.6
	Violative sample %	100	100	100	100	100	66.6
	SE ±	0.15	0.01	0.04	0.07	0.09	0.08
Total OCs		1.1	0.51	0.63	0.58	1.24	0.33
Total loading/Kg fruit		5.79	0.51	1.44	1.09	1.97	0.33
Frequency of Detection		9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)
Violation samples		9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)

Table 1: *Pesticides Residues in greenhouses cucumber fruits collected from markets in Khartoum state*

The highest frequency of violation (> MRLs) corresponds to dimethoate and heptachlor (100%) followed by omethoate (66.6%). The highest residue load per Kg fruit was found in fruits collected from Khartoum North (5.79 mg/kg) followed by Omdurman (1.97 mg/kg) and the least load was found in fruits collected from Khartoum (1.49 mg/kg).

These findings are similar to those of Hammad., *et al.* [8,9] and Elbashir., *et al.* [10] who found that the pyrethroid residue levels in tomato fruit exceeded the MRL. The current study agrees with Hammad., *et al.* [8] who found that tomato sample collected from a greenhouse in a different location in Khartoum state contain lambdacyhalothrin and imidacloprid residues levels higher than the maximum residue levels established by either Codex Alimentarius [11] or the European Union (EU). These findings are similar to studies conducted in the National Research Center, Dokki Cairo, Egypt (1999) for monitoring of pesticides residues where residue of BHC, lindane, dieldrin, heptachlor epoxide and DDT derivatives were found in the range of 0.009, 0.003, 0.006, 0.008 and 0.083 mg/kg; respectively in tomatoes and tomato products. This high level of pesticide residues in cucumber fruit in Sudan raise questions over the safety of food commodities sold across the country.

Washing of fruits seems to decrease the levels of residue load of some pesticides. The reduction corresponds mainly to the level of heptachlor and sometime dimethoate. The current result agrees with of Robertson and Jaskukle [12], that washing can cause a significant reduction in pesticides residues especially in smooth surface fruits as in the case of tomato. He added that 90% of the surface residues could be removed by washing with water. The reduction corresponds to heptachlor and sometimes dimethoate. The current result agrees with the report of Albach and Lime [13] who mentioned that washing of orange fruits sprayed with malathion, parathion and dimethoate eliminated from 8 - 35% of the residue percent in the unwashed fruits.

Level of pesticide residue in open fields cucumber

The results (Table 2) indicated that pesticides residues were detected in 100% of open fields samples collected from central vegetable markets. Dimethoate and heptachlor were detected in all samples analyzed, while diazinon, chlorpyrifos, and endosulfan beta were not detected. Endosulfan alpha was detected in some samples at a frequency of detection ranging from ND to 33.3%, while omethoate was detected at a frequency ranging from ND to 66.6%. The highest level detected corresponds to heptachlor (2.12 mg/kg) followed by dimethoate (1.52 mg/kg). Awad., *et al.* [14] reported that 94.7% of cucumbers and tomatoes samples collected from greenhouses were found contaminated by diazinon, malathion, chlorpyrifos, ethephon, profenofos, and oxyfluorfen. The residues measured in both of them were above maximum residue limits (MRLs) set by Codex Alimentarius.

Pesticides	Parameters	Location					
		Khartoum North		Khartoum		Omdurman	
		Unwashed fruits	Washing water	Unwashed fruits	Washing water	Unwashed fruits	Washing water
Diazinon	Average	ND	ND	ND	ND	ND	ND
	Range	-	-	-	-	-	-
	Median	ND	ND	ND	ND	ND	ND
	Sample tested + ve%	ND	ND	ND	ND	ND	ND
	Violative sample %	ND	ND	ND	ND	ND	ND
	SE ±	0.0	0.0	0.0	0.0	0.0	0.0
Omethoate	Average	0.52	ND	ND	ND	ND	ND
	Range	ND - 0.89	-	-	-	-	-
	Median	0.75	ND	ND	ND	ND	ND
	Sample tested + ve%	66.6	ND	ND	ND	ND	ND
	Violative sample %	66.6	ND	ND	ND	ND	ND
	SE ±	0.13	0.0	0.0	0.0	0.0	0.0
Dimethoate	Average	1.34	ND	1.09	0.24	0.61	0.20
	Range	1.11 - 1.49	ND	0.73 - 1.52	ND - 0.75	0.48 - 0.86	ND - 0.65
	Median	1.33	ND	1.08	0.7	0.62	0.6
	Sample tested + ve%	100	ND	100	33.3	100	33.3
	Violative sample %	100	ND	100	33.3	100	33.3
	SE ±	0.04	0.0	0.09	0.12	0.04	0.1

Chlorpyrifos	Average	ND	ND	ND	ND	ND	ND
	Range	-	-	-	-	-	-
	Median	ND	ND	ND	ND	ND	ND
	Sample tested +ve%	ND	ND	ND	ND	ND	ND
	Violative sample %	ND	ND	ND	ND	ND	ND
	SE ±	0.0	0.0	0.0	0.0	0.0	0.0
OPs Total		1.86	ND	1.09	0.24	0.61	0.2
Endosulfan alpha	Average	0.29	ND	ND	ND	ND	ND
	Range	ND- 0.99	ND	ND	ND	ND	ND
	Median	0.83	ND	ND	ND	ND	ND
	Sample tested +ve%	33.3	ND	ND	ND	ND	ND
	Violative sample %	33.3	ND	ND	ND	ND	ND
	SE ±	0.15	0.0	0.0	0.0	0.0	0.0
Endosulfan beta	Average	ND	ND	ND	ND	ND	ND
	Range	ND	ND	ND	ND	ND	ND
	Median	ND	ND	ND	ND	ND	ND
	Sample tested + ve%	ND	ND	ND	ND	ND	ND
	Violative sample %	ND	ND	ND	ND	ND	ND
	SE ±	ND	0.0	ND	0.0	ND	0.0
Total Endo-sulfan		0.29	ND	ND	ND	ND	ND
Heptachlor	Average	0.93	0.5	1.24	0.5	1.07	0.16
	Range	0.4 - 1.15	0.44 - 0.55	0.57 - 2.12	0.45 - 0.55	0.53 - 1.07	ND - 0.45
	Median	1.07	0.5	1.06	0.49	1.01	0.45
	Sample tested +ve%	100	100	100	100	100	33.3
	Violative sample %	100	100	100	100	100	33.3
	SE ±	0.09	0.02	0.25	0.01	0.08	0.08
Total OCs		1.22	0.5	1.24	0.5	1.07	0.16
Total loading/Kg fruit		3.08	0.5	2.33	0.74	1.68	0.36
Frequency of Detection		9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)
Violation samples		9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)

Table 2: *Pesticides Residues levels in openfields cucumber fruits samples collected from markets in Khartoum state.*

Although chlorpyrifos was reported as the main contaminated of vegetable in many places in the world [15], yet it was not detected in any the sample analyzed in the current study, the questionnaire and interviews with some farmers indicated that this pesticide was not used in the study area.

The highest frequency of violation (> MRLs) corresponds to dimethoate and heptachlor (100%) followed by omethoate (66.6%). The highest residue level per Kg fruit was found in fruits collected from Khartoum North (3.08 mg/kg) followed by Khartoum (2.33 mg/kg) and the least load was found in fruits collected Omdurman (1.68 mg/kg).

A study from the Brazilian monitoring program showed that 48.3% of the 13,556 samples analyzed from 2001 to 2010 were positive for at least one pesticide residue, apple, papaya, grape, and sweet pepper were the crops with the highest percentage of positive samples (around 80%) [16]. Dutch program carried out between 2003 and 2005 reported that 30% of the vegetable and fruit samples contain organophosphorus compounds.

Washing of fruits seems to decrease the levels of residue load of some pesticides. The reduction corresponds to heptachlor and sometimes dimethoate. The current result agrees with Robertson and Jaskukle [12], that washing can cause a significant reduction in pesticides residues especially in smooth surface fruits as in the case of tomato. He added that 90% of the surface residues could be removed by washing with water. The reduction corresponds to heptachlor and sometimes dimethoate. The current result agrees with the report of Albach and Lime [13] who mentioned that washing of orange fruits sprayed with malathion, parathion and dimethoate eliminated from 8 - 35% of the residue percent in the unwashed fruits.

Conclusion

Pesticides residues were found in all sample analyzed. Heptachlor and dimethoate were found in all samples from both greenhouses and open fields at levels exceeding MRLs. The highest levels detected from greenhouses samples correspond to omethoate followed by dimethoate, while heptachlor and dimethoate scored the highest levels in open fields samples. The highest residue load was found in fruit collected from Khartoum North in both greenhouses and open fields samples. Washing seems to decrease the residues level, especially for heptachlor and dimethoate.

Conflicts of Interest

The authors declare no competing financial interest.

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