Pesticide Residues Detected on Tomato and Cucumber Fruits Grown in Greenhouse farms in Khartoum State, Sudan

Awad O. Mohamed^{*1}, Adam A. Mater², Ahmed M.A. Hammad³, Abd Elaziz S.A. Ishag⁴, Elsadig M. El Tayeb⁵, Asia A. Dahab⁶

***Corresponding author**; E-mail: awadomer51@hotmail.com

Abstract: The study was aimed to assess the level of pesticide residues in tomatoes and cucumbers vegetables grown in greenhouse farms in Khartoum State (Sudan) and compare them with the maximum residue limits set by Codex Alimentarius. A total of 19 samples of cucumbers (14) and tomatoes fruits (5) were randomly collected from 14 greenhouse farms under study. The levels of pesticide residues were determined by gas chromatography equipped with a flame ionization detector (GC-FID). The results indicated that 94.7% of the samples were contaminated by diazinon, malathion, chlorpyrifos, ethephon, profenofos, and oxyfluorfen. The residues measured in cucumber fruits samples ranged from 0.05 to 167.19 mg kg⁻¹, and that measured in tomato fruit ranged from 2.55 to 136.87 mg kg⁻¹, and all of them were above maximum residue limits (MRLs) set by Codex Alimentarius. The highest level of diazinon residues was detected in cucumber samples collected from the greenhouse farms MB (25.68 mg kg⁻¹), Eltiseen (24.63 mg kg⁻¹), and Elzawaya (10.58 mg kg⁻¹) were exceeded the MRLs of cucumber (0.1 mg kg⁻¹) by 257, 246 and 106 folds, respectively. However the corresponding values of malathion resides that detected in tomato fruits samples collected from the farms YAY (39.7 mg kg⁻¹), MB (9.45mg kg⁻¹) by 79, 47, 42 and 41 folds, respectively. These high levels of pesticide residues detected in both vegetables raise a great public health concern as they can pose serious adverse effects to consumers.

Keywords: Tomato fruits, Cucumbers, Pesticide residue, Greenhouse, Khartoum State.

1. INTRODUCTION

Vegetables are considered as protective supplementary foods as they contain large quantities of minerals, vitamins, and essential amino acids, which are required for normal functioning of the metabolic processes. They are important to neutralize the acids produced during digestion and also useful as "roughage" (Shanmugavelu 1989). Tomato (*Lycopersicon Esculentum*) is the second-most important vegetable in the world after potato (Dorais et al. 2008). The world tomato production was 152 million tons in 2009. The main tomato producers were China, the USA, India, Turkey, Egypt, Italy, Iran, Spain, Brazil and Mexico (75% of the world production) (Heuze et al. 2015). Tomato is the main source of health-promoting compounds due to the balanced mixture of antioxidants including vitamins C and E, lycopene, beta-carotene, lutein and flavonoids (50), amino acids, proteins, fatty acids and carbohydrates (Hauffman and Bruce 2002, Heeb 2005). Since tomato fruit play an important role in human health (Chapagain and Wiesman 2004), strategies for

^{1, 2} Faculty of Public Health, Alzeim Alazhari University, Khartoum North, Sudan

^{3, 4} Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Khartoum, SD 3114, Sudan

^{1,5,6} Directorate General of PHC, Federal Ministry of Health, Khartoum, Sudan

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increasing fruit production and quality are of great interest to producers (Gruda 2005). Cucumber (Cucumis sativus) is a widely cultivated plant/belonging to the family Cucurbitaceae or commonly called cucurbits. Cucumber is commonly used for salads and pickles while squash and others are used for cooking. Cucumber (C. sativus L.) is one of the most important economic crops; the economic importance of this crop appears in both local consumption and export. Cucumber is grown either in the open field or under protected agriculture houses. The purpose of growing crops under protected house conditions is to extend their cropping season and to protect them from adverse conditions as well as diseases and pests. Cucumber plants are affected by several fungal pathogens, such as Fusarium oxysporum an important pathogen (Ahmed 2010). Vegetables also attract a wide range of pests and diseases and require intensive pest management. Vegetables such as tomatoes, country beans, cabbage, cauliflower, and cucumber may receive higher doses of pesticides (Ali et al. 2002). Pesticides applied to food crops in the field can leave potentially harmful residues. Organochlorine pesticides, in particular, can persist in foodstuffs for a considerable period. If crops are sprayed shortly prior to harvest without an appropriate waiting period, even organophosphate residues can persist up until the food is in the hands of the consumer (Stephen et al. 2011). Pesticide residues remain in the inner part of vegetable in the shape of residues, though not documented how much active material could be imminent in vegetables even after they are washed and cooked (Agnihotri 1999; Kabir et al. 2008). However, food safety problems caused by pesticide residues in vegetables have become one of the top issues of public interest (Zhang et al. 2013). Though it is sometimes thought that residues are destroyed if food is properly washed and cooked, this is not always the case. Washing and cooking may reduce pesticide residues in food; boiling may remove only 35-60% of organophosphate residues and 20-25% of organochlorines (Bull 1992). Residues above tolerance limits do occur in cooked food. Consumption of contaminated food is an important route of human exposure to pesticide residues and may pose a public health risk (Macintosh et al. 1996). Many of these chemical residues, especially derivatives of chlorinated pesticides, exhibit bioaccumulation which could build up to harmful levels in the body as well as in the environment (Walter 2009). Consequently, pesticide exposure can cause a variety of human health problems, both chronic and acute in both farmers and consumers. Major health impacts from chronic exposure include cancers, reproductive and endocrine disruption, neurological damage, and immune system dysfunction (EPA 2007, Moses 1999, Sanborn et al. 2004). The World Health Organization (WHO) and the United Nations Environment Program estimated that nearly 4.0 million people suffer from severe pesticide poisoning and its rate is 2-3 per minute, with approximately 20,000 workers dying from exposure every year, the majority in developing countries (Kishi et al. 1995, Pimental et al. 1992, Rosenstock et al. 1991, Sarker et al. 2002).

Sudan has a great potential to produce good quality fruits and vegetables. This is because of its large areas of fertile soil, an abundant amount of water from rivers, rains, and underground water, the suitable wide range of climate which allows variability of crops. Many types and cultivars of fruits and vegetables can be produced almost all the year round due to the climatic variations plus available land and water. This large potential could supply both local and export markets. The most important vegetables are onions and tomatoes followed by potatoes, okra, cucumbers, eggplant, watermelons, pumpkins and a number of leafy vegetables. Vegetables are grown in small plots with pumped water including the national corporations such as Gezira Scheme where about 30,000 ha are devoted to vegetables (Elbashir 2013). Greenhouse cucumber production is very popular in many areas of the world. The cucumber is a warm season crop with the required growing condition of 80° F to 85° F and plenty of sunlight. Greenhouse seedless cucumbers have a high nutrient requirement and grow very rapidly when supplied with sufficient nutrient and have very large leaves (Hochmuth 2012). A greenhouse may simply be a protective structure supporting by their shade or screen cloth with or without side curtains, or it may be a permanent structure in which the internal environment (i.e., air temperature and movement, relative humidity, carbon dioxide content ... etc.) can be controlled. In the recent greenhouse systems, hydroponic growing systems are employed (Jensen 1997). The primary advantage with greenhouses are that any crop can be grown in any season of the year depending on the market demand; excellent quality of the produce. In general, greenhouses facilitate the control of environmental conditions and provide protection against heavy rain and excess irradiation (Moller et al. 2004). Previous works were carried out on tomato grown in greenhouse and found some of them contaminated with several insecticides (Hammad et al. 2015; 2017). Therefore, this study was aiming to determine the levels of pesticide residues on vegetables grown by the greenhouse farms in Khartoum State when they are ready for transportation to the markets.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

The technical grade pesticide standards were used for standardizations. Analytical standards of diazinon, malathion, chlorpyrifos, ethephon, profenofos, and oxyfluorfen (~99% pure) were obtained from the Plant Protection Directorate (Ministry of Agriculture, Sudan). The standards were stored in a freezer at - 10°C. Standard solutions of 2 mg/ml⁻¹ of these pesticides were made by dissolving 20 mg from each of the analytical standards in 10 ml of n-hexane. All solvents used were of analytical grade or similar quality. The solvents used (acetone, dichloromethane, toluene, and n-hexane) were HPLC grade (Scharlau, Spain). Other reagents such as anhydrous, Florisil® (60-100) mesh and sodium chloride were purchased from Alwaldain Co., Sudan.

2.2 Study area/setting:

Khartoum State is the capital of Sudan and it is the largest state population. The estimated population of the state is 7, 413, 239 inhabitants. The state is administratively divided into seven localities. It is bordering Gezira and White Nile States in the south, Northern State and River Nile in the north, Gedarif and Kassala States in the east and North Kordufan in the west. Both Blue Nile and White Nile congregate to form the River Nile in the state. The population depends on government employment, trade and agricultural activities. Quite a sizable number of people migrate to the capital from all other states.

The state is heavily busy with mixed farming along the Nile banks and in rural artificially irrigated huge areas. The usual diet in Khartoum depends very much on different varieties of vegetables which are mostly grown in Khartoum State and nearby areas of other states.

2.3 Sample size and sampling technique:

The desired sample size was calculated through the following equation:

$$n = \frac{N}{1 + e(N-1)}$$

Where:

- n = the required sample size
- N =the total population (49 farms)
- e = error allowed by using (95%) confidence level = 0.05.
- by substituting the given values of N and e in the equation; then:

n = 49/1 + (0.05)*(49 - 1) = 14.4, by rounding then n = 14 farms

For distributing the desired sample size (selected farms between the localities of Khartoum state), the proportion according to the magnitude of each locality from the total population had been used as shown in Table 1.

Locality	No. of Farms	No. of Samples
Umbada	17	5
Omdurman	3	1
Karri	2	1
Sharg Elneel	5	1
Bahri	12	3
Jabal Awliya	8	2
Khartoum	2	1
Total	49	14

Table 1: Distribution of the Samples by Localities

All farms of each locality had been listed and given serial numbers, and then by using simple random sampling technique, 14 farms had been selected.

The most grown vegetables during the study period were cucumber and tomato. A total of 19 cucumbers and tomato fruit samples were collected from the fourteen selected farms during the growing season of 2016/2017 in the seven localities of Khartoum State, Sudan. Fourteen samples of cucumber were collected from greenhouse farms as following: in Bahri locality; Khartoum Bank (Abu Haleima), AlHaya Al Arabia and Khartoum University farms; Jebel Awliya: Ziyada and MK farms; Khartoum locality: MSE farm; Umbada locality: AM, Elsha'er, YAY, ME and MEE farms; SE: Elzawaya farm; Umdorman locality: Elwalda farm; and Karari locality: Eltiseen farm. The five samples of tomato fruits were collected from these farms; AM, Elsha'er, YAY, ME and MEE of Umbanda locality. Each selected farm had been visited during the samples collection and one sample of 1-2 kg was collected randomly from each vegetable (tomato and cucumber). The collection process was done from the amount ready to be transported to the market. The collected samples had been kept in polythene bags, labeled and immediately taken to the pesticides laboratory of the Faculty of Agriculture, University of Khartoum for further analysis. Each sample was finely chopped using a pre-cleaned knife and mixed thoroughly to be homogenized prior to the extraction.

2.4 Extraction and Partitioning

Extraction was done according to the methods of Specht and Winkelman (1980) and Pang et al (1999). Forty grams per sample were blended with 5 ml water and 100 ml acetone in a high-speed chemical resistance blender (National Analytical Corporation, Mumbai, India) for two minutes. The extract was collected in an Erlenmeyer flask and filtered through a fast rate filter paper (Whatman No. 1) in a Buchner funnel. The Erlenmeyer flask was rinsed with a little water and cleaned with acetone and the extract was filtered. The combined filtrates were collected in an Erlenmeyer flask for partitioning.

Extracts from each sample were transformed into a 500 ml separation funnel. Forty ml of dichloromethane and 10 ml of saturated NaCl solution were added. The mixtures were carefully shaken for 2 minutes with an open top to reduce pressure and left to stand for 10 minutes to allow separation of layers. The organic layer was collected and then re-extracted with 50 ml of dichloromethane. The combined extracts of dichloromethane were filtered through cotton wool and mixed with 25 gm of anhydrous sodium sulfate which was added to improve the extraction of polar pesticides and for its moisture absorbing ability. The products were then collected in 500 ml round-bottom flasks. Extracts were again refiltered through cotton wool and a 3 cm layer of anhydrous sodium sulfate in a separation funnel. The solvent was removed to dryness by a rotary evaporator (Buchi, Postfach, Switzerland) operating under vacuum at a temperature of 40°C. Dried extracts were dissolved in 10 ml of n-hexane and kept in closed vials at -10°C for clean-up and pesticide residue analysis.

2.5 Cleanup

Sample cleanup followed the methods of Specht and Winkelman (1980) and Pang et al (1999). Sample cleanup was done using a solid phase extraction (SPE) column containing Florisil[®] and anhydrous sodium sulfate. The column was first rinsed with a few ml of hexane. Extracts from each sample were added as soon as the hexane dried in the top of the Florisil[®] layer and was then eluted by a 200 ml of toluene: acetone in a 19:1 mixture. Elutes were concentrated to dryness by rotary evaporation. The dry powder was dissolved in 10 ml of hexane, transferred to a 10 ml volumetric flask and stored at -10° C for subsequent residue analysis by GC-FID.

2.6 Chromatographic instrumentation

The Gas chromatography instrument (Shimadzu Model -2010, Japan), was fitted with a DB-5 (5% phenylmethyl polysiloxane) capillary column (30 m × 0.25 mm i.d., 0.25 μ m film thickness), coupled with a flame ionization detector (FID), was employed under the following operating conditions: injection temperature of 280°C; FID temperature of 300°C; column temperature of 250°C; N2 flow rate at 1.5 ml min⁻¹ as the carrier gas; the N2/air makeup gas flow rate was 30 ml min⁻¹; and splitless injection with the opening of the splitter 0.5 min after injection. The column temperature was started at 80°C for 1 minute, and then was increased to 150°C at a rate of 15°C min⁻¹, followed by a final increase to 250°C at a rate of 10°C min⁻¹until the end of the sample analysis. The total retention time was 40 minutes. For all samples the injection volume was 1 μ L.

2.7 Preparation of standard solutions of pure pesticides

Technical standards solutions (2 mg ml⁻¹) of diazinon, malathion, chlorpyrifos, ethephon, profenofos, and oxyfluorfen were made by dissolving 20 mg from each of the analytical standards in 10 ml n-hexane, then three concentrations of the standard solution of each pesticide were prepared. These were stored in the dark at 4° C in order to be used to create calibration curves. The concentrations of the individual pesticide residues in tomato and cucumber samples are presented in Table 3. The concentration of pesticide residues were determined using external calibration and the formula below:

Concentration in mg kg⁻¹ = $= ABV_t/V_iW_s$

Where:

A= response factor (1/slope from calibration curve)

B = peak area

 $Vt = extract volume in \mu L$

Vi= volume injected in μL

Ws= weight of tomato or cucumber sample (kg)

The limit of detection (LOD) for diazinon, malathion, chlorpyrifos, ethephon, profenofos, and oxyfluorfen were determined from the signal-to-signal ratio using the equations:

LOD = 3*SD (standard deviation) of intercept/slope

The LODs were calculated for all mentioned pesticides and ranged between 0.01 and 0.20 mg kg⁻¹. The R^2 ; regression coefficient values, were between 0.998 and 0.999 (Table 2).

Table 2: Retention Time, R2 and LOD for pesticides screened for tomato and cucumber fruit samples by GC-FID

Pesticide name	Retention time	Break Area	R2	LOD (mg/kg-1)	
diazinon	diazinon 7.476 51469 0.9		0.9980	0.05	
malathion	n 13.325 2019836		0.9985	0.017	
chlorpyrifos	chlorpyrifos 13.801		0.9981	0.001	
ethephon	15.845	293517	0.9968	0.18	
profenofos 16.718		229920	0.9990	0.20	
oxyfluorfen	17.185	3470768	0.9981	0.01	

LOD; limit of detection, R2; regression coefficient

3. RESULTS AND DISCUSSION

The presence of pesticide residues is a concern for consumers because pesticides are known to have potentially harmful effects to other non-targeted organisms than pests and diseases. The major concerns are their toxic effects such as interfering with the reproductive systems and foetal development as well as their capacity to cause cancer and asthma (Gilden et al. 2010).

A total of nineteen samples (five tomato and fourteen cucumber fruits) were collected from 14 greenhouse farms in the seven localities of Khartoum State (Sudan) and then were analyzed by using Gas chromatographic (GC) methods. Tables 3 and 4 show that all samples of the cucumber collected from the farms were contaminated with different pesticides, 12 samples (85.7%) had ethephon residues at levels ranged from $17.42 - 167.19 \text{ mg kg}^{-1}$, 5 samples (35.7%) contaminated with diazinon at the level of residues ranged of $0.21 - 25.86 \text{ mg kg}^{-1}$ and all were exceeding the MRLs (0.1 mg kg⁻¹) established for cucumber by Codex Alimentarius (FAO/WHO, 2009), 4 samples (28.6%) contaminated with malathion at the level of residues ranged between $7.87 - 9.45 \text{ mg kg}^{-1}$ and all are more than recommended MRLs (0.2 mg kg⁻¹); 2 samples (14.3%) contaminated with Chlorpyrifos at level of residues: 6.65 and 7.00; 2 samples (14.3%) contaminated with oxyfluorfen at level of residues: 0.05 and 9.50 mg kg⁻¹. No MRLs set for ethephon, chlorpyrifos, and oxyfluorfen in cucumber by Codex until the time of this study. These results were higher than the results of the study conducted in Khartoum in 2014 (Hammad et al. 2015) and the results of other studies (Tahany et al. 2011, Chauhan et al. 2012, Elbashir et al. 2013). The cucumber pesticides residues measured in our findings were higher than that found in other studies (Azadeh et al. 2016, Bozena et al. 2015).

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Table 3: Concentrations and MRLs of Five Pesticide Residues in Tomato and Cucumber Fruits Samples Collected from the Selected Greenhouses in Khartoum State

District	Farm Name	Type of vegetable	No. of Samples Collected	Name of Pesticide Found Pesticide Ievels (mg/kg)		No. of samples with pesticide concentration exceeded MRL mg/kg	Times of exceeding MRL	
Bahri	Khartoum Bank (Abu Haleima)	Cucumber	1	1 Ethephon 25.15 NA				
Bahri	AlHai'a Al Arabia	Cucumber	1	Chlorpyrifos	6.65	NA		
Jebel Awliya	Ziyada	Cucumber	1	Diazinon Malathion	0.21	0.1	1	2
Jehel Awliva	МК	Cucumber	1	Ethenhon	106.89	NA	-	
Bahri	Khartoum University	Cucumber	1	Ethephon	132.09	NA		
Khartoum	MSE	Cucumber	1	Ethephon	147.50	NA		
				Diazinon	2.55	0.5		5
Umbada	AM	Tomato	1	Ethephon	136.87	2	1	68
Umbada	Elsha'er	Cucumber	1	Ethephon	161.37	NA		
Umbada	AM	Cucumber	1	Ethephon	134.36	NA		
Umbada	YAY	Cucumber	1	Ethephon	167.19	NA		
Sharq ElNeel	Elzawaya		1	Diazinon	10.58	0.1		106
		Cucumber		Malathion	8.28	0.2	1	41
				Ethephon	58.14	NA	1	
	Elwalda	Cucumber	1	Diazinon	1.25	0.1		12
Umdorman				Ethephon	17.42	NA	1	
				Oxyfluorfen	0.05	NA	1	
IImbada	ME	Cucumber	1	Diazinon	25.68	0.1		257
				Malathion	9.45	0.2		47
omoudu				Chlorpyrifos	7.00	NA		
				Ethephon	106.17	NA	1	
Umbada	MEE	Cucumber	1	Ethephon	28.34	NA		
				Oxyfluorfen	9.50	NA		
				Diazinon	24.63	0.1		246
Karari	Eltiseen	Cucumber	1	Malathion	8.46	0.2		42
				Ethephon	107.39	NA	1	
Umbada	ME	Tomato	o 1	Ethephon	109.60	2		55
				Profenofos	11.62	10	1	
Umbada	MEE	Tomato	1	ND				
Umbada	Elsha'er	Tomato	1	Ethephon	12.01	2	1	6
Umbada	YAY	Tomato	1	Malathion	39.70	0.5		/9
Tatal				Eurephon	108.88	2	1	
Total			19				9	
%							4/%	

NA: Not Available in Codex Alimentarius; ND: Not detected

Table 4: Pesticide Residues (mg/kg) in Cucumber and Tomato Collected from Study areas, Khartoum

Vegetable	# Samples	Insecticide Name	# Sample contaminated	%	Range of Residues	MRLs	# Samples exceeded
					(mg/kg)		MRLs
Cucumber	14	Ethephon	12	85.7	17.42 - 167.19	NA	0
		Diazinon	5	35.7	0.21 - 25.86	0.1	5
		Malathion	4	28.6	7.87 - 9.45	0.2	4
		Chlorpyrifos	2	14.3	6.65 - 7.00	NA	0
		Oxyfluorfen	2	14.3	0.05 - 9.50	NA	0
		Profenofos	0	0.0			0
Tomato	5	Ethephon	4	80.0	12.01 - 136.87	2	4
		Diazinon	1	20.0	2.55	0.5	1
		Malathion	1	20.0	39.7	0.5	1
		Chlorpyrifos	0	0.0	0	0	
		Oxyfluorfen	0	0.0	0	0	
		Profenofos	1	20.0	11.62	10	1

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The result in Table 4 showed that 4 out of 5 samples (80%) of the tomatoes were contaminated with deferent pesticides as following: 4 samples have ethephon residues at levels ranged between: $12.01 - 136.87 \text{ mg kg}^{-1}$ which were higher than the maximum residue limits established for ethephon in tomatoes (MRLs:2 mg kg⁻¹), 1 sample (20%) contaminated with diazinon at the level of residue 2.55 mg kg⁻¹ which exceeded the MRL (0.5 mg kg⁻¹) set for tomatoes by Codex, 1 sample (20%) contaminated with malathion at level of residue 39.7mg kg⁻¹ which was more than MRL (0.5 mg kg⁻¹), 1 sample (14.3%) contaminated with profenofos at level of residue 11.62 mg kg⁻¹ which was more than MRL (10 mg kg⁻¹). No Chlorpyrifos and oxyfluorfen residues had been detected in undertaken tomato fruit samples. One of the analyzed tomato fruit samples was free from pesticides residues.

Generally, the results of analyzed samples revealed that one sample had contained no residue while the remaining 95% (18 out of 19) of the samples had one or multiple residues of more than one pesticide found in the same sample (Table 3). A single residue was detected in 47% of the samples, and two, three, and four residues in 26%, 16%, and 5% of the samples, respectively. No sample was contaminated with more than four pesticide residues.

The combinations of two pesticides residues detected in the same sample were found in three samples of tomato fruits as follows: diazinon and ethephon in one sample collected from AM farm, profenofos, and ethephon in one sample collected from ME farm and malathion and ethephon in one sample collected from YAY farm (Tables 3 and 4).

In addition, the same combinations of two pesticides residues or more detected in the same sample were found in five samples of cucumber as follows: diazinon and malathion in one sample collected from Ziyada farm, diazinon, malathion and ethephon in two sample collected from Elzawaya and Eltiseen farms, diazinon, oxyfluorfen, and ethephon in one sample collected from Elwalda farm and diazinon, malathion, chlorpyrifos, and ethephon in one sample collected from ME farm (Table 3 and 4).

It was noted that ethephon had been used excessively by 78.6% (11 out of 14) of the selected greenhouses farms.

As reported by (Allen et al. 2015), with the intensive use of pesticides in greenhouses, crops grown under these protected environments may be prone to an increased level of pesticide residue than similar crops grown in the open field. Also, farmers tend to apply pesticides too close to harvest because of lack of adequate knowledge regarding the safe and judicious use of pesticides (Jallow et al. 2017), potentially contaminating the crop prior to sending their produce to the market.

The occurrence of multiple residues in some of the samples analyzed is likely to be a consequence of the application of different types of pesticides to protect a crop against different insect pests and diseases, especially vegetable crops in greenhouse environments where the incidence of pests can be extremely high (Berlinger et al. 2002). Also, the occurrence of multi-residue pesticide contamination in different commodities has been reported in other investigations (Allen et al. 2015, Osman et al. 2011).

From the results of this study, it is believable to state that farmers were not following proper safety measures to use the pesticides in correct dosages and at standard pre-harvest intervals as mentioned on the bottles of the pesticides. The high pesticide residue levels detected in some samples of this study would suggest that these pesticides have been used comprehensively, which might lead to serious health problems not only to the farmers but also to the consumers.

4. CONCLUSION

This study revealed the presence of pesticides residues in tomatoes and cucumbers grown in the greenhouse farms in Khartoum (Sudan) at the levels higher than the maximum residue limits (MRLs) set by Codex Alimentarius for these two fruits. 95% of tomato and cucumber samples contained pesticide residues. From the results of this study, it is believable to state that farmers were not following proper safety measures to use the pesticides in correct dosages and at standard preharvest intervals as mentioned on the label of pesticides containers. The high pesticide residue limits detected in the study indicated that pesticides have been extensively applied, which might lead to serious health problems not only to the farmers but also to the consumers.

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Conflict of interest

The authors have declared no conflict of interest.

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