

Determination of Organochlorine and Pyrethroid Pesticide Residues in Some Vegetables by QuEChERS Method and Gas Chromatography Triple Quadrupole Mass Spectrometry

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Abstract: *Amaranthushybridus* (spinach), *hibiscus esculentus* (okra) and *Telfairiaoccidentalis* (fluted pumpkin leaves) are three staple vegetables commonly consumed by residents of Minna, Nigeria. The cultivation of these vegetables is accompanied by the use of pesticides and other agrochemicals. Most of these pesticides are resistant to physical, chemical and biological degradation and remain as residues accumulating in both aquatic and terrestrial food webs causing some diverse health effects to humans and animal. The concentration of organochlorine and pyrethroid pesticide residues in *Amaranthushybridus* (spinach), *hibiscus esculentus* (okra) and *Telfairiaoccidentalis* (fluted pumpkin) sampled from seven farms in Minna, Nigeria were investigated using gas chromatographytriple quadrupole mass spectrometry. The analysis showed the presence of cypermethrin in concentration range of 0.51 to 9.95 µg/mL in two samples of spinach. The presence of heptachlor was however not confirmed in these samples. None of the pesticides under investigation was detected in any of the okra and fluted pumpkin samples.

Keywords: *Amaranthushybridus*, Gas chromatography/mass spectrometry, *Hibiscus esculentus*, Pesticides, *Telfairiaoccidentalis*.

1. Introduction

Pesticides are widely used in fruits and vegetables because of their susceptibility to insects and disease attacks [1]. Pesticide residues in food commodities are a fast growing global problem, raising a number of environmental concerns such as potential toxicity to humans and other animals [2]. Most pesticides are resistant to physical, chemical and biological degradation and accumulate in both aquatic and terrestrial food webs [3]. Over 98% of sprayed insecticides and 95% herbicides reach a destination other than their target species, including non- target species, air, water and soil.

More than 700 pesticides are registered for use in the world and more continue to persist in the environment even though they are no longer being applied [4]. According to the Stockholm Convention on Persistent Organic Pollutants (POPs), 10 of the 12 most dangerous and persistent organic chemicals are pesticides [5]. Persistent pesticides are highly toxic, causing an array of diverse effects, notably death, diseases and birth defects among humans and animals [6].

Pesticide residues are very small amounts of pesticides that can remain in or on a crop after harvesting or storage and make their way into food chain [7]. They can remain even when pesticides are applied in the right amount and at the right time. Pesticide residue refers not only to the active ingredient but also to any derivatives of pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance [8]. Organochlorine pesticides (OCPs) were widely used during the last century for agricultural and non-agricultural purposes throughout the world. Even though they have been banned in most countries, due to their high persistence in the environment, their residues are still found in many matrices [9]. Pyrethroids have greater photostability, enhanced insecticidal activity, and relatively low toxicity as compared with organochlorine and organophosphorus pesticides [1]. Improved analytical protocols that are rapid and require minimum effort are continually being sought to test foods for pesticide residues. In addition, increasing attention is being devoted to the development of sample preparation methods which can be integrated with the separation and detection steps, thereby permitting integration of the entire analysis scheme. Such an approach will facilitate the routine analysis of large numbers of samples [10]. The method of extracting pesticide residues from food samples and preparing them for analysis is a time consuming, expensive, and labour intensive process. To address this problem, Anastassiades and his colleagues [11] introduced an improved extraction procedure known as Quick, Easy, Cheap, Effective, Rugged and Safe, the QuEChERS (pronounced “catchers”) based on work done and published by the US Department of Agriculture Eastern Regional Research centre in Wyndmoor PA. It is an extraction method for pesticides in fruits and vegetables, coupled with a clean-up method that remove sugars, lipids, organic acids, sterols, proteins, pigments and excess water. The technique

offers a user-friendly alternative to traditional liquid-liquid and solid phase extractions by employing the dispersive solid phase extraction (dSPE). QuEChERS technique has resulted in a practical reliable method for pesticide residues at low detection limits with good precision and accuracy for over 150 pesticides in a wide range of fruits and vegetables [12].

The list of pesticides used on vegetables in West African countries include organochlorine and organophosphorus pesticides declared by WHO as obsolete or banned [13]. It has also been observed by some authors that literature on pesticides residues in vegetables and other foodstuffs from Nigeria are sparse [14,15]. Spinach, okra and fluted pumpkin leaves are three staple vegetables commonly used in making soups by residents of Minna, Nigeria. The vegetables are grown in Minna and also brought to the town from neighbouring villages. Some of the pesticides determined include those used for the cultivation of vegetables in the area. It is the aim of this study to determine the presence of organochlorine and pyrethroid pesticides residue in the three vegetables sampled from seven selected farms in Minna Niger State, Nigeria.

2. Experimental

2.1 Reagents

Acetonitrile and glacial acetic acid of analytical grade were used. Packets of pre-weighed extraction salts each containing 6.0g anhydrous $MgSO_4$, 1.5g anhydrous sodium acetate (NaOAc) were obtained from Restek United Kingdom Ltd. Pre-packed mini centrifuge tubes (2mL) each containing 150mg of $MgSO_4$, 50mg primary, secondary amine (PSA), and 50mg graphitized carbon black (GCB) also from Restek UK Ltd. The certified pesticides standards used were 608 calibration mix (for heptachlor, aldrin, endrin), AOAC QuEChERS QC spike mix (for lambda-cyhalothrin) and cypermethrin isomers mix purchased from Restek Bellefonte, Pennsylvania U.S.A.

2.2 Sampling

The samples that were used for the research were spinach (*Amaranthushybridus*), okro (*Hibiscusesculentus*) and fluted pumpkin leaves (*Telfairiaoccidentalis*). These vegetables were taken from seven selected farms in (Okada, Kpakungu, Dutsenkura, Keterengwari, Chanchaga, Mandela and Saukakahuta) areas of Minna. An imaginary W was drawn on the particular area in the farm where the samples are to be taken, then primary samples were collected at intervals along the four arms of the W following standard procedures [16].

2.3 Sample Processing

Samples were cut coarsely with a knife and kept in separate labelled polythene bags in a freezer at around $-18^{\circ}C$ overnight prior to milling. Each sample was separately homogenized by grinding the frozen vegetable in a ceramic mortar.

2.4 QuEChERS Sample Extraction and Clean-up

Extraction of samples for the analysis was according to the AOAC International Official method 2007.01 [17]. The solvent used for the analysis was 1% acetic acid in acetonitrile. 15 g each of the homogenized samples were weighed in 50ml polypropylene tubes and labelled. 15 ml of the solvent and 6g anhydrous magnesium sulphate and 1.5 g anhydrous sodium acetate were added into the extraction tube. The tube was then capped and shaken immediately and vigorously for one minute. After vortex the tube was centrifuged for five minutes at 3000 rpm for phase separation. Using the extracts resulting from the phase separation, 1 ml of the supernatant was transferred into a 2 ml polypropylene tube containing pre-weighed dSPE sorbents: 50 mg primary secondary amine (PSA), 150 mg magnesium sulphate ($MgSO_4$) and 50 mg graphitized carbon black (GCB). Each tube was labelled, capped, shaken vigorously for 2 minutes and then centrifuged for 5 minutes at 3000 rpm to separate the solid materials. Extracts were carefully transferred to amber coloured vials and kept in the refrigerator prior to analysis by GC/MS.

2.5 Preparation of Calibration Curve

Stock solutions of the pesticides standards were prepared and then serially diluted to produce different concentrations of pesticides. Working solutions were prepared by appropriate dilution with 1% acetic acid in acetonitrile. Standard solutions of the pesticides were run on GC/MS triple quadrupole and mean peak areas were plotted against concentrations to obtain calibration curves of individual pesticides. GC/MS analysis was conducted at Materials Characterization Centre, Inc. University of Puerto Rico.

2.6 GC/MS Conditions

The GC/MS system comprises of BrukerSciion 451 gas chromatography equipped with electronic pressure control for split/splitless injector. A capillary fused silica column Rxi-5ms (30m x 0.25mm in length

and 0.25µm film thickness) was used due to its low bleed and high inertness characteristics. Aliquots of 1µL of samples were injected into the GC. The oven temperature was held at 70°C for 3 min then ramped at 8°C min⁻¹ to 270°C which was held for 5min. Carrier gas was high purity helium (99.999%) at a constant flow rate of 1mLmin⁻¹

The MS BrukerSciion triple quadrupole was operated in electron impact ionization (EI (+) external ion volume control 70eV). The mass range was 50-600m/z and the MS workstation software was used for data acquisition. The MS was used in full scan and then in selective ion monitoring (SIM) mode and three ions were selected and monitored for each pesticide. The Scion triple quadrupole (TQ) detector was used because of its high performance and reliability. It uses an innovative “lens free,” elliptical ion-path design that delivers ultra-high sensitivity and chemical noise reduction. EPA/NIST library of about 60,000 stored spectra was used for identification of the pesticides, a correlation reference of 999 was obtained showing how well the sample peak mass spectrum matches that of the standards. Identified pesticides were quantified using the external standard method of comparing sample peak areas with those of the pesticide standards under the same conditions.

3. Results And Discussion

The retention time widow and the ion mass used for the SIM mode are shown in TABLE 1.The chromatograms and mass spectra of the pesticides standards are as given in fig 1, 2 and 3.

Table 1: Retention times and the ion mass detected for each pesticide

Pesticides	Retention Time (min)	Ion Mass Detected (a.m.u)
Heptachlor	25.531	65, 100, 272
Aldrin	26.393	81, 263, 345
Endrin	29.360	66, 91, 263
Lambda-cyhalothrin	32.985	141, 181, 197
Cypermethrin	35.145 35.295 35.478	163, 181, 209

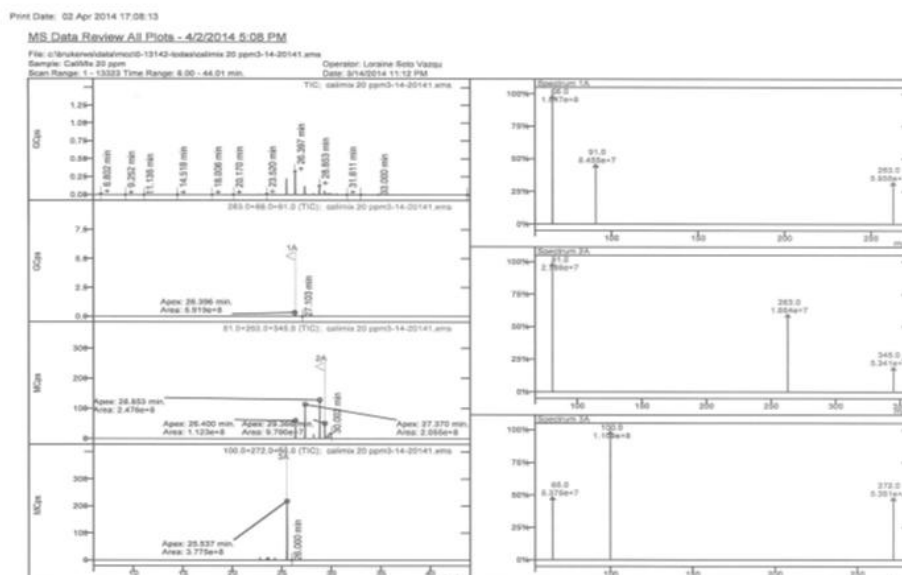


Figure 1: Chromatogram and mass spectra of heptachlor, aldrin, endrin Mix standard (SIM mode)

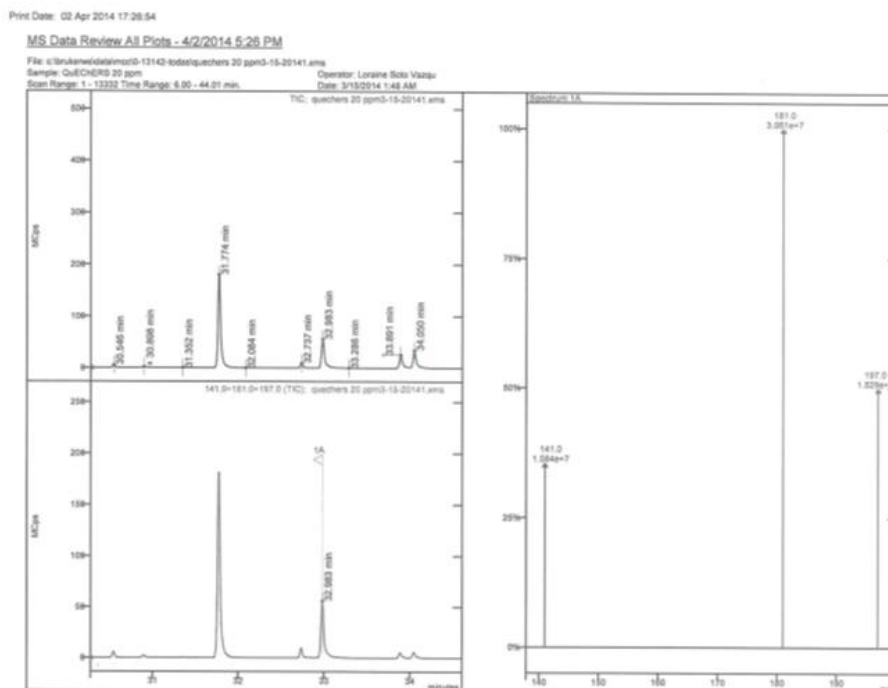


Figure 2: Chromatogram and mass spectra of lambda-cyhalothrin (SIM mode).

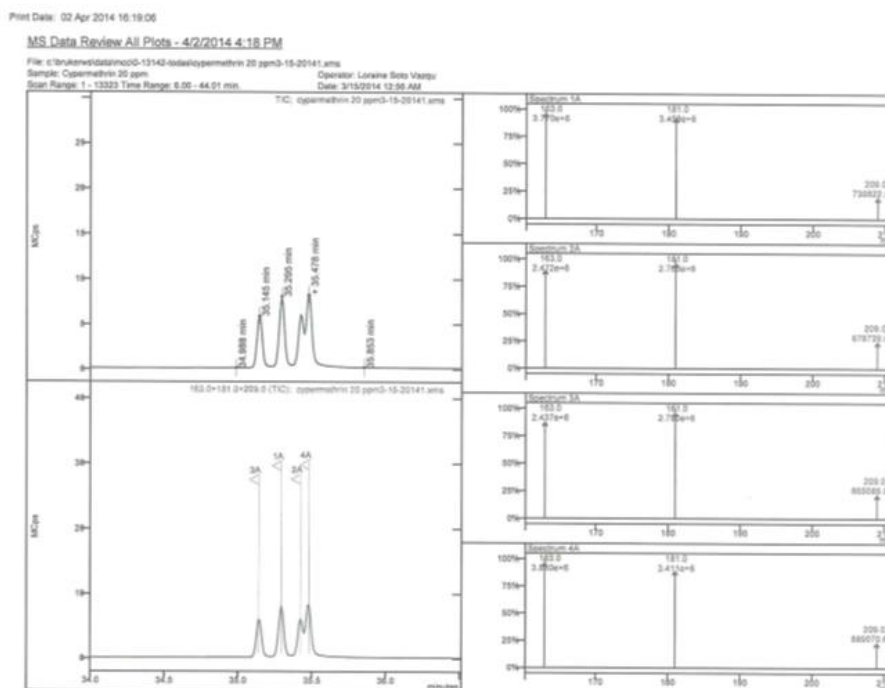


Figure 3: Chromatogram and mass spectra of cypermethrin isomers (SIM mode).

Cypermethrin was detected in spinach samples from farm E and F and heptachlor was also detected in sample fractions of spinach from farm E and F. No pesticide residue was detected in spinach samples from other farms. The okra and fluted pumpkin samples from all farms did not indicate the presence of any of the pesticides. Chromatograms and mass spectra of the spinach samples that contain pesticide residues are shown in fig 4 and 5.

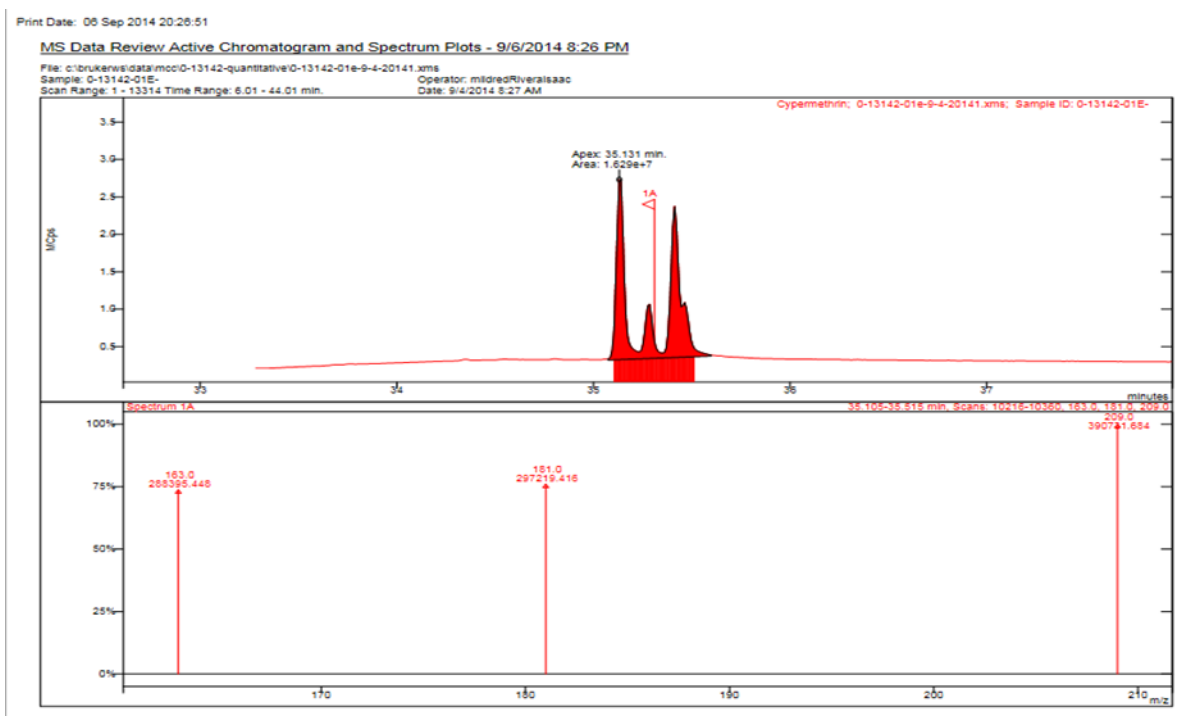


Figure 4: Chromatogram and mass spectra of spinach from farm E

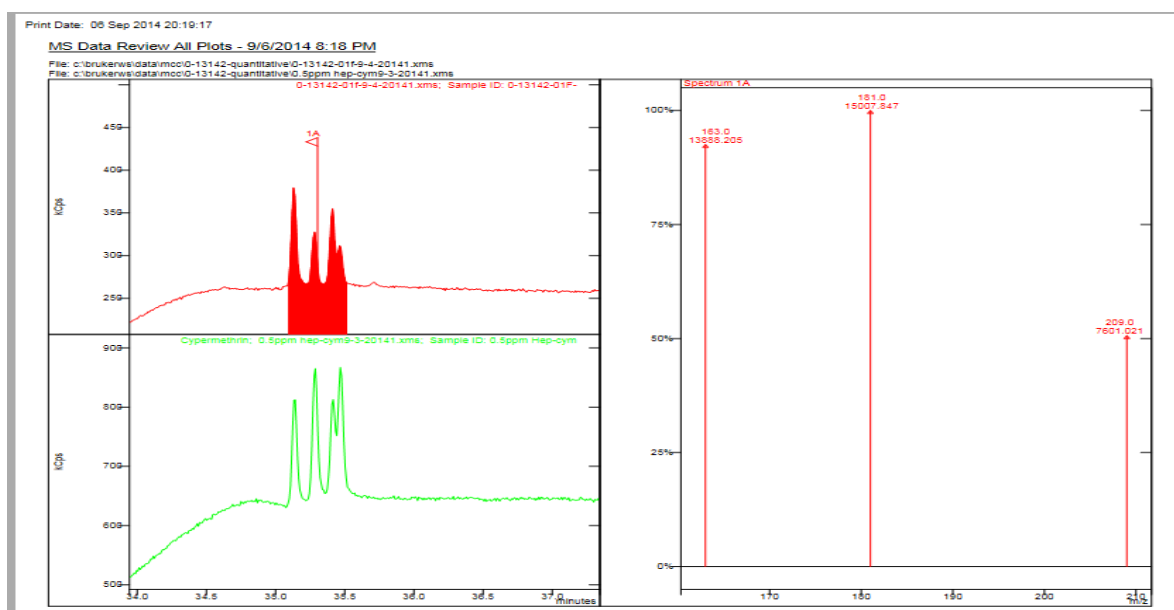


Figure 5: Chromatogram and mass spectra of spinach from farm F

To estimate the relative concentration of cypermethrin and heptachlor in spinach samples E and F, GC/MS analysis was repeated in duplicate using the SIM quantitative test for the pesticides. The linearity of the testing was obtained in the concentration range of 0.25, 0.50, 5.0, 10.0 and 20 µg/mL of organic extract. The ion ratio (quantifier/qualifier) were found to be consistent to the expected pattern over the entire calibration range used. The average response factors for the calibrations range had a RSD ≤ 15%, demonstrating the method's precision for analysing the pesticides in the samples. Fig 6 gives the calibration curve for cypermethrin. The presence of heptachlor was not confirmed because only the qualifier ion 65 a.m.u was found at the retention time that corresponds to heptachlor. Other relevant ions (100 and 272) were not detected, or if present they are below the detection limit of ≤ 0.05 µg/mL. The results obtained for the detected pesticides are given in TABLE 2. TABLE 3 gives the number of samples for this study and those with positive results.

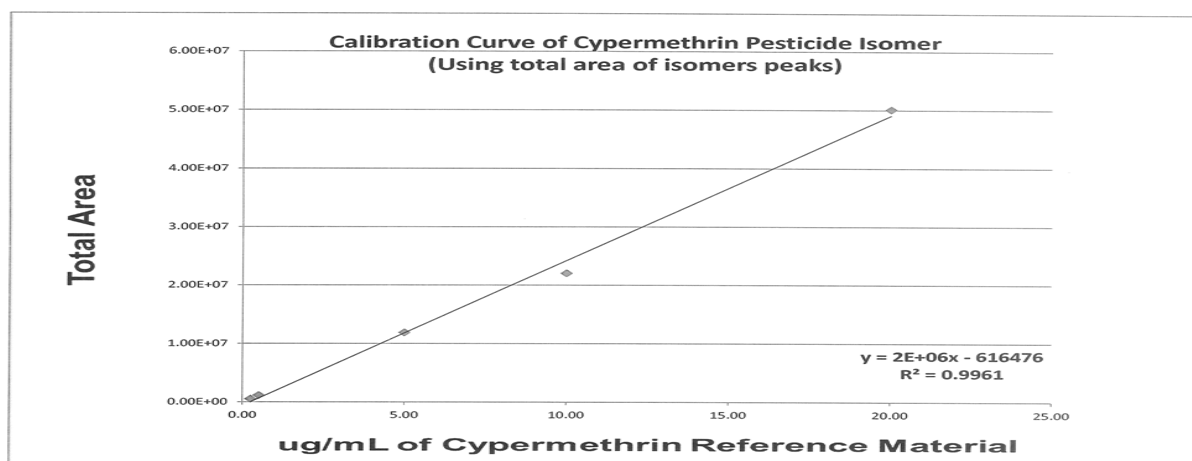


Figure 6: Cypermethrin calibration curve

Table 2: Pesticides Residue Result for Spinach Samples

Farm	Retention Times (min)	Ion Mass (a.m.u)	Pesticide	Pesticide Extract (µg/mL)	Average
A	Nil	ND	ND	ND	
B	Nil	ND	ND	ND	
C	Nil	ND	ND	ND	
D	Nil	ND	ND	ND	
E	35.14, 35.41, 35.29	163, 181, 209	Cypermethrin	8.65 ± 1.30	
F	25.52	65	Heptachlor	BDL (≤ 0.05)	
	35.14, 35.41, 35.29	163, 181, 209	Cypermethrin	0.60 ± 0.09	
G	25.52	65	Heptachlor	BDL (≤ 0.05)	
	Nil	ND	ND	ND	

Table 3: Names of farms, pesticides, vegetables, number of samples and number with detected pesticide residues.

Farms	Pesticides	Vegetables	No. Analyzed	Samples with Residues
Okada road(A)	Heptachlor	Spinach	7	2
Kpakungu (B)	Aldrin	Okra	7	-
Dutsenkura (C)	Endrin	Fluted Pumpkin	7	-
Keterengwari (D)	Cypermethrin			
Chanchaga (E)	Lambda-cyhalothrin			
Mandela (F)				
Sauk aka huta (G)				
Total	5	3	21	2

The FAO/WHO Codex Alimentarius commission gave the maximum residue level (MRL) for cypermethrin on leafy vegetables as 0.7µg/mL [18]. The spinach sample from farm E has value of 8.65±1.30µg/mL which is above the MRL, but the value (0.60±0.09µg/mL) from farm F is below the WHO maximum residue limit. The value from farm E calls for concern since it well exceeds the WHO limit, this may be due to non-observance of certain recommended agricultural practices like respecting the safety interval between the last pesticide application and the harvest of the crop or using the recommended rate of pesticide [19]. Washing of agricultural produce is also known to reduce the levels of residues, which can be dissolved or

physically dislodged from the raw product [19]. The low value ($0.60 \pm 0.09 \mu\text{g/ml}$) of farm F is also significant and should not be neglected since spinach is a vegetable eaten regularly in this part of the country. None of the samples run for this study gave retention times that corresponds to that recorded for lambda-cyhalothrin standard, indicating the absence of the pesticide or if present it was beyond the detection limit of this procedure. The presence of heptachlor was not confirmed during the SIM mode quantitation analysis of the samples. Only the qualifier ion 65 was found at the retention time that corresponds to heptachlor reference material. Other relevant ions (100 and 272) were not detected. The reason for this result could be the fact that the use of organochlorine pesticides has been scaled down due to the ban on their use in response to the Stockholm Convention on the use of persistent organic pesticides (POPs) [20].

4. Conclusion

Result of this study reveal that a pyrethroid(cypermethrin) was only present in some spinach samples and it was not detected in any of the okra and fluted pumpkin leaves. The level of cypermethrin in one of the samples was above the WHO MRLs which calls for concern. Although cypermethrin level was below the WHO MRLs in another sample, the presence and eventual biomagnification may pose a health risk considering that spinach is widely consumed in Minna. Heptachlor an OCP was only detected in some spinach samples but their level was below quantitation. Aldrin, endrin and lambda-cyhalothrin were also not detected in any of the samples analysed. It is essential to monitor the level of pesticides residues on a large scale in varieties of foodstuffs in order to minimize health hazards associated with pesticides.

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