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Detection Of Pendimethalin and Cypermetrin Residues in Locally Produced Tomato Using QuEChERS-HPLC Analysis

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ABSTRACT
This study investigated the levels of pendimethalin and cypermetrin residues in tomato sold in Malete market, Moro Local Government Area of Kwara State. Tomatoes were randomly collected from five different vendors in Malete market and analysis was performed using the QuEChERS (Quick, Easy Cheap, Effective, Rugged and Safe) method followed by chromatographic analysis using high performance liquid chromatography (HPLC) coupled to ultra-violet (UV) detector. Method validation of the study showed a linearity of the analytes which ranges from 5 – 500 µg/kg, with correlation coefficients greater than 0.999. The average recovery ranges from 75.6 to 111 % with relative standard deviation (RSD) from 2.74 to 9.03 %. The results indicated the presence of cypermethrin in analyzed samples at concentrations lower than the permissible maximum residue levels.
Keywords: Sample preparation, Pesticide residues, HPLC-UV, QuEChERS.

INTRODUCTION
In order to increase food production, many types of pesticides including herbicides have been developed. Pesticides are used to control pests and plant diseases that can cause to damage plants and reduce agricultural production (Fenoll *et al.*, 2007). The need for increase in agricultural food production is due to the greater demands for food, by the ever increasing world population (Abdulra'uf *et al.*, 2012a).
Pendimethalin (N - (1 - ethylpropyl) - 3, 4 – dimethy - 2, 6 - dinitrobenzenamine), a member of 2, 6 dinitro aniline herbicides, is widely used as a pre-emergence herbicide for selective control of weeds in crops (Tandon, 2008). Cypermethrin (*R S*) – alpha-cyano – 3 - phenoxybenzyl (1 *RS*) - cis, trans – 3 - (2, 2-dichlorovinyl) - 2, 2 - dimethylcyclopropanecarboxylate, is an alpha-cyano type II pyrethroid, which has been found to cause neurotoxicity in mammals and insects, is also used as insecticide due to their high insecticidal potency and relatively few side effects on birds and mammals (Muccio *et al.*, 1997). Cypermetrhin has long rudimental time and undergoes relatively rapid biotransformation and excretion in mammals (Cheng *et al.*, 2009; Zhang *et al.*, 2009).

The continued use of these pesticides in agriculture has resulted in environmental pollution and their bioaccumulation in the food chain. Due to their uses and potential harm to public health, several organizations including the Food and Agricultural Organization, the World Health Organization established and Codex Alimentarius Commission, the European Union Council Directives (European Union, 2011, 2012), set the Maximum Residue Level (MRL) for pesticides and other contaminants in food of plant and animal origin. The Codex Committee on Pesticide Residues and other regulatory standards were established based on an international legal framework guiding the trade and use of pesticides (Abdulra'uf *et al.*, 2016). Therefore, there is a need to develop an effective and efficient analytical method to determine their presence in food and the environment in order to determine if their presence conforms to the set limits of MRL.

Several methods have been developed for the analysis of pesticide residues in fruit and vegetable and most of these methods rely on solvent extraction and cleanup process which is time consuming and make use of toxic solvents. Microextraction techniques such as solid phase

microextraction, SPME (Arthur *et al.,* 1992), microextraction in packed sorbent, SBSE (Abdel-Rehim, 2004), liquid phase microextraction, LPME (Abdulra'uf *et al.,* 2012b) were developed to reduce or completely eliminate the use of toxic solvent, and these methods were found to be environmentally friendly and required less time however with some limitations (Sarafraz-Yazdi and Amiri, 2010).

Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method (Anastassiades *et al.,* 2003) developed recently is also less time consuming, with high selectivity and sensitivity, has gained popularity due to its safety, simplicity, affordability, effectiveness and efficiency (Sirhan *et al.,* 2011). In the present study, a simple, affordable, and reliable extraction method is developed for the analysis of cypermethrin and pendamethalin in tomato samples, using high performance liquid chromatography coupled to ultra violet detector (HPLC-UV). This choice of pesticides was informed by the fact that they are widely used by vegetable farmers in Ilorin, Kwara State, Nigeria.

MATERIALS AND METHODS
Reagents and Solutions
Cypermethrin and Pendimethalin standards of analytical grade (99%) at 100 µg/mL were purchased from Sigma Aldrich (St. Luis, MO, USA). A working standard solution containing the pesticides was prepared daily by diluting the stock standard in methanol to a concentration of 10 µg/mL and kept at 4 ℃ before use. Aliquot of 50 µL of the working standard solution was used to spike 5 mL of water for method development. A 5 g of the sample matrix was also spiked with a known amount of the working standard solution to concentration between 50, 100 and 150 µg/kg used for method optimization and validation studies. A concentration range of 10 – 500 µg/kg was prepared directly in the sample matrix for calibration purpose and method validation.

Sample Collection and Preparation
All tomato samples were collected from Malete Markets for multi-residue and multiclass pesticide residues analysis and the sample where kept in a freezer at 4℃ prior to analysis. The samples used for calibration and recovery studies were first analyzed to ensure the absence of the target pesticide residues (Cortés-Aguado *et al.,* 2008).

For modified QuEChERS method, 100 g of prewashed tomato sample was weighed, finely chopped and homogenized using a blender. Then 5 g aliquot of the homogenized sample was placed in a separate 20 mL polypropylene tube and left for 1hr at room temperature. This was followed by addition of 10 mL of acetonitrile and was manually shaken for 1 minute. $MgSO_4$ (2 g) and 0.5 g of NaCl were then added and shaken after which the mixture was centrifuged for 5 minutes at 5000 rpm. The supernatant was collected and filtered with a 45 µm polypropylene syringe filter. Exactly 20µL of the filtrate was then injected into the HPLC.

HPLC Analysis
A bulk scientific HPLC (BLC2O series) isocratic system coupled to UV-detector with variable wavelength, located in the Chemistry Department of Kwara State University, Malete, was used for the analysis of extracted pesticides. Chromatographic separation was carried out on Pinnacle DBAQ C_{18} column (250×4.6mm i.d, 5µm) Restek USA. HPLC analyses were performed at room temperature with a flow rate of 1 mL/min and injection volume of 20 µL. The detector wavelength was set at 290 nm. The mobile phase constituted methanol-water (80:20, %v/v), three (3) replicate injections were made for each sample and calibration curves were derived from a matrix matched solution. The average area of the elution peaks were plotted against the standard concentrations.

Method Validation
Method validation is a quality assurance step, which is used to determine if the in-house method developed is suitable for its intended purpose. It is essential to ensure optimal utilization of

analytical procedure (Chan, 2011). The analytical figures of merit were validated using external standard prepared in matrix-matched calibration standard. The calibration curve (Figure 1) of each pesticide was constructed using matrix sample spiked at eight different concentrations with the working standard solution. The prepared concentrations ranged from 5 to 500 µg/kg, and the peak area obtained for each analyte and the external standard was used to construct a calibration curve by plotting the peak area of each analyte as a function of concentration. Each concentration point was analyzed in triplicate in three different sample matrices. The intra- and inter-day precision, accuracy, selectivity and sensitivity, limit of detection (LOD), limit of quantitation (LOQ) and average recovery were determined briefly defined as follows:

Intra- and Inter-day precision describe the closeness or agreement between a series of

independent measurement obtained when an analytical method is applied in replicate to multiple sampling of homologous samples specified in terms of relative standard deviation (RSD). Intra-day precision describes the precision under the same experimental condition over a short period of time, while inter-day precision describes precision obtained under the same experimental condition carried out on different days. The intra-day precision was determined by performing three extractions in single day, while inter-day precision was determined based on three extractions per day for three days. A one-way single factor ANOVA was used to estimate the variance, which gives the total sum of square, between group mean square (BMS) and within group mean square (WMS). The BMS estimate the variance associated with the intra-day precision (within-day) and inter-day precision (between-day) variances:

$$\% \ RSD \ (Intra-day) = \frac{\sqrt{WMS}}{Average \ Relative \ Recovery} \ x \ 100$$

$$\% \ RSD \ (Inter-day) = \frac{\sqrt{\left(\frac{BMS-WMS}{N}\right)+WMS}}{Average \ Relative \ Recovery} \ x \ 100$$

Where: RSD = relative standard deviation; WMS = within group mean square; BMS = between group mean square; N = Number of replicate

LOQ is the lowest concentration of analyte that can be quantitatively determined with an acceptable level of accuracy and precision. LOD is the lowest concentration of analyte that can be detected but not necessarily quantified as an exact value under the optimized experimental conditions (ICH-Topic Q2 (R1), 2006). The LOQ was estimated based on the signal-to-noise ratio of 10:1, while the LOD was estimated based on the signal-to-noise ratio of 3:1 both of which were calculated using the following equations:

$$LOQ = \frac{10\sigma}{S} \qquad LOD = \frac{3\sigma}{S}$$

Where σ is the standard deviation of the response; S is the slope of the calibration curve.

RESULTS AND DISCUSSION
Analytical Method Validation
Analytical methods was validated in terms of linearity, accuracy, intra- and inter-day precision, limit of detection (LOD) and limit of quantification (LOQ) using the optimized parameters.

Linearity and Calibration Curve
The calibration parameters are as presented in Table 1. Linearity of the method was tested using standard solution of the target pesticides in concentration range of 5 – 500 µg/kg using a matrix-matched external standard calibration curve. The peak area was plotted against the concentration of the analyte. The calibration

curve (Figures 1a and 1b) were linear over the tested concentration with correlation coefficients greater than 0.99.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)
The LOQ and LOD determined using the standard deviation of the y-intercept of the regression line is shown in Table 1. The LOQ

was found to be 12.06 and 21.04 µg/kg, while the LOD was 3.62 and 6.31 µg/kg, for pendimethalin and cypermethrin respectively. The figures of merit obtained in this study were found to be comparable with values reported in related studies for the analysis of pesticide residues in fruits and vegetables using different extraction techniques (Abdulra'uf et al., 2012a)..

Table 1: Linearity and Calibration Parameters

Pesticide	Linear Range (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	MRL (µg/kg)	Linearity curve equation	r^2
Pendimethalin	5 – 500	12.06	3.62	50	y = 19.692x + 202.85	0.9998
Cypermethrin	5 – 500	21.04	6.31	500	y = 20.069xx + 49.307	0.9995

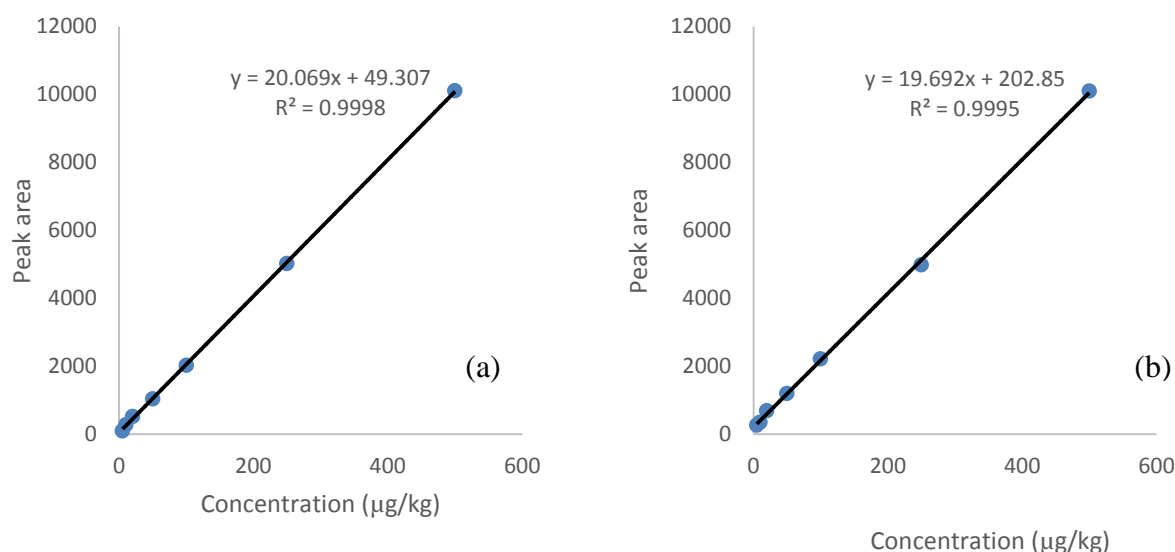LOQ (limit of quantitation); LOD (limit of detection); MRL (maximum residue level)

Figure 1: Calibration Curves of (a) Pendimethalin and (b) Cypermethrin

Recovery, Intra- and Inter-day Precision
The accuracy of the developed method was determined by estimation of the recovery of the selected pesticides spiked in the tomato matrix using three different concentrations (50, 100 and 150 µg/kg) and analysed in triplicate. As shown in Table 3, the relative recovery of the spiked sample ranged from 75.6 to 111 % which falls within acceptable limits according to SANCO guidelines (SANCO, 2011).

The results obtained for the recovery and precision studies were in accordance with acceptable practice (ICH-Topic Q2(R1), 2006)..

However, better recovery and precision were achieved at higher spiked level. All parameters were validated based on the method validation parameters of the European Union (SANCO, 2011).
Also presented in Table 3 is the precision of the developed method. The intra-day precision ranged from 2.74 – 8.54% and 4.86 – 5.84 % for pendimethalin and cypermethrin respectively, while the inter-day precision was found to range between 2.98 – 9.03 % and 4.74 – 6.03 % for pendimethalin and cypermethrin respectively, calculated based on the analysis of variance presented in (Tables 2a & 2b.)

Table 2: Summary of analysis of variance for (a) pendimethalin and (b) cypermethrin

(a) Pendimethalin						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	3	278	92.666	50.333		
Column 2	3	281	93.666	65.333		
Column 3	3	296	98.666	69.333		
			95.9993			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	62	2	31	0.5027	0.6282	5.1432
Within Groups	370	6	61.6666			
Total	432	8				
(b) Cypermethrin						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	3	139	46.333	1.333		
Column 2	3	136	45.333	9.333		
Column 3	3	131	43.666	4.333		
			45.111			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10.8889	2.0000	5.4444	1.0889	0.3950	5.1433
Within Groups	30.0000	6.0000	5.0000			
Total	40.8889	8.0000				

Table 3: Accuracy, Intra-and Inter-day Precision of the Developed Method

Pesticide	Spiked (µg/kg)	Recovery Accuracy (%)	Intra-RSD (%)	Inter-RSD (%)
Pendimethalin	50	96.8	2.74	2.98
	100	85.6	8.26	7.74
	150	105	8.54	9.03
Cypermethrin	50	90.8	4.96	4.98
	100	75.6	4.86	4.74
	150	111	5.84	6.03

The average recoveries and inter- and intra-day relative standard deviation were found to be acceptable according to the SANCO guideline (SANCO, 2011), which stated that the method performance criteria of average recovery should be in the range of 70 – 120 % with relative standard deviation (RSD) less than or equal to 20% (SANCO, 2011)..

Analysis of Real Sample

The presence of cypermethrin in all the tomato samples analyzed but were found to be below the maximum residue levels, while pendimethalin were not detected. The absence of pendamethalin could be as a result of the fact that it was used as a pre-emergence herbicide and must have been transported to the subsoil. The use and sales of pesticides is controlled and monitored by National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria. The agency adopts the CODEX limits and (when necessary) the MRLs of the importing country (Keri, 2009).

CONCLUSION

A simple, rapid and inexpensive sample preparation procedure using QuEChERS technique has been successfully developed. This is based on partitioning of the target analyte in acetonitrile /water mixture. It can be recommended as an alternative method to time-consuming and multistep solid phase extraction method. The study revealed the presence of cypermethrin in all the analyzed real samples, while pendimathalin was not detected in the samples. The detected pesticide was found at concentrations lower than the maximum residue levels. This shows that the farmers have been using the pesticides based on the recommended dosage and therefore the tomatoes sold in Malete market are save for consumption.

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