

Multi-residue levels of organophosphorus pesticides in cocoa beans from some cocoa farms in Ekiti state, Nigeria

O. A. Ibigbami^{1,2*}, A. F. Aiyesanmi¹, E. I. Adeyeye², A. O. Adebayo¹ and R. D. Aladesanwa³

¹Department of Chemistry, The Federal University of Technology, PMB 704, Akure, Nigeria

²Department of Chemistry, Ekiti State University, PMB 5363, Ado- Ekiti, Nigeria

³Department of Crop, Soil, and Pest Management, The Federal University of Technology, PMB 704, Akure, Nigeria

Abstract

This study investigated the level and occurrence of 13 organophosphorus pesticides (OPPs) residues in cocoa beans from some cocoa farms in Ekiti state, Nigeria. The pesticides in the cocoa beans were extracted and cleaned up on silica gel adsorbent. A gas chromatograph coupled with flame photometric detector (FPD) was used to quantify the pesticides. The reliability of the analytical method was examined by spiking the beans with known concentration of the pesticides and analysed following similar procedure of extraction and clean-up. The percentage of pesticides recovered ranged from 88% to 92%. Dichlorvos, mevinfos, diazinon, chlorpyrifos, dimethoate and parathion were detected with varying concentration in the bean samples whilst phosphamidon, pirimiphos-methyl, fenthion, bromophos, isofenfos, diclofenthion and ethion showed no detectable level. The concentration of OPPs ranged from ND - 58µg/kg and ND - 85µg/kg for the dry and wet seasons respectively. Analysis of variance (ANOVA) showed no significant variation ($p > 0.05$) in the levels of all detectable pesticides among the sampled farms whilst dichlorvos, diazinon, dimethoate and chlorpyrifos showed significant difference between both seasons. Residual levels of these detectable pesticides were below the maximum residue limits (MRLs) set by the European Union in food. Hence, the cocoa beans from the study areas are considered safe for consumption.

Keywords: Organophosphorus; Pesticides; Residue; Cocoa bean; Gas chromatography

Introduction

Cocoa (*Theobroma cacao*) is a small (4-8 m tall) perennial tree crop that primarily comes from three tropical regions- Southeast Asia, Latin America, and West Africa. The cocoa fruit is an egg-shaped red to brown pod that contains 30 – 40 seeds, each of which is surrounded by bitter-sweet white pulp. When the seeds are fermented and dried in the sun they are brownish red, and known as cocoa beans the principal ingredient of chocolate. Cocoa has a high food value as it contains as much as 20% protein, 40% carbohydrate, and 40% fat. It also contains theobromine, an alkaloid closely related to caffeine and phenols and flavonoids, antioxidants that can inhibit cancer and cardiovascular diseases. Cocoa beans are good sources of potassium, magnesium and iron. Nigeria is ranked fourth largest producer of cocoa beans in the world where cocoa is mostly grown in fourteen of the thirty six states. The size of cultivated area vary across states but an average cocoa farmer in Nigeria has a farm size of 2.5ha (Oguntade, 2003). On the whole, the average delivered per farmer in Nigeria is less than 5 bags per season (roughly 300 kg per hectare of cocoa) (Nwachukwu *et al.*, 2010). Nigeria cocoa production depends on pesticides to attain

acceptable levels of crop production. Hence, in obtaining good quality and high productivity of cocoa fruits, the cultivation of cocoa receive frequent application of various contact and systemic pesticides throughout the cropping season. Therefore, thousands of tonnes of pesticides (active ingredients) particularly OPPs are used annually, despite the ban in the use and production of OCPs globally. The present study aims at determining the levels and occurrence of thirteen OPPs (diazinon, mevinfos, dichlorvos, dimethoate, diclofenthion, phosphamidon, pirimiphos-methyl, chlorpyrifos, parathion, fenthion, isofenfos, bromophos and ethion) in cocoa beans from Ekiti state, south western Nigeria.

Materials and methods

Study area

The study area for the study covers eighteen cocoa-producing communities in Ekiti State, South-western Nigeria.

*Corresponding author e-mail: olayinkaibigbami@yahoo.co.uk

They include Ijero, Aramoko, Ido-Ile, Ikoro, Igede, Esure, Ise, Emure, Orun, Igbara-Odo, Ilawe, Ilupeju, Ire, Ayedun, Ipawo, Usi and Ifaki. Ekiti state where the samples were collected lies between latitude $7^{\circ} 25' - 8^{\circ} 2' N$ and longitude $5^{\circ} 00' - 6^{\circ} 00' E$. Ekiti State covers about 6,353 km² land area. Fig. 1 shows the map of Ekiti State indicating the Local Government Areas where the samples were collected.

Sample collection and preparation

Four cocoa trees were randomly selected from each area of the farm where two ripe pods were harvested from each of the cocoa trees and combined to make composite samples. Three samples were collected in each farm making a total of 54 cocoa beans samples for each season. The beans were allowed to ferment for 3-5 days before being sun dried and dehulled, grind in agate mortar and later blended with Excella Mixer Blender (Kanchan International Limited) to fine particles. The samples were then packed in glass sample bottles prior to further analysis.

Na₂SO₄ were mixed together in a pre-cleaned 250 mL conical flask. Fifty millimeters (50 mL) mixture of acetone and n-hexane (1:1 v/v) were mixed with the sample. This was followed by sonication in a high frequency ultrasonic bath for 10-15 minutes. The extract was then decanted into round bottom flask. The extraction process was repeated with additional 50 mL (acetone and n-hexane mixture), sonicated and allowed to settle and decanted into the same round bottom flask. The combined extract was concentrated to 2 mL using a rotary evaporator. The extract was re-dissolved in 5 mL n-hexane and later concentrated to 2 mL in a rotary evaporator at 40 °C.

The clean-up involved the use of a column of about 15 cm (length) x 1cm (internal diameter) packed with 2 g of activated silica gel and 1 g anhydrous Na₂SO₄ on top of the silica gel (adsorbent). The column was conditioned with 15 mL n-hexane prior to clean up. The extract was introduced into the column and eluted with 20 mL of n-hexane and

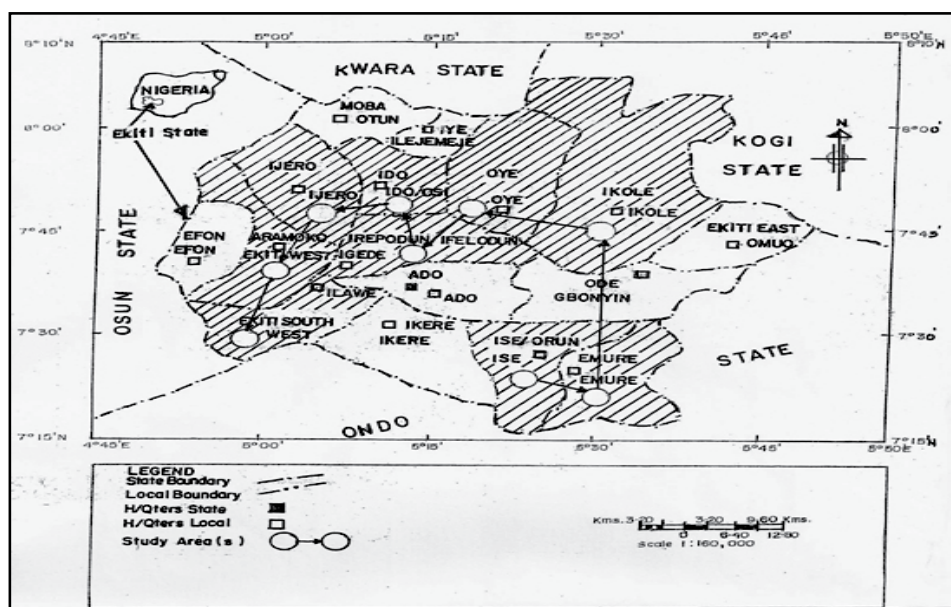


Fig. 1. Map of Ekiti State showing the position of the study areas

Source: Min. of Lands, Urban and Regional Planning, Ado-Ekiti/Dept. of Geography and Planning Science, Cartographic Unit, Ekiti State University (EKSU), Ado-Ekiti.

Extraction procedure and clean-up

Extraction of pesticides from cocoa beans samples was carried out by EPA 3550C method as described by USEPA (2000). About 20 g of each sample and 20 g of anhydrous

diethyl ether (1:1 v/v). The eluate was concentrated to dryness on the rotary evaporator and recovered into 2 mL n-hexane. The extract was transferred into glass GC vials for subsequent injection into the GC. The OPPs in the extracts were determined by a Gas Chromatograph (GC) coupled with flame photometric detector (FPD).

Gas chromatographic conditions

The gas chromatography conditions for the analysis were as follows: GC model: Agilent 7693 Autosampler; the carrier gas flow rate was 2.7 ml/min; Injector temperature: split injection: 20:1; carrier gas: nitrogen; inlet temperature: 250 °C; column type: HP5 MS; column dimension: 30 m x 0.32 mm x 0.25 µm; oven programme: initial temperature at 60° C for 1 minute, first ramping 10 °C/min for 14 min (200 °C); maintained for 2 min; second ramping at 10 °C/min for 8 min (280 °C); maintained for 3 min; detector: flame photometric detector (FPD); detector temperature: 250° C; hydrogen pressure: 22 psi; compressed air: 35 psi. The total run time was 28 minutes.

Identification, quantification and quality control

The validation of the analytical method was performed by the accuracy, precision, linearity, limit of detection (LOD) and quantification (LOQ). All the analysis was carried out using the same blank samples. Similar volume of solvents (n-hexane/acetone) and anhydrous sodium sulphate used in the extractions were subjected to similar extraction and clean-up procedures to detect any possible traces of the studied pesticides.

To determine the validity of the methodology, a recovery study was performed using standard addition method. Three samples were spiked with the mixture of pesticides standard solutions (1, 2 and 5 µg/L). The spiked samples were allowed to stand for some hours and then extracted, cleaned up and analysed as described in the method above. The results showed that the mean recovery values ranged from 88 to 92%. This indicated that the analytical procedures were reliable, reproducible and efficient. Precision of the method was evaluated through the relative standard deviation associated with pesticides measurements during recovery. Standard solutions of OPPs were run in GC-(FPD) under set chromatographic conditions and mean peak areas were plotted against concentrations to obtain calibration curves of individual pesticides. Stock solutions of organophosphorus (dichlorvos, mevinfos, diazinon, chlorpyrifos, dimethoate, diclofenthion, phosphamidon, pirimiphos-methyl, parathion, fenthion, isofenfos, bromophos and ethion) were prepared and stored in amber coloured bottles at 4° C in a refrigerator where working standard solutions were prepared fresh before use. Under the set chromatographic conditions, standard calibration curve was prepared for each OPP. The signatory retention time for each OPPs was used as confirmatory

indicator. Linearity was determined by plotting calibration curve with standard solution in n-hexane containing four different concentrations (0.1, 0.25, 0.5, 1.0ng/µL).

The limit of detection (LOD) and quantification (LOQ) were calculated according to standard guidelines (Taylor, 1987; Saadati *et al.*, 2013). All reagent used were of spectra purity.

Statistical analysis of the data

Data generated in the study were subjected to statistical analysis to test for spatial and seasonal variations with analysis of variance (ANOVA) using SPSS 15.0 package. One level of confidence limit ($p = 0.05$) was considered in the interpretation of the statistical results.

Results and discussion

Residues of dichlorvos, mevinfos, diazinon, dimethoate, chlorpyrifos and parathion were detected in the cocoa bean samples with varying concentration among the farm locations. The detected residues reflected the type of pesticide that has been used in the respective farm. The mean concentration of organophosphorus pesticide residues in the total samples were presented in Table I whilst Table II showed the level for both seasons. The concentration of OPPs in the cocoa beans samples ranged from ND - 58.1 µg/kg with average concentration of ND - 10.8 µg/kg during the wet season whilst the dry season ranged from ND - 85.4 µg/kg with average concentration of ND - 5.96 µg/kg. Six (6) OPPs were detected generally in the cocoa beans samples with concentration ranged of 0.79 µg/kg (parathion) to 85.4 µg/kg (diazinon) whilst phosphamidon, primiphos-methyl, fenthion, bromophos, isofenfos and ethion showed no detectable level.

The mean TOPPs in the samples ranged from 1.09-29.1 µg/kg for wet season while the dry season ranged from ND-36.9 µg/kg. Out of the total 108 cocoa beans samples, 59.2% contained dichlorvos, 5.56%, 13.9%, 11.1% and 6.48% had mevinfos, diazinon, dimethoate and chlorpyrifos respectively while 0.92% contained parathion. The contamination levels of the beans using the mean organophosphorus pesticides were in the order: diazinon >dichlorvos>dimethoate>chlorpyrifos>mevinfos> parathion whilst in terms of occurrence of the pesticides: dichlorvos> diazinon >dimethoate> chlorpyrifos> mevinfos> parathion. The non-detectable OPPs in most of the beans samples

Table I. Mean concentration ($\mu\text{g}/\text{kg}$) of organophosphorus pesticides residues in the cocoa beans samples

Sampling farm	Dichlorvos	Mevinfos	Diazinon	Dimethoate	Diclofenthion	Phosphamidon	Pirimiphos-methyl	Chlorpyrifos
ARA	8.10 ^{ab} ±4.01	ND	3.97 ^{ab} ±4.15	ND	ND	ND	ND	ND
AYD	9.93 ^{ab} ±4.01	ND	ND	1.92 ^a ±1.75	ND	ND	ND	ND
EMR	4.36 ^{ab} ±4.01	0.553 ^a ±0.34	2.40 ^{ab} ±4.15	2.31 ^a ±1.75	ND	ND	ND	ND
EMU	2.24 ^{ab} ±4.01	ND	ND	ND	ND	ND	ND	ND
ESR	2.86 ^{ab} ±4.01	ND	14.9 ^b ±4.15	ND	ND	ND	ND	ND
IDE	7.18 ^{ab} ±4.01	0.520 ^a ±0.34	3.87 ^{ab} ±4.15	ND	ND	ND	ND	1.19 ^{bc} ±0.64
IFK	10.3 ^{ab} ±4.01	0.393 ^a ±0.34	ND	ND	ND	ND	ND	ND
IGD	0.878 ^a ±4.01	0.295 ^a ±0.34	ND	1.38 ^a ±1.75	ND	ND	ND	ND
IGR	4.16 ^{ab} ±4.01	1.10 ^a ±0.34	4.53 ^{ab} ±4.15	2.68 ^a ±1.75	ND	ND	ND	ND
IJR	10.8 ^{ab} ±4.01	ND	2.07 ^{ab} ±4.15	ND	ND	ND	ND	2.24 ^{bc} ±0.64
IKO	6.89 ^{ab} ±4.01	0.295 ^a ±0.34	9.85 ^{ab} ±4.15	3.83 ^a ±1.75	ND	ND	ND	0.972 ^{ab} ±0.64
ILJ	9.63 ^{ab} ±4.01	ND	ND	1.17 ^a ±1.75	ND	ND	ND	ND
ILW	1.09 ^a ±4.01	ND	ND	ND	ND	ND	ND	ND
IPW	8.90 ^{ab} ±4.01	ND	ND	4.08 ^a ±1.75	ND	ND	ND	ND
IRE	15.5 ^b ±4.01	ND	6.06 ^{ab} ±4.15	0.517 ^a ±1.75	ND	ND	ND	ND
ISE	2.13 ^{ab} ±4.01	ND	0.583 ^a ±4.15	ND	ND	ND	ND	ND
ORN	1.70 ^a ±4.01	ND	ND	ND	ND	ND	ND	ND
USI	12.4 ^{ab} ±4.01	ND	4.90 ^{ab} ±4.15	4.28 ^a ±1.75	ND	ND	ND	2.993 ^c ±0.64

Table I. Mean concentration ($\mu\text{g}/\text{kg}$) of organophosphorus pesticides residues in the cocoa beans sample (contd.)

Sampling Farm	Parathion	Fenthion	Isofenfos	Bromophos	Ethion	TOPP
ARA	ND	ND	ND	ND	ND	12.1 ^{ab} ±7.00
AYD	ND	ND	ND	ND	ND	11.8 ^{ab} ±7.00
EMR	0.132 ^b ±0.03	ND	ND	ND	ND	13.7 ^{ab} ±7.00
EMU	ND	ND	ND	ND	ND	2.24 ^a ±7.00
ESR	ND	ND	ND	ND	ND	21.3 ^{ab} ±7.00
IDE	ND	ND	ND	ND	ND	11.6 ^{ab} ±7.00
IFK	ND	ND	ND	ND	ND	11.9 ^{ab} ±7.00
IGD	ND	ND	ND	ND	ND	2.56 ^a ±7.00
IGR	ND	ND	ND	ND	ND	20.4 ^{ab} ±7.00
IJR	ND	ND	ND	ND	ND	14.6 ^{ab} ±7.00
IKO	ND	ND	ND	ND	ND	21.7 ^{ab} ±7.00
ILJ	ND	ND	ND	ND	ND	10.8 ^{ab} ±7.00
ILW	ND	ND	ND	ND	ND	1.09 ^a ±7.00
IPW	ND	ND	ND	ND	ND	14.6 ^{ab} ±7.00
IRE	ND	ND	ND	ND	ND	22.6 ^{ab} ±7.00
ISE	ND	ND	ND	ND	ND	4.38 ^{ab} ±7.00
ORN	ND	ND	ND	ND	ND	2.48 ^a ±7.00
USI	ND	ND	ND	ND	ND	26.8 ^b ±7.00

Data are presented as mean \pm S.E. (n = 6.). Value within column with the same superscript letter (s) are not significantly different (p > 0.05) using Duncan multiple range test.

Table II. Mean concentration ($\mu\text{g}/\text{kg}$) of organophosphorus pesticides residues in cocoa beans for both seasons

Season	Dichlorvos	Mevinfos	Diazinon	Dimethoate	Diclofen- thion	Phosphami- don	Pirimiphos- methyl	Chlorpyrifos	Parathion
Wet	10.8 ^b ±1.34	0.351 ^b ±0.11	ND	0.099 ^a ±0.58	ND	ND	ND	0.822 ^a ±0.215	0.015 ^a ±0.01
Dry	2.45 ^a ±1.34	ND	5.96 ^b ±1.38	2.36 ^b ±0.58	ND	ND	ND	ND	ND

Table II. Mean concentration ($\mu\text{g}/\text{kg}$) of organophosphorus pesticides residues in cocoa beans for both seasons (contd.)

Season	Fenthion	Isofenfos	Bromophos	Ethion	TOPP
Wet	ND	ND	ND	ND	1.34 ^a ±2.34
Dry	ND	ND	ND	ND	11.8 ^a ±2.34

Data are presented as mean \pm S.E (n=54). Value within column with the same superscript letter (s) are not significantly different ($p > 0.05$) using Duncan multiple range test.

Table III. Mean concentration ($\mu\text{g}/\text{kg}$) of organophosphorus pesticides residues in cocoa beans samples for both seasons

Sampling farm	Dichlorvos		Mevinfos		Diazinon		Dimethoate		Diclofenthion	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
ARA	12.11 ^{a-c} ±5.68	4.10 ^{a,b} ±5.68	ND	ND	ND	7.93 ^a ±5.87	ND	ND	ND	ND
AYD	19.9 ^{a-c} ±5.68	ND	ND	ND	ND	ND	ND	3.83 ^a ±2.47	ND	ND
EMR	2.09 ^a ±5.68	6.63 ^{a,b} ±5.68	1.11 ^{a,b} ±0.48	ND	ND	4.80 ^a ±5.87	0.593 ^a ±2.47	4.03 ^a ±2.47	ND	ND
EMU	1.09 ^a ±5.68	3.40 ^a ±5.68	ND	ND	ND	ND	ND	ND	ND	ND
ESR	5.71 ^{a,b} ±5.68	ND	ND	ND	ND	29.8 ^b ±5.87	ND	ND	ND	ND
IDE	14.3 ^{a-c} ±5.68	ND	1.04 ^{a,b} ±0.48	ND	ND	7.73 ^a ±5.87	ND	ND	ND	ND
IFK	18.3 ^{a-c} ±5.68	2.27 ^a ±5.68	0.787 ^{a,b} ±0.48	ND	ND	ND	ND	ND	ND	ND
IGD	0.623 ^a ±5.68	1.13 ^{a,b} ±5.68	0.590 ^a ±0.48	ND	ND	ND	ND	2.77 ^a ±2.47	ND	ND
IGR	4.17 ^{a,b} ±5.68	4.15 ^{a,b} ±5.68	2.21 ^b ±0.48	ND	ND	9.07 ^a ±5.87	1.19 ^a ±2.47	4.17 ^a ±2.47	ND	ND
IJR	17.4 ^{a-c} ±5.68	4.13 ^{a,b} ±5.68	ND	ND	ND	4.13 ^a ±5.87	ND	ND	ND	ND
IKO	13.8 ^{a-c} ±5.68	ND	0.590 ^a ±5.68	ND	ND	19.7 ^{a,b} ±5.87	ND	7.67 ^a ±2.47	ND	ND
ILJ	16.4 ^{a-c} ±5.68	2.87 ^a ±5.68	ND	ND	ND	ND	ND	2.33 ^a ±2.47	ND	ND
ILW	2.18 ^{a-c} ±5.68	ND	ND	ND	ND	ND	NDL	ND	ND	ND
IPW	14.3 ^{a-c} ±5.68	3.47 ^a ±5.68	ND	ND	ND	ND	ND	8.17 ^a ±2.47	ND	ND
IRE	26.5 ^c ±5.68	4.47 ^{a,b} ±5.68	ND	ND	ND	13.2 ^{a,b} ±5.87	ND	1.03 ^a ±2.47	ND	ND
ISE	1.90 ^{a-c} ±5.68	2.37 ^a ±5.68	ND	ND	ND	1.17 ^a ±5.87	ND	ND	ND	ND
ORN	ND	3.40 ^a ±5.68	ND	ND	ND	ND	ND	ND	ND	ND
USI	23.2 ^{b,c} ±5.68	1.70 ^a ±5.68	ND	ND	ND	9.80 ^a ±5.87	ND	8.57 ^a ±2.47	ND	ND

Table III. Mean concentration ($\mu\text{g}/\text{kg}$) of organophosphorus pesticides residues in cocoa beans samples for both seasons (contd.)

Sampling Farm	Phosphamidon		Pirimiphos- methyl		Chlorpyrifos	
	Wet	Dry	Wet	Dry	Wet	Dry
ARA	ND	ND	ND	ND	ND	ND
AYD	ND	ND	ND	ND	ND	ND
EMR	ND	ND	ND	ND	ND	ND
EMU	ND	ND	ND	ND	ND	ND
ESR	ND	ND	ND	ND	ND	ND
IDE	ND	ND	ND	ND	ND	ND
IFK	ND	ND	ND	ND	2.37 ^{a,b} \pm 0.91	ND
IGD	ND	ND	ND	ND	ND	ND
IGR	ND	ND	ND	ND	ND	ND
IJR	ND	ND	ND	ND	4.49 ^{b,c} \pm 0.91	ND
IKO	ND	ND	ND	ND	1.94 ^{a,b} \pm 0.91	ND
ILJ	ND	ND	ND	ND	ND	ND
ILW	ND	ND	ND	ND	ND	ND
IPW	ND	ND	ND	ND	ND	ND
IRE	ND	ND	ND	ND	ND	ND
ISE	ND	ND	ND	ND	ND	ND
ORN	ND	ND	ND	ND	ND	ND
USI	ND	ND	ND	ND	5.99 ^c \pm 0.91	ND

Table III. Mean concentration ($\mu\text{g}/\text{kg}$) of organophosphorus pesticides residues in cocoa beans samples for both seasons (contd.)

Sampling Farm	Isofenfos		Bromophos		Ethion		TOPP	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
ARA	ND	ND	ND	ND	ND	ND	12.1 ^{a,b} \pm 9.91	12.0 ^{a,b} \pm 9.91
AYD	ND	ND	ND	ND	ND	ND	19.9 ^{a,b} \pm 9.91	3.83 ^{a,b} \pm 9.91
EMR	ND	ND	ND	ND	ND	ND	11.9 ^{a,b} \pm 9.91	15.5 ^{a,b} \pm 9.91
EMU	ND	ND	ND	ND	ND	ND	1.09 ^a \pm 9.91	3.40 ^{a,b} \pm 9.91
ESR	ND	ND	ND	ND	ND	ND	5.72 ^{a,b} \pm 9.91	36.9 ^b \pm 9.91
IDE	ND	ND	ND	ND	ND	ND	15.4 ^{a,b} \pm 9.91	7.73 ^{a,b} \pm 9.91
IFK	ND	ND	ND	ND	ND	ND	21.5 ^{a,b} \pm 9.91	2.27 ^{a,b} \pm 9.91
IGD	ND	ND	ND	ND	ND	ND	1.21 ^a \pm 9.91	3.90 ^{a,b} \pm 9.91
IGR	ND	ND	ND	ND	ND	ND	23.5 ^{a,b} \pm 9.91	17.4 ^{a,b} \pm 9.91
IJR	ND	ND	ND	ND	ND	ND	21.0 ^{a,b} \pm 9.91	8.27 ^{a,b} \pm 9.91
IKO	ND	ND	ND	ND	ND	ND	16.1 ^{a,b} \pm 9.91	27.4 ^{a,b} \pm 9.91
ILJ	ND	ND	ND	ND	ND	ND	16.4 ^{a,b} \pm 9.91	5.20 ^{a,b} \pm 9.91
ILW	ND	ND	ND	ND	ND	ND	2.18 ^{a,b} \pm 9.91	ND
IPW	ND	ND	ND	ND	ND	ND	14.3 ^{a,b} \pm 9.91	15.0 ^{a,b} \pm 9.91
IRE	ND	ND	ND	ND	ND	ND	26.5 ^{a,b} \pm 9.91	18.7 ^{a,b} \pm 9.91
ISE	ND	ND	ND	ND	ND	ND	1.90 ^a \pm 9.91	6.87 ^{a,b} \pm 9.91
ORN	ND	ND	ND	ND	ND	ND	1.90 ^a \pm 9.91	3.07 ^{a,b} \pm 9.91
USI	ND	ND	ND	ND	ND	ND	29.1 ^{a,b} \pm 9.91	24.0 ^{a,b} \pm 9.91

Data are presented as mean \pm S.E. (n=3). Value within column with the same superscript letter(s) are not significantly different ($p > 0.05$) using Duncan multiple range test. ND = Not detected; Detectable limit for OPPs= 0.02 $\mu\text{g}/\text{kg}$; IJR= Ijero; IPW= Ipawo; ISE= Ise; ORN= Orun; ARA=Aramoko; IDE= Ido-Ile; USI= Usi; IFK= Ifaki; IGD= Igede; IGR= Igbara-Odo; EMR=Emure I; EMU= Emure II; AYD=Ayedun;IPH=Ipawo;IRE=Ire;ILJ=Ilupeju;ILW=Ilawe;ESR=Esure.

implies that these OP pesticides might have not been used on the study farms or possibly used and have degraded due low persistence and fast biodegradable nature of OPPs in the environment.

Dichlorvos mean concentration ranged from 0.878 $\mu\text{g}/\text{kg}$ (IGD) to 15.5 $\mu\text{g}/\text{kg}$ (IRE). For mevinfos, the mean concentration ranged from ND - 1.10 $\mu\text{g}/\text{kg}$ (IGR). Mevinfos contaminations were in order: IGR > EMU > IDE > IFK > IKO = IGD. Diazinon mean concentration ranged from ND - 14.9 $\mu\text{g}/\text{kg}$. Diazinon contamination in the beans were in the order: ESR > IKO > IRE> USI > IGR>IDE>ARA > EMU> IJR> ISE. The present study showed low level of diazinon as compared to the level reported by Aikpokpodion *et al.* (2012) where 40% of the cocoa beans from Ondo state contained diazinon with concentration range of non-detected to 170 $\mu\text{g}/\text{kg}$ and average concentration of 120 $\mu\text{g}/\text{kg}$ as compared to 13.9% in this study. The value reported in this study is comparably higher than those reported by Frimpong *et al.* (2012) in fermented dried cocoa beans samples from Tema and Tekoradi warehouses in Ghana. Aiyesanmi and Idowu (2012) reported a diazinon concentration of 117 $\mu\text{g}/\text{kg}$, the level that is significantly higher than those reported in this study. No sample exceeded EU MRL of 100 $\mu\text{g}/\text{kg}$ for diazinon in food. The diazinon average residue concentration of 21.5 $\mu\text{g}/\text{kg}$ is also below MRL EU (100 $\mu\text{g}/\text{kg}$ for diazinon) in food and CODEX (2005) maximum residue limit (MRL) for diazinon in food substances. However, despite this observation, the long term consumption of such trace concentrations in food substances have been implicated as the major source of chronic exposure to pesticide with residues now commonly found in human tissues. Diazinon have the tendency to accumulate in human system, this was confirmed by a study carried out by Sosan *et al.* (2008) where pesticides residues were found in six human blood serum. For dimethoate, mean concentration ranged from ND to 4.28 $\mu\text{g}/\text{kg}$ (USI). The contamination pattern for dimethoate were in the order: USI > IPW > IKR > IGR > EMU> AYD > IGD> ILJ> IRE. The mean dimethoate level (10.2 $\mu\text{g}/\text{kg}$) recorded in this study is lower than (22.3 $\mu\text{g}/\text{kg}$) reported by Frimpong *et al.*, (2012). The average concentration of dimethoate is below EU maximum residue limit (50 $\mu\text{g}/\text{kg}$) of dimethoate in food.

Chlorpyrifos showed detectable levels in four farms. The highest concentration (13.1 $\mu\text{g}/\text{kg}$) of chlorpyrifos was found in USI while IJR had the least (3.64 $\mu\text{g}/\text{kg}$). Chlorpyrifos contaminations were in order: USI> IJR> IFK> IKO. The level of chlorpyrifos reported in this study was comparably lower than what was reported by Frimpong *et al.* (2012). All the samples collected were below EU MRL of 100 $\mu\text{g}/\text{kg}$ for chlorpyrifos in cocoa beans.

In general, phosphamidon, pirimiphos-methyl, fenthion, isofenfos, bromophos, diclofenthion and ethion proved not to have probably been used in cocoa production in Ekiti state. Considering the levels of OPPs residues in cocoa beans against the European Union showed that the cocoa beans produced in Ekiti will not pose any significant threat. OPPs residues above allowable limits in cocoa beans have potential detrimental effects on human health depending on the frequency of exposure and/or the potency toxicity of the pesticide (US EPA, 2000).

Analysis of variance (ANOVA) in SPSS 15 was used to assess the level of OPPs in the cocoa beans samples. The results showed no significant variation ($p > 0.05$) in the levels of all detectable pesticides except chlorpyrifos from the sampling farms. Seasonal variation showed no significant difference ($p > 0.05$) in parathion and TOPP whilst dichlorvos, mevinfos, diazinon, dimethoate and chlorpyrifos showed significant variation in both seasons.

Conclusion

The study revealed the presence of six (6) OPPs (dichlorvos, mevinfos, diazinon, dimethoate, chlorpyrifos and parathion) at varying concentration with diazinon and dichlorvos as highest concentration and the most occurring pesticides in the cocoa beans samples whilst phosphamidon, pirimiphos-methyl, fenthion, isofenfos, diclofenthion, bromophos and ethion showed no presence. The detectable pesticides showed low levels in the cocoa beans samples while most OP in the samples generally shows no detection. The levels of OPPs obtained in this study showed that the cocoa beans from this area are safe for consumption as none of the samples exceeded the European Union and Codex Alimentarius standards for the detectable OPPs in food.

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