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DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN DRIED COCOA

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ABSTRACT. Organochlorine pesticide residues in dried cocoa beans from selected towns in Ondo and Osun States, Nigeria were determined. Four towns Ondo and Idanre (Ondo State) and Ife and Ilesa (Osun State) were selected to represent cocoa producing areas in the states. Samples were extracted and cleaned-up on silica gel adsorbent. Pesticide residues were detected using Gas Chromatography – Mass Spectrometry (GC-MS). Five organochlorine pesticides were detected in the samples from Ondo state which includes alpha-HCH, beta-HCH, delta-HCH, endrin and *p p'* DDT. The levels of *p p'* DDT (0.108 mg/kg and 0.107 mg/kg) detected in samples from Ondo and Idanre towns were lower than the EU MRL (0.5 mg/kg), while other detected organochlorine pesticide residues from the State were higher than the EU MRL. In Osun State, alpha-HCH, lindane, dieldrin, endrin, heptachlor-epoxide, endosulfan I and *p p'* DDT were organochlorine pesticide residues detected, with lindane (0.074 mg/kg), endosulfan I (0.099 mg/kg) and *p p'* DDT (0.235 mg/kg) being lower than EU MRLs. Higher levels of organochlorine pesticide residues than the EU MRLs suggest that the produce were not safe for human consumption because of the tendency of the pestsides to persist for a long period of time and as well bioaccumulate within the environment. The produce can also face trade threat in international market. Measures were suggested to ameliorate this situation.

Introduction

Cocoa (*Theobroma cacao*) is a very important export crop and the largest non oil foreign exchange earner for Nigeria. Nigeria produced an estimated 225,000 tonnes cocoa for export between 2012 and 2013 to become the fourth largest producer of cocoa behind Cote d'Ivoire, Ghana and Indonesia [1]. Cocoa bean attracts high demand worldwide especially by developed countries and the total world production increased by 13% from 4.3 million MT to 4.8 million MT between 2008 and 2012 representing an average year-over-year increase of 3.1% [2].

Cocoa like other crops is associated with some pests and diseases and this is a major factor causing low yield in the growing regions [3]. The brown mirid (*Sahlbergilla singularis* Haglund) is the most serious insect pest of cocoa in Nigeria [4], among other insect pests. Yield loss to pests and diseases in cocoa is estimated at 30 to 40% worldwide [5]. In order to mitigate the yield losses to pests and diseases, cocoa farmers tends to use different types of pesticides. Pesticide is a chemical used to prevent, destroy, or repel pests [6]. In cocoa farms, pesticides used are grouped into insecticide, fungicides, and herbicides.

Nevertheless, as important as pesticides uses are in cocoa production in Nigeria, they also pose some environmental problems in areas where they are used. Organochlorine pesticide for instance can persist in the environment for many years and cause surface and underground contamination of

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water [7, 8]. Among the inherent problems in cocoa pesticides applications are toxicity and phytotoxicity, mismanagement and maintenance of equipment, poor availability of pesticide and equipment, lack of safety measures, poor extension services, wrong dosage of pesticides, misuse of pesticide and low government intervention [9]

The use of any chemicals in food crop production may leave some residue in food chain, especially agricultural commodities. Adverse effects on human health resulting from pesticides residues in food after application are of great importance to concerned authority. Possible health risk due to pesticide residues in the diet has deeply modified the strategy for the crop protection, with emphasis on food quality and safety in a more sustainable way leading to the strict compliance with Maximum Residue Limits (MRLs) of pesticide residues in food commodities [10].

There are various organizations that set MRLs, such as European Commission (EC), Codex Alimentarius, World Health Organisation (WHO), Food and Agriculture Organisation (FAO) or National governments in many countries of the world such as Australia, Canada, Japan, USA, India, Israel, Korea and some others. Pesticide residue in food products can be determined by prescribing the tolerance level in food production chain. Among the terms used in setting the tolerance levels of pesticide residue in food are Acceptable Daily Intake (ADI), Minimum Residue Level (MRL) and No Observable Adverse Effect Level (NOAEL) [10]. Thus, the knowledge of pesticide residues in cocoa beans from Nigeria determines the fate of the product in the world market. Hence, the objectives of this study include to determine the levels of pesticide residues in dry cocoa beans in the study areas and to compare the determined pesticide residues with the European Union Maximum Residue Levels (EU MRLs).

Materials and Methods

The experiment was carried out in the laboratories of the Departments of Crop and Environmental Protection, Pure and Applied Chemistry, LAUTECH, Ogbomoso and Oupearl Scientific Laboratory Services, Lagos, Nigeria.

Study areas

The study areas were cocoa stores in Ondo and Osun states, Nigeria. Cocoa is mostly grown in fourteen of the thirty-six states in Nigeria. Aside Cross River state in the South-South Nigeria, most of cocoa growing states are found in the South-Western Nigeria, with Ondo state taking the lead followed by Osun state. National Bureau of Statistics in year 2012 rated Ondo state as the top producer of cocoa in Nigeria with the capacity to produce about 90,000MT of cocoa bean between 2006-2010 production years. In the same rating, Osun state came second with the capacity of producing 75,000MT. However, the study areas were selected base on already stated production capacity.

Sample collection and sampling procedure

Dried cocoa beans ready for export were collected from six different cocoa stores in each study area between December, 2013 and January, 2014. In Ondo state, two different cocoa growing towns were selected and the towns were Ondo, and Idanre out of which six notable cocoa stores in each of the towns were considered for the study. In Osun state, Ile-Ife and Ilesa towns were selected. Two kilograms each of dried cocoa beans were randomly taken from bags of cocoa beans ready for export in the stores. Samples were bagged separately and transported to the laboratory for analysis.

Composite samples were prepared from the entire samples collected from each town by combining the samples together, stirred thoroughly and 1kg of the sample measured out to represent a town. The composite samples were bagged separately in polyethylene bags.

Reagents and chemicals

Dichloromethane and n-Hexane solvents were purchased from Sigma-Aldrich Co. (Steinheim, Germany). Anhydrous magnesium sulphate and sodium chloride were used for the cleanup. Pesticide standards (purity > 97.0%) were purchased from Sigma-Aldrich Co (Steinheim, Germany).

A total of fifteen organochlorine pesticides (OCP) residues were studied. They include α- BHC, β- BHC, δ- BHC, Lindane, Chlorothalonil, Heptachlor, Aldrin, Heptachlor-epoxide, Endosulpan I, Dieldrin, Eldrin, Endosulfan II, *p,p′* DDD, Endosulfan sulphate and *p,p′* DDT.

Sample preparation

Samples were prepared following a procedure [11], with little modification. Dry cocoa beans samples were separately milled using electric kitchen blender. 10g of each sample was measured in using sensitive weighing scale into 60ml volume beaker and 40ml dichloromethane (DCM) was added. The samples were sonicated for 30min in a (360 W selecta ultrasonic bath) and filtered using Watman 1 filter paper. The procedure was repeated three times for each of the sample.
The extracts were subjected to gel permeation chromatography using bio-beads S-X3 pack glass column (380 x 22 mm i.d) with 300ml of hexane: dichloromethane in 1:1 volume. The portions containing lipids was collected separately and discarded. The left over portion was collected, concentrated and divided into saturates and the pesticides (Organochlorine and Organophosphate). The extract were later subjected to fractionation using glass column packed with silica-alumina in the ratio of 2:1 into two fractions of aliphatic hydrocarbons and the pesticides, using 7:3 volumes of hexane/dichloromethane.
The resulting extracts were concentrated using rotary evaporator at 30^0C to approximately 1ml. The concentrated extracts were transferred into 2ml volume clean chromatographic vials and reduced to 0.5ml under gentle stream of nitrogen and subjected to gas chromatographic determination.

Gas chromatographic detection

The gas chromatography was coupled to mass spectrometry using Shimadzu Model Q 2010 GC-MS (Shimadu, Japan) fitted with HP-5MS column of fused silica (30x0.25x0.25mmid film thickness) and the carrier gas used was helium (99.99%). The injection was splitless and the split time was 1 min after injection using auto-sampler and the injection temperature was 250^0C the pesticides.
The temperature programme was from 100^0C (held for 1min) to 200 ^{0}C at 10^0C/min (held for 2 min) and to 280 ^{0}C at 10^0C/min (held for 2 min). The pesticide residues were identified by comparing the retention time of sample peaks with that of the standards. Concentration levels detected were compared with the EU MRLs

Results

Concentrations of organochlorine pesticide residue detected in samples from Osun state were presented in Table 1. α-HCH (0.084 mg/kg), dieldrin (0.077 mg/kg), endrin (0.733 mg/kg) and endosulfan I (0.099 mg/kg) pesticide residues were present in samples from Ile-Ife. The chromatographs of the detected peaks were presented in figure 1 and 2. α-HCH, dieldrin and endrin were with levels higher than the EU MRLs (0.01 mg/kg, 0.05 mg/kg and 0.01 mg/kg), while endosulfan I contained concentration lower than the EU MRL (0.10 mg/kg). Lindane (0.074 mg/kg), heptachlor-epoxide (0.124 mg/kg) and *p,p′*-DDT (0.235 mg/kg) were recorded in samples from Ilesa town in Osun State with only heptachlor-epoxide (0.124 mg/kg) being higher than the EU MRL (0.02 mg/kg).
Results presented in table 2 show the levels of organochlorine pesticide residues in cocoa samples from Ondo State. Samples from Ondo town contained residues of alpha-HCH (0.104 mg/kg), endrin (0.499 mg/kg) and *p,p′*-DDT (0.108 mg/kg), with levels of alpha-HCH and endrin being higher than the EU MRLs (0.01 mg/kg and 0.01 mg/kg). *p,p′*-DDT was detected at level being lower than the EU MRL (0.50 mg/kg). Residues of beta-HCH (0.099 mg/kg), δ-HCH (0.170 mg/kg), endrin (0.399 mg/kg), endosulfan II (0.421 mg/kg) and *p,p′*-DDT (0.107 mg/kg) were recorded in samples from Idanre town. Levels of beta-HCH, delta-HCH, endrin and endosulfan II were higher than the EU MRLs (0.01 mg/kg, 0.02 mg/kg, 0.01 mg/kg and 0.421 mg/kg), but *p,p′*-DDT level lower than the EU MRL (0.50 mg/kg). The chromatographs of the detected peaks were presented in figure 3 and 4.

Table 1: Concentrations of organochlorine pesticide residues in dried cocoa bean samples from Osun State compared with the EU MRL

Pesticides	Retention Time Ile-Ife	Retention Time Ilesa	Conc. [mg/kg] Ile-Ife	Conc. [mg/kg] Ile-Ife	EU MRL [mg/kg]
Alpha-HCH	10.547	ND	0.084	ND	0.01
Beta-HCH	ND	ND	ND	ND	0.01
Delta-HCH	ND	ND	ND	ND	0.02
Lindane	ND	11.291	ND	0.074	1.00
Chlorothalonil	ND	ND	ND	ND	0.10
Heptachlor	ND	ND	ND	ND	0.02
Aldrin	ND	ND	ND	ND	0.05
Dieldrin	16.857	ND	0.077	ND	0.05
Endrin	17.265	ND	0.733	ND	0.01
Heptachlor-epoxide	ND	15.111	ND	0.124	0.02
Endosulfan I	16.014	ND	0.099	ND	0.10
Endosulfan II	ND	ND	ND	ND	0.10
Endosulfan sulphate	ND	ND	ND	ND	0.10
p,p’-DDD	ND	ND	ND	ND	0.50
p,p’-DDT	ND	18.437	ND	0.235	0.50

Table 2: Concentrations of organochlorine pesticide residues in dried cocoa bean samples from Ondo State compared with the EU MRL

Pesticides	Retention Time Ondo	Retention Time Idanre	Conc. [mg/kg] Ondo	Conc. [mg/kg] Idanre	EU MRL [mg/kg]
Alpha-HCH	10.567	ND	0.104	ND	0.01
Beta-HCH	ND	11.075	ND	0.099	0.01
Delta-HCH	ND	11.917	ND	0.170	0.02
Lindane	ND	ND	ND	ND	1.00
Chlorothalonil	ND	ND	ND	ND	0.10
Heptachlor	ND	ND	ND	ND	0.02
Aldrin	ND	ND	ND	ND	0.05
Dieldrin	ND	ND	ND	ND	0.05
Endrin	17.255	17.250	0.499	0.399	0.01
Heptachlor-epoxide	ND	ND	ND	ND	0.02
Endosulfan I	ND	ND	ND	ND	0.10
Endosulfan II	ND	17.585	ND	0.421	0.10
Endosulfan sulphate	ND	ND	ND	ND	0.10
p,p’-DDD	ND	ND	ND	ND	0.50
p,p’-DDT	18.403	18.349	0.108	0.107	0.50

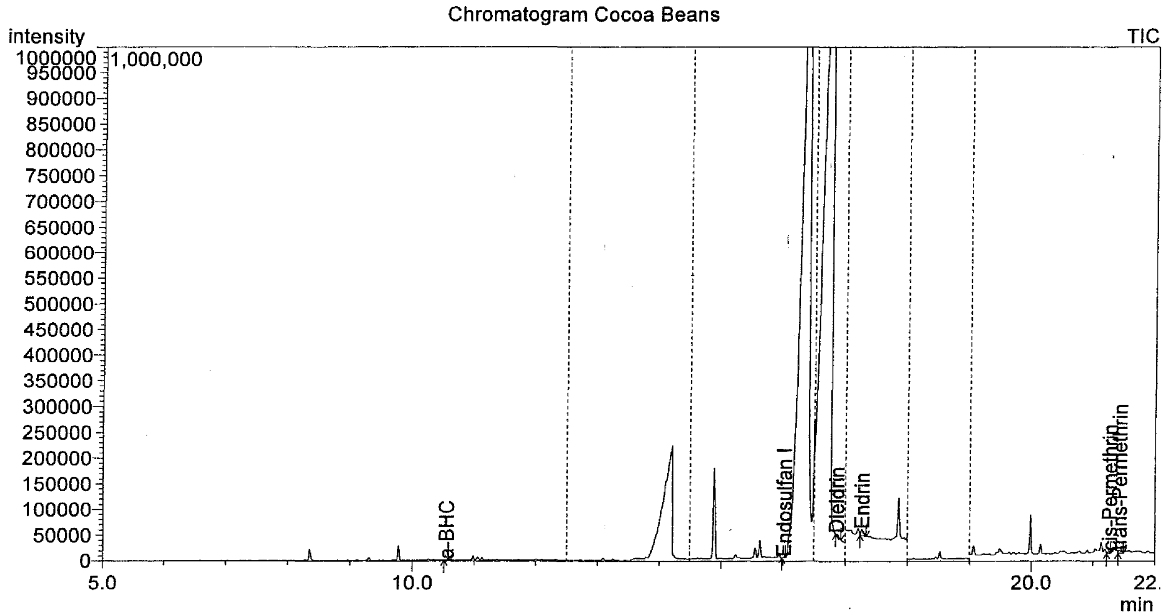

Figure 1: The chromatograph of organochlorine and pyrethroid pesticides peaks in dried cocoa beans samples from Ile-Ife town.

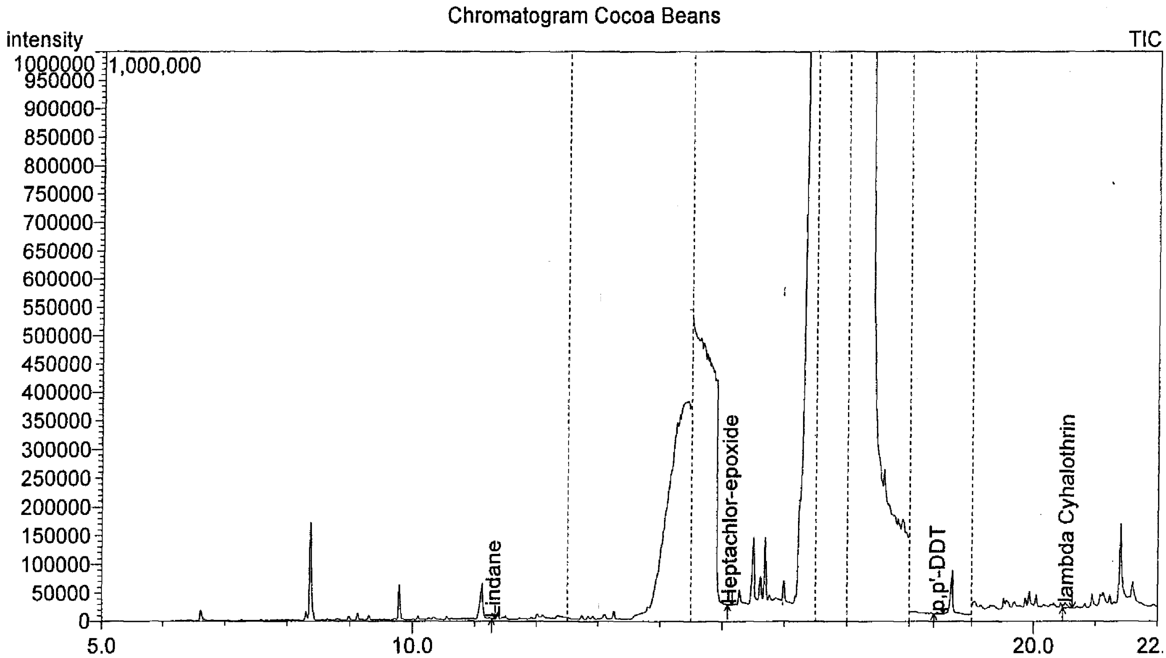

Figure 2: The chromatograph of organochlorine and pyrethroid pesticides peaks in dried cocoa beans samples from Ilesa town

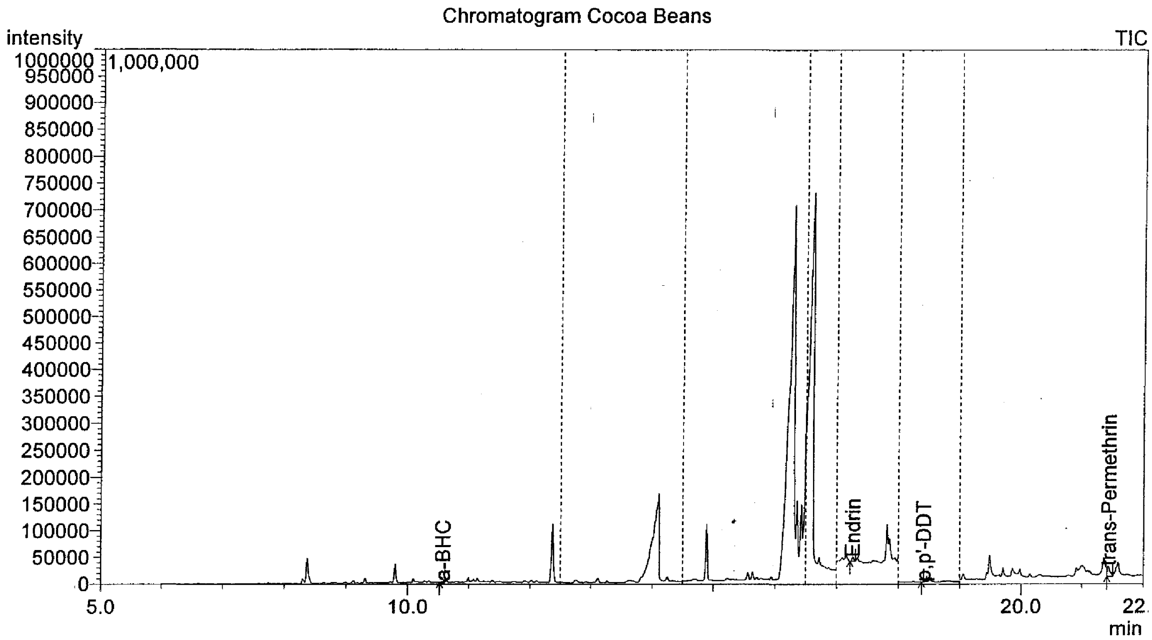

Figure 3: The chromatograph of organochlorine and pyrethroid pesticides peaks in dried cocoa beans samples from Ondo town.

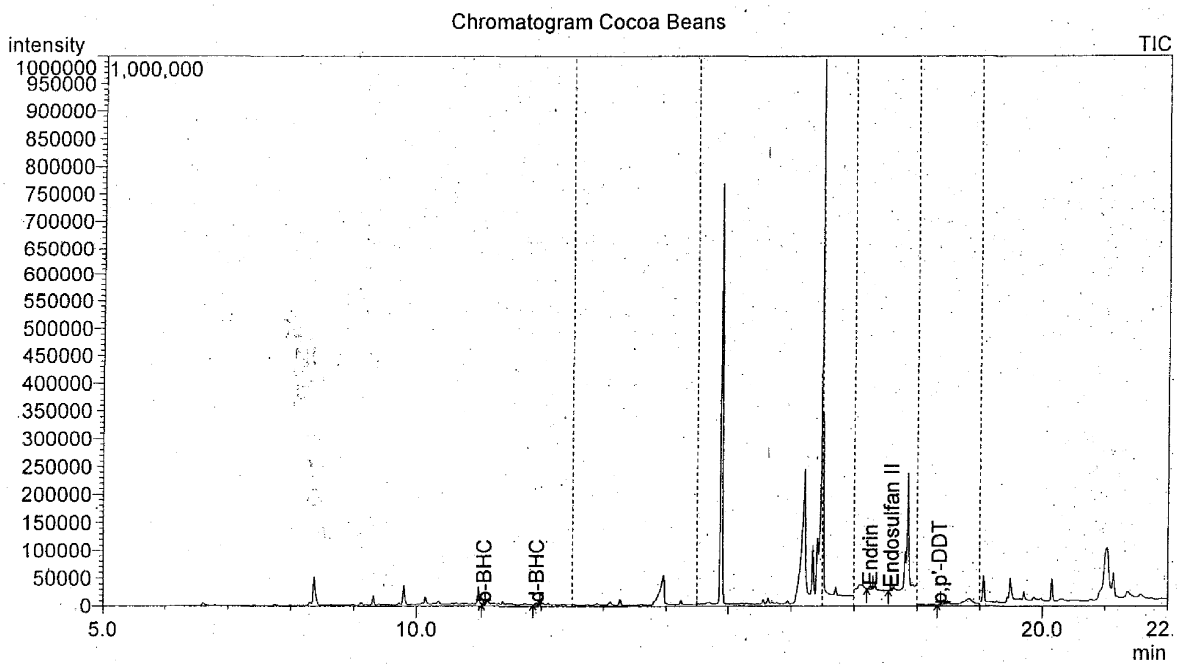

Figure 4: The chromatograph of organochlorine and pyrethroid pesticides peaks in dried cocoa beans samples from Idanre town

Discussion

The organochlorine pesticide residues detected in the samples from Osun State include α-HCH, Lindane, Dieldrin, endrin, heptachlor- epoxide, endosulfan I and *p,p′*-DDT. Out of these pesticides detected in the samples from the State, α-HCH, Lindane, endosulfan I and *p,p′*-DDT belong to WHO/EPA category II pesticides, which are moderately hazardous (WHO, 2010), while dieldrin, endrin and heptachlor- epoxide are categorized as obsolete pesticides by the same classification. These insecticides have been banned for use in cocoa production by the European Union [12]

The presence of these detected organochlorine insecticides revealed that cocoa farmers in Osun State are using banned pesticides. Although, the presence of DDT, lindane, α- HCH and endosulfan I may be as a result of long time application rather than recent, as organochlorine insecticides are highly persistent in the environment. The levels of other insecticides not detected in this study suggest gradual phasing out of organochlorine pesticides. On the circulation of banned pesticides in Nigerian market, marketers of the pesticides are blamed for not supporting the ban as a result of government inadequate information on the newly approved pesticides [13].
Among the pesticides detected in the state, α-HCH, Dieldrin and endrin were with levels higher than the EU MRLs. By implication, such cocoa product may not be accepted in the European Union (EU) market. However, residue of pesticides that have tendency of phasing out in 2-3years may be accepted in some European Union market [12].
Similar to insecticide residues detected in samples from Osun State, α-HCH, endrin and DDT were found in samples from Ondo State. Other samples recorded in sample from Ondo State are β- HCH, δ-HCH and endosulfan II. This suggests that similar pesticides are used by cocoa farmers in the two states. DDT levels detected in Ondo States are lower than the EU MRL. DDT is highly used by cocoa farmers in Ondo State [14] confirming the detected levels the pesticide in the study areas. Lower concentration of DDT detected in the state suggests gradual phasing out of the pesticides. The acceptability of cocoa produced from the state in the international market is subjected to recorded levels of pesticide residues accepted in the European Union (EU), being the largest consumer of cocoa produced in the world [1].
p,p'–DDT is the active ingredient of the pesticide named dichlorodiphenyltrichloroethane (DDT). The pesticide can easily degrade into dichlorodiphenyltrichloroethane (DDD) and dichlorodiphenyltrichloroethane (DDE), which are more persistent than DDT itself. *p,p'*–DDE degrade from *p,p'*-DDT may persist for more than seven years in human being [15]. In line with the former, DDD a metabolite of DDT was not detected in the samples from the two states, establishing the fact that the DDT usage might have been stopped or applied at a very low concentration. This explains the phasing out of DDT in the study areas as its metabolites (DDD and DDE) were not recorded. Lindane, which is made from gammehexachlorocyclohexane marketed as Gammalin 20EC® in Nigeria is acutely toxic to man as it half-life is only 20 hours after exposure [16].
Aldrin, dieldrin and endrin are similar in structures and were used as insecticides in 1950s to the mid 1970s. Aldrin was not detected in the samples from both state but its metabolite, dieldrin was found in samples from Osun State. Aldrin is rarely found in the environment because it easily metabolized to dieldrin [16]. Endrin is a stereoisomer of dieldrin and could rapidly metabolize in the environment [16]. This however suggests that endrin could be found in an environment where dieldrin was previously applied. Dieldrin and endrin were both detected in sample from Ile-Ife, Osun State but with the level of endrin being higher than the level of dieldrin, suggesting gradual degradation of dieldrin to endrin in the environment where samples were collected.
Organochlorine pesticides are generally highly persistent and lipophilic in nature. DDT has also been reported to have been associated with animal tissue and milk [17]. This implies that human exposure to organochlorine pesticides could result in bioaccumulation of pesticide residues in the tissues. Diazion, endosulfan, propoxur and lindane organochlorine pesticides which have been reported to have been detected in the blood serum of 42 out of the 76 cocoa farmers examined from south-western Nigeria [18] confirmed the potentials of the pesticides to bio-accumulate in human tissues. By implication, farmers in the study areas and the consumers of their farm produce may possess some residues of this class of pesticide in their body system and hence this necessitates a prompt action to prevent further contamination of the food chain.
However, human exposure to organochlorine pesticides could result in some health challenges. Thyroid hormonal imbalance, hormonal related cancers such as in breast and prostate; childhood developmental disorders and diabetes are major effects of organochlorine pesticides on human health [18]. In line with this, β-HCH and dieldrin, which were also found in this study, were significantly associated with prostate cancer prevalence [19]. Type 2 diabetes in the American

population is associated with human exposure to organochlorine pesticides [20]. This however suggests that cocoa farmers and the consumers of their produce may suffer from some of these deadly diseases if efforts are not taken to reduce human exposure to the pesticides.

Conclusion

Cocoa samples from the two states contained banned organochlorine pesticides, indicating that such pesticides detected are still in use or might have been used in time past. The levels of pollutant detected in the two states are similar, suggesting that same pesticide brands are used in the states. Also, most of the detected levels were higher than the EU MRLs, suggesting that cocoa produce from the two states may not be accepted in international market.

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