Organochlorine Pesticide Residue Levels and Potential Human Health Risks in Kolanuts from Selected Markets in Osun State, Southwestern Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author MBS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author JAOO reviewed the study and assisted tremendously in the extraction protocols of the OCPs from the samples. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Organochlorine Pesticide (OCP) residue were qualitatively and quantitatively determined using Gas Chromatography-Electron Capture Detector (GC-ECD) and their potential health risks assessed in kolanut (*Cola nitida*) samples from selected markets in Osun State, Southwestern Nigeria. Health risk estimates were analyzed using Estimated Average Daily Intake (EADI) and Hazard Index (HI) with risk categorized for non-carcinogenic health effects. A total of 17 OCPs were identified and their residues were detected in all the kolanut samples analyzed. The results showed that the mean concentration of *p,p*-DDE, Dieldrin, Endosulfan, and Lindane were 0.480±1.106 mg kg^{-1}, 0.310±0.442 mg kg^{-1}, 0.243±0.144 mg kg^{-1} and 0.044±0.041 mg kg^{-1} respectively. Among the OCPs analyzed, *p,p*-DDE and α-HCH were the dominant isomers for DDT and HCH, respectively. The Principal Component Analysis (PCA) results showed that out of the 17 OCPs detected, *p,p*-DDE,

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endosulfan sulphate, dieldrin, and methoxychlor were major compounds primarily contributing to variation in the detected OCPs residues in the kolanut samples. The concentrations of all the analyzed OCP observed in the nuts were found to be higher than the recommended maximum residual limits (MRLs), the maximum concentration of a pesticide residue legally permitted to remain in food after it has been treated with the pesticide. The percentage above the MRL ranges from 28 to 100%. The analysis of health risk estimates revealed that the HI values for γ-HCH (1.907), heptachlor (15.700), aldrin (20.3), dieldrin (40.3) and endrin (2.925) were above value of 1 suggesting a potential for chronic toxicity to the regular consumers of kolanuts obtained from these markets. The current situation could be therefore considered a serious public health problem. Hence, a continuous monitoring of pesticide residues in kolanuts and a tighter regulation of pesticides used on stored produce are recommended.

1. INTRODUCTION

Kola is the second most important indigenous cash crop in Nigeria. It is estimated that the country produces 70% of world's kolanuts with an annual production of 200,000 metric tonnes of fresh nuts mostly from the Southwest Nigeria, which accounts for about 88% of the produce [1,2]. However, the bulk of the nuts produced are marketed and consumed in the Northern parts of the country [3]. Kolanut contains a substantial amount of Caffeine, *Theobromine*, *Kolatin* (a heart trinculant) and *Thophylline* as the active ingredients. The properties of these constituents make eating kola to induce strength, alertness and concentration in an individual [1,4].

The kola weevils, *Balanogastris kolae*, Desbr. and *Sophrorhinus* spp Olivier are the most destructive field-to-store insect pests of kolanuts. The weevils are capable of causing 30-70% damage on the stored nuts while 100% damage has been recorded in cases of late harvest [5,6]. In order to overcome the insect pest problems and prevent stored nuts from attack by the weevils, kola nuts farmers and traders use various types of pesticides including banned ones to ward off pests from their kola nuts [7]. Many of these pesticides including organochlorines and their active metabolites are known to cause severe health conditions like neurological damage, hypertension, cardiovascular diseases and skin disorders in humans [8,9]. Organochlorine pesticides pose substantial short and long-term health risks [9,10]. They are known to disturb the biological and physiological functions of erythrocytes and lymphocytes [11].

Dietary intake constitutes the most common and principal pathway of exposure to pesticides [12] and human exposure to pesticides through contaminated food intake can cause chronic and acute toxicities. Thus, regular surveys and monitoring programmes of pesticide residues coupled with risk assessments have been carried out and reported in many developed countries worldwide [13,14,15,16,17] and this is done in order to protect consumer health, improve the management of agricultural resources and prevent economic losses [18].

In West Africa, many studies have reported contamination levels of pesticide residues in fruits and vegetables [19,20,21,22,23,24] and in maize and cowpea [25]. In Nigeria, several studies have also reported high levels of pesticide residues in fruits, vegetables and other food crops produce [26,27,28,29,30,31], on cocoa [32] and on kolanut [33] as well as seafood products [34].

These studies have not really assessed the potential health risks associated with these products. For instance, a study [33] reported residues levels of two OCPs; heptachlor and endosulfan on kolanut samples without evaluating the related health risk resulting from consumption of the contaminated kolanuts. To guarantee that any consumed product is sufficiently safe, evaluation of pesticide levels and assessing the potential risk to public health is imperative [35]. Thus, the objectives of the study were to evaluate the concentrations of pesticide residues in kolanuts collected from selected markets in Southwestern Nigeria, compare the detected levels with the maximum residue limits (MRLs) of pesticides as well as the acceptable daily intakes (ADIs) and also estimate the potential health risks associated with each of the organochlorine pesticides in the kolanut samples.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Kolanut samples were randomly purchased from popular markets in Ile-Ife, Modakeke and Erin Osun in Osun State, Southwestern Nigeria. The markets were known to be major wholesale and retail markets for processed kolanuts both for local consumption and packaging for transportation to the northern part of the country where bulk of kolanut produced are consumed. The purchased kolanuts according to the traders have been preserved with synthetic insecticides to protect the nuts from attack of weevils and also to increase the shelf life of the product. A total of twenty-five samples were collected. The samples were wrapped in aluminium foils before they were packed in black polyethylene bags, labeled and taken to the laboratory. In the laboratory, the nuts were chopped with a clean stainless steel knife to facilitate proper drying of the samples carried out at an ambient temperature. When a constant weight was attained, each of the samples was ground to a homogenous powdered form with an agate mortar and pestle. Each of the powdered samples was then packed in ziploc bags, labeled and then stored in a refrigerator at 4°C prior to further analysis.

2.2 Extraction

All reagents and chemicals were of analytical grade and were used as received. The reagents used in this study were Dichloromethane (GFS Chemicals, Columbus), n–Hexane (Ultrafine Limited, Marlborough House, London), Acetone (GFS Chemicals, Columbus), Silica gel 60–200 mesh (Labtech Chemicals), and anhydrous sodium sulphate (Merck, Germany). Extraction of pesticide residues in kolanuts was done following the method described by [36]. From each powdered sample, 20 g was weighed into a Whatman extraction thimble pre-extracted with n-hexane and dichloromethane (DCM) to remove extraneous organic contaminants that might be adhering to the surface or pores of the thimble. The extraction was carried out with Soxhlet extractor for an average period of 5 hours using DCM as the extracting solvent. The extract was concentrated by distilling-off the solvent (DCM) to about 3 mL using a rotary evaporator at about 41°C. The concentrated extract was cooled down to room temperature and then concentrated further to about 2 mL under a stream of high purity (99.99%) nitrogen. The reduced extract was then preserved for chromatographic clean-up before Gas Chromatographic analysis.

2.3 Clean-up

A column of about 15 cm (length) x 1 cm (internal diameter) was packed first with glass wool and then with about 7.5 g activated silica gel prepared in a slurry form in DCM. About 5 g of anhydrous sodium sulfate was placed at the top of the column to absorb any water in the sample or the solvent. Pre-elution was done with 15 mL of DCM, without exposing the sodium sulfate layer to air, so as to prevent the drying up and cracking of the packed silica gel adsorbent. The reduced extract was run through the column and allowed to sink below the sodium sulfate layer. Elution was done with 3 x 10 mL portions of DCM. The eluate was collected and the accompanying solvent was evaporated to dryness under a stream of analytical grade nitrogen (99.99%). The dried eluate was reconstituted with 1 mL 2, 2, 4 – trimethylpentane (isooctane) in readiness for instrumental analysis.

2.4 Instrumental Analysis

With the aid of a micro syringe, 1 µL of the 1.0 mL purified and reconstituted fraction was injected into the injection port of a gas chromatograph coupled with a ^{63}Ni electron capture detector (GC-ECD, Hewlett Packard 5890 series II). The column consisted of a DB-5 fused silica capillary column (30 m × 0.32 mm i.d. × 0.25 µm film thickness). The temperatures of the injector and detector were 250°C and 300°C, respectively held for 4 mins oven temperature programme started from 50°C (1 min) and continued at 20°C to 150°C and at 5°/min to 280°C held for 4 mins. The injection was carried out on a splitless injector at 200°C and the purge activation time was 30s. The carrier gas was N_2 at 30 mL/min; and the splitless flow rate was 19.6 mL/min. The run time was 26 minutes. Quantification of the OCPs was based on external calibration curves prepared from the standard solutions of each of the OCPs.

2.5 Quality Control

The three major quality control protocols used to ascertain the accuracy and precision of the results obtained were blank determination, recovery experiment and triplicate determination.

2.5.1 Blank determination

The background value of OCPs in the DCM was determined by injecting the spectra grade DCM into the GC and no peak was seen.

2.5.2 Recovery experiment

For the GC-ECD analysis of the OCPs, several runs were made for each of the samples in order to correct deviation in the retention time of each of the residues present in each of the samples that were analyzed, which occurred as a result of the bleeding in the column of the GC after it had been used for the first run on each of the samples. This bleeding in the column causes the retention time of each of the residues in a sample to increase because it delays the time of passage of every compound (residue) passing through it. Hence, the column was changed for every set of runs performed on the GC and the retention time of all residues in each of the samples were ensured to be constant for every run. The reliability of the analytical procedures used was tested in terms of percentage recovery (%R) using standard addition method since no certified pesticide reference materials were available during the course of this study. A 20 g sample of pulverized kolanuts was divided into two. One part was spiked with 10 ppm standard mixture consisting of some of the available organochlorine insecticides of interest, and thoroughly mixed, while the other (control) group was left unspiked. The samples were taken through the extraction and clean-up protocols, following the procedures of [36]. Also, 10 mL of the standard 1000 mg/L mixture of the OCPs, in spectra grade n- hexane, was put into a clean, oven-dried sample bottle. This was dried at ambient temperature by passing a stream of high purity nitrogen gas through it, and the residue reconstituted using 2, 2, 4 - trimethylpentane. With the aid of a microsyringe, 1.0 µL of each of the spiked, unspiked (control) and standard mixture was injected into the GC column for GC-ECD analysis. The recoveries of OCPs were determined by comparing the peak areas of the OCPs after spiking with those obtained from the evaporated standard residues.

2.5.3 Triplicate determination

This was done in order to determine the standard deviation, and hence, the error margins involved in each of the OCP residue congener determined.

2.6 Response Factor (RF)

The response factor of the standard OCPs was obtained by the method of [37]. This was determined by analyzing 1.0 µL of 1000 ppm stock solution of the standard mixture containing the internal standard (I.S.). The internal standard used for this work was hexachlorocyclobenzene (HCB). The response factor for a sample peak is defined by the expression:

$$RF = \frac{\text{Peak area of OCPs}}{\text{Peak area of I.S.}}$$

2.7 Human Health Risk Assessment

Pesticide residues levels were compared with MRLs recommended by [38]. Maximum residue limit of a pesticide is the maximum concentration of its residue that is legally permitted to remain in food after it has been treated with the pesticide [39]. Health risk indices of the residues detected were computed using the data obtained and food consumption assumptions since the consumption data play a major role in assessing the dietary risk of residues in food. The health risk estimates for the pesticide residues in kolanut was computed using two basic standard indices: the Estimated Average Daily Intake (EADI) and the Hazard Quotient (HQ). Estimated Average Daily Intakes (EADIs) of a pesticide residue and food consumption assumption were used to determine long term health risks to consumers. The food consumption rate for kolanut in Nigeria is quoted to be 0.013 kg d^{-1} [40]. For each type of exposure, the EADI was obtained by multiplying the mean residual pesticide concentration (mg kg^{-1}) in the food of interest and the food consumption rate (kg d^{-1}) [21,25]. The EADIs expressed as mg/kg/day was compared with already established Acceptable Daily Intake (ADI) to assess the long-term risk from exposure to the pesticide residues through kolanut consumption since the ADI is based on exposure over a lifetime [41]. The HQ was used to assess the risks associated with the non-carcinogenic and carcinogenic health effects. In this study, the non-carcinogenic health effects were evaluated by dividing the EADI by their corresponding values of ADI [42], assuming average adult's body weight of 60 kg. When the health risk index >1, the food involved is considered a risk to the consumers; when the index <1, the food involved is considered acceptable [25,43].

2.8 Statistical Analysis

The results obtained from the chromatographic analysis were summarized using descriptive

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statistics (mean, range, minimum, maximum and standard deviations) and Principal Component Analysis (PCA) (SAS version 9.2 software [44].

3. RESULTS AND DISCUSSION

Table 1 shows the percentage recovery (%R) values of various analytes in kolanut samples. Recovery values of OCPs ranged from 84.26% α-Chlordane to 102.23% Dieldrin. The %R values obtained were within the 70-110% range for acceptable recovery values as stipulated by the EU Guidelines for evaluating accuracy and precision of a method [45]. The mean residual concentrations of organochlorine pesticide residues detected in kolanut samples are presented in Table 2.

The total concentration of HCH, Cyclodienes and DDT were 0.387 mg kg^{-1} 1.466 mg kg^{-1} and 0.776 mg kg^{-1} respectively. The results from this study have shown that all the kolanut samples were contaminated by all the 17 organochlorine pesticides analyzed with cyclodienes subgroup being the most frequently detected. All the HCH isomers were detected in the nuts with a mean concentration of 0.208 and 0.032 for α-HCH and δ-HCH respectively. γ-HCH, the major component of lindane was detected at a mean level of 0.044 mg kg$^{-1.}$ The HCH isomers (α, β, γ-HCH) detected have also been reported in animal products [34,46]. The higher

concentration of α-HCH isomer recorded in this study compared to other isomers may be attributed to the relatively high volatility of this isomer whose uptake increases via deposition from the atmosphere or by direct treatment with pesticides [47]. The γ-HCH has been known to dominate pesticides market for many years before its ban. The detection of appreciable amounts of γ-HCH (lindane) was reported in *S.lycopersicum* and *C. annum* samples commercially sold in Ota, Ogun State, Nigeria [27]. Studies have suggested a potential association between exposure to at least one organochlorine pesticide and breast cancer risk. The risk of breast cancer was found to increase with higher adipose tissue concentrations of the pesticide lindane (in which β-HCH is commonly present) in a case–control study of a female population recruited in Southern Spain [48].

Table 1. Response factor (RF) and percentage recovery (%R) for OCPs

OCPs	RF	(%R)	LOD (µg/mL)
HCB	-	93.65	0.094
Aldrin	1.572	95.32	0.031
α-Chlordane	1.761	84.26	0.023
Dieldrin	1.125	102.23	0.071
Heptachlor	1.212	93.47	0.094
Endrin	1.413	88.69	0.112
p,p'-DDT	0.893	96.91	0.078

Table 2. Mean concentration of organochlorine pesticide residues detected in kolanut (*Cola nitida*) and EU MRL

Pesticide	Mean ± SD (mg kg^{-1})	Range (mg kg^{-1})	MRL (mg kg^{-1})
α-HCH	0.208 ± 0.406	ND – 1.725	0.01
β-HCH	0.103 ± 0.118	ND – 0.623	0.01
Υ-HCH	0.044 ± 0.041	0.011 – 0.157	0.01
δ-HCH	0.032 ± 0.029	0.017 – 0.127	0.01
∑HCH	0.387 ± 0.594		
Heptachlor	0.121 ± 0.087	0.074 – 0.436	0.01
Heptachlor epoxide	0.089 ± 0.092	0.018 – 0.375	0.01
Aldrin	0.156 ± 0.202	0.023 – 0.729	0.01
Dieldrin	0.310 ± 0.442	0.069 – 1.979	0.01
Endrin	0.045 ± 0.059	0.002 – 0.192	0.01
Endrin Aldehyde	0.208 ± 0.201	ND – 1.097	0.01
α –Endosulfan	0.076 ± 0.053	0.022 – 0.245	0.1
β-Endosulfan	0.243 ± 0.144	0.136 – 0.667	0.1
Endosulfan sulphate	0.218 ± 0.278	0.124 – 1.523	0.1
∑Cyclodienes	1.466 ± 1.558		
p,p-DDD	0.050 ± 0.012	0.036 – 0.081	0.05
p,p-DDE	0.480 ± 1.106	0.019 – 5.718	0.05
p,p-DDT	0.108 ± 0.098	0.039 – 0.392	0.05
Methoxychlor	0.138 ± 0.234	ND – 0.919	0.01
∑ DDT	0.776 ± 1.450		
Total OCP burden	**2.269 ± 3.602**		

SD = Standard Deviation; MRL = Maximum residue limit

Table 3. Proportion of organchlorine pesticide residues detected in kolanut in relation to total OCP burden and percent above EU-MRL

Pesticide	% of total OCP burden	% above EU-MRL
α-HCH	7.92	76.0
β-HCH	3.92	88.0
Υ-HCH	1.52	100.0
δ-HCH	1.22	100.0
∑HCH	14.58	-
Heptachlor	4.61	100.0
Heptachlor epoxide	3.39	100.0
Aldrin	5.94	100.0
Dieldrin	11.81	100.0
Endrin	1.71	68.0
Endrin Aldehyde	7.92	96.0
α –Endosulfan	2.90	28.0
β-Endosulfan	9.26	100.0
Endosulfan sulphate	8.30	100.0
∑Cyclodienes	55.80	-
p,p-DDD	1.90	44.0
p,p-DDE	18.29	92.0
p,p-DDT	4.11	68.0
Methoxychlor	5.26	76.0
∑ DDT	29.56	-

Table 4. Principal component analysis (PCA) for organochlorine pesticide residues detected in kolanut (*Cola nitida*)

Pesticide	Eigen vectors	
	PC 1	PC 2
α-HCH	-0.048	**0.84**
β-HCH	0.004	**0.162**
Υ-HCH	0.022	0.007
δ-HCH	-0.001	0.021
Heptachlor	-0.004	0.046
Heptachlor epoxide	0.043	0.122
Aldrin	0.007	0.098
Dieldrin	**0.204**	**0.476**
Endrin	0.017	0.005
Endrin Aldehyde	0.160	-0.029
α –Endosulfan	-0.006	0.006
β-Endosulfan	0.077	0.064
Endosulfan sulphate	**0.224**	-0.054
p,p-DDD	0.002	0.014
p,p-DDE	**0.921**	-0.044
p,p-DDT	0.048	-0.029
Methoxychlor	**0.142**	-0.065
Proportion of Eigen	**77%**	**9%**
Value (%) cumulative	**77**	**86**
proportion		

PC = Principal Component

Among the cyclodiene group, dieldrin recorded the highest mean concentration of 0.310 mg kg^{-1} and the least detected was heptachlor epoxide

with a mean residual concentration of 0.089 mg kg^{-1}. The cyclodienes group which include dieldrin, aldrin, endrin and endosulfan have been reported in some food products in Nigeria [30,31,34]. The mean concentration of aldrin was 0.156 mg kg^{-1}, while the mean concentration of endosulfan metabolite gave 0.076 mg kg^{-1} α-endosulfan, 0.243 mg kg^{-1} β-endosulfan and 0.218 mg kg^{-1} endosulfan sulphate with total endosulfan concentration of 0.537 mg kg^{-1}. The highest mean residual endosulfan was observed from this study and all the endosulfan levels detected were above the MRL. This result corroborates the findings that reported highest concentration of endosulfan in all samples of kolanuts from Ogun, Osun, and Oyo states [33], thus confirming that the insecticide was the most frequently used in Southwestern Nigeria for the preservation of kolanuts. This also may be attributed to the proliferation of the insecticides which are available under various trade names such as Endocel 35EC®, Thionex 35EC®, Endocap 625EC® in Nigeria market as a broad spectrum insecticide with contact and stomach activity against numerous sucking and biting insect pests of cocoa, coffee, citrus, other fruit trees, cowpea, soyabean, maize and vegetables. Endosulfan is also available as a mixture of endosulfan with deltamethrin marketed under the trade name Cracker 282EC®. Farmers use the insecticide as a replacement for the banned insecticide, lindane. This new trend may have been responsible for the frequent and high detection of this OCP in the nuts. High levels of endosulfan have also been reported in cocoa bean samples [32] and blood serum of cocoa farmers [49]. The clinical short term endosulfan toxicity is dominated by the potential of this pesticide to induce convulsions and other central nervous system signs and symptoms [50,51] while the permanent neurological damage has been reported following endosulfan-induced convulsions [52]. The concern about the adverse health effects of the insecticide led to the total worldwide ban of endosulfan due to concerns that it is a carcinogen, teratogen and a male reproductive toxicant. Although Nigeria also joined in the campaign by placing a ban on the sale and use of the insecticide since 2008 but despite the ban and restrictions, it is still available under different trade names. The mean concentration levels of *p,p*-DDD, *p,p*-DDE and DDT were 0.050 mg kg^{-1} 0.480 mg kg^{-1} and 0.108 mg kg^{-1} respectively. Of all the OCPs analyzed, *p,p*-DDE recorded the highest concentration and δ-HCH the least concentration. Among all the metabolites of DDT detected in this work, *p,p-*

DDE had the highest concentration. The *p,p*-DDE and α –HCH were reported as the dominant isomers for DDT and HCH in pinenut, chestnut and walnut in China [18]. However, the concentration of *p,p*-DDE (0.480 mg kg^{-1}) detected in our study is much lower than the concentration (4.3 mg kg^{-1}) detected in pinenut samples in China [18] and 5.1 mg kg^{-1} in nuts from Hong Kong [53]. The high concentration of *p,p*-DDE among the DDTs in the kolanut samples could be as result of the direct usage and/or presence and persistence of the insecticide in the environment or because of DDT metabolites to *p,p*-DDE over time. Although DDT has also been banned in the country, its residual concentration in the nuts could be attributed to the pesticide that is possibly available under different chemical name. In northern Nigeria, *o, p*-DDE, *p,p*'-DDD, *o,p*'-DDD, *p,p*'-DDT, dieldrin and aldrin were detected in water melon [54] while Endrin and DDT have also been detected in bean samples both on field (pre storage) and stored samples (post harvest) [29].

The proportion of OCP residues detected in kolanut in relation to the total OCP burden and percent above EU-MRL is presented in Table 3. Among the 3 sub-groups of OCPs analyzed, the cyclodienes accounted for 55.8%, DDT 29.56% and HCH isomers 14.58% of the total OCPs burden. Considering the individual pesticides detected, the *p,p*-DDE recorded the highest percentage of 18.29% while dieldrin accounted for 11.81%. The least recorded was δ-HCH (1.22%). All the pesticides detected were found to be above their respective MRLs with 100% of γ-HCH, δ-HCH, heptachlor, heptachlor epoxide, aldrin, dieldrin, β-endosulfan and endosulfan sulphate detected above respective MRLs. However, only 44% and 28% of *p,p*-DDD and α-endosulfan respectively, detected in the samples were above their respective MRLs. Results of the principal component analysis (PCA) of the total OCP residues detected in kolanut samples indicated that the first two principal axis were of importance in explaining variation in the OCP residues in kolanut samples. The first PC axis (PC1) explained 77% of the variation and PC2 explained 9%, both explaining a total of 86%. Any component with relatively high Eigen vector under each PC is considered to be loaded on the PC. On this basis, of all the 17 OCPs analyzed, *p,p*-DDE, endosulfan sulphate, dieldrin, and methoxychlor had high Eigen vectors on PC1 (>0.10), indicating that they were major compounds primarily contributing to variation in the OCP residues in the kolanut samples (Table

4). In the same vein, α-HCH and β-HCH had high Eigen vectors under PC2 indicating that the compounds were of secondary importance in their contribution to the OCP residues in the kolanut samples. Clearly, all the OCPs detected occurred at concentrations above their respective MRLs indicate that high quantities of these chemicals were used on the nuts in the course of preserving the product against insect pests. Since the insecticides were not manufactured purposely for the control of storage insect pests, the processors and traders applied the insecticides on the nuts using their initiatives without any measure or standard for its use on the product. Similar observations were made by [55] on beans from Lagos market. Residues of pesticides in food are influenced by the storage, handling and processing that occur between harvesting of raw agricultural commodities and consumption of prepared foodstuffs [56]. It would have been expected that the contamination from pesticides will reduce during the processing, but unfortunately kolanuts are eaten raw without any form of processing and the situation is worsened by the direct application of the pesticides on kolanuts during storage. It is also unlikely that the traders would allow some sufficient period of time between application and sale of the nuts because of economic gain considerations.

The ADIs, EADIs and corresponding Hazard Indices (HI) as well as the Health Risk Estimation of systemic effects associated with OCP residues in kolanut are presented in Table 5. The HI values showed that γ-HCH (1.91), heptachlor (15.7), aldrin (20.3), dieldrin (40.3), endrin (2.93) in kolanut were >1. However, HIs in α-HCH (0.33), α-endosulfan (0.16), β-endosulfan (0.53), endosulfan sulphate (0.03), DDT and its metabolites (*p,p*-DDE *p,p*-DDD) and methoxychlor (0.36) were less than 1. The analysis of health risk estimates revealed that γ-HCH, heptachlor, aldrin, dieldrin and endrin levels exceeded the reference dose indicating that these insecticides are still being used in our environments and suggesting a great potential for systemic toxicity for the consumers of the kolanuts. Other detected pesticides posed neither an acute nor chronic dietary risk as the Hazard Index values were all below 1 implying a non-carcinogenic health risk to consumers of nuts from these markets. The high value of the Hazard Index of dieldrin when compared with the eigen vector (0.204) in the PCA analysis supported the contribution of the insecticide to the overall OCPs in kolanut samples. Aldrin, dieldrin, endrin, and heptachlor are listed in

Table 5. Health risk estimation for organochlorine pesticide residues in kolanut (*Cola nitida*)

Pesticide	ADI (mg kg^{-1}d^{-1})	EADI ((mg kg^{-1}d^{-1})	Hazard Index	Health risk
α-HCH	0.008	2.70 x 10^{-3}	0.3375	No
β-HCH	NA	1.34 x 10^{-3}	-	-
Ɣ-HCH	0.0003	5.70 x 10^{-4}	1.9067	Yes
δ-HCH	0.0030	4.16 x 10^{-4}	0.1387	No
Heptachlor	0.0001	1.57 x 10^{-3}	15.700	Yes
Heptachlor epoxide	NA	1.16 x 10^{-3}	-	-
Aldrin	0.0001	2.03 x 10^{-3}	20.300	Yes
Dieldrin	0.0001	4.03 x 10^{-3}	40.300	Yes
Endrin	0.0002	5.85 x 10^{-4}	2.9250	Yes
Endrin Aldehyde	NA	2.70 x 10^{-3}	-	-
α –Endosulfan	0.0060	9.88 x 10^{-4}	0.1650	No
β-Endosulfan	0.0060	3.16 x 10^{-3}	0.5270	No
Endosulfan sulphate	0.0060	2.83 x 10^{-3}	0.4720	No
p,p-DDD	0.0200	6.50 x 10^{-4}	0.0330	No
p,p-DDE	0.0200	6.24 x 10^{-3}	0.3120	No
p,p-DDT	0.0200	1.40 x 10^{-3}	0.0700	No
Methoxychlor	0.0050	1.79 x 10^{-3}	0.3580	No

NA = ADI not available for β-HCH, Heptachlor epoxide and Endrin Aldehyde

Annex A of the Stockholm Convention, meaning that most countries in the world consider these chemicals to be so dangerous to humans and the environment that the chemicals should not be used [57].

In order to reduce health risks, the age long and old non-chemical control measures should be encouraged among kolanut farmers, processors and traders. For instance, cultural practices involving early harvesting of mature kola pods, prompt removal of fallen and hanging mature pods at the end of the season, as well as the removal of dead and moribund pods between crops have been suggested as effective and economic methods of reducing the level of insect pest infestation in kola [58]. Also, a minimal level of weevil damage (35.4%) was recorded by [59] on kolanuts which were obtained from timely harvested pods, when compared with 58.3% - 83.3% damage of nuts obtained from pods whose harvest were delayed. This will go a long way in reducing the use of pesticides against these pests of the nuts and thereby improving the safety of the produce being consumed by the Nigerian populace.

4. CONCLUSION

The findings from this study showed that the kolanuts were highly contaminated with the investigated pesticides with unacceptable exposure risk and the likely co-occurrence of other pesticides most especially those ones that are not analyzed in the study may further increase the risk of the consumers of these produce. The *p,p*-DDE, endosulfan sulphate, dieldrin, and methoxychlor were the primary pesticide components contributing to OCP residues in kolanut. Kolanut is yet to be listed in the international market for export, 90% of the products is consumed locally in Nigeria. Being a local crop, the kolanut farmers may not give the required attention since it does not go through the international market that may reject the product as a result of high pesticides residues. Thus, there is high need to give urgent attention to farmers on the use of chemicals for protection against storage insects. Regular public enlightenment campaign especially among kolanut processors and traders will go a long way in addressing the possible problems that could arise due to poor knowledge and adverse health hazards associated with OCP residues in kolanut during processing and storage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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