Appraisal of Pesticide Residues in Kola Nuts Obtained from Selected Markets in Southwestern, Nigeria

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Authors’ contributions
This work was carried out in collaboration between all authors. Author PEA designed the experiment, wrote the manuscript. Author OOO facilitated sample collection while author SA made the sample collection. All the authors read the manuscript and made necessary contributions.

ABSTRACT

Aims: To assess the level of pesticide residues in kola nuts.
Study Design: Kola nuts were purchased in open markets within South Western, Nigeria.
Place and Duration of Study: The samples were obtained in markets within Oyo, Osun and Ogun States, Nigeria between November and December, 2012.
Methodology: Kola nuts were sun-dried and pulverized. 3 g of each of the pulverized samples was extracted with acetonitrile saturated with hexane. Each of the extracts was subjected to clean-up followed by pesticide residue determination using HP 5890 II Gas Chromatograph.
Results: Result show that, 50% of kola nuts samples obtained from Oyo State contained chlordane residue ranging from nd – 0.123 µg kg⁻¹; all the samples from Osun State had chlordane residue ranging from 0.103 to 0.115 µg kg⁻¹ while 70% of kola nuts from Ogun State had chlordane residues (nd – 0.12 µg kg⁻¹). All samples from Ogun, Osun and Oyo States had endosulfan residues while 30 and 20% of kola nuts obtained from Osun and Ogun States had alachlor residues respectively.
Conclusion: The presence of alachlor (herbicide), endosulfan and chlordane residues in kola nuts is an indication that, kola nuts processors use hazardous pesticides in the...
storage of fresh kola nuts. This practice can pose serious health threat to the consumers of the commodity.

Keywords: Kola nuts; pesticide residues; endosulfan; alachlor; chlordane.

1. INTRODUCTION

The word ‘kola’ is a collective name for the seed of four tree species belonging to *sterculeacea* family. Kola is one of the major tree crops grown in Nigeria. The two species that are of commercial importance include *Cola nitida* and *Cola acuminata* [1]. Nigeria accounts for about 70% of the total world production of kola nuts [2]. About 90% of the kola produced in Nigeria is consumed within the country while 10% is exported [3]. The cultivation of kola in Nigeria is ecologically limited to the rain forest zones of the southern part of Nigeria and riverine areas of the savannah region [4].

Kola nuts are widely used as a stimulant and raw material for soft drinks. A good example is in the production of coca-cola and pepsi-cola in which kola was thought to be the original formulation ingredient [5]. Recently, kola nuts have been used in the production of kola wine and in the manufacture of certain drugs [6,7]. Kola nut is also used in the social functions while its tannin content qualifies it as a good source of dyes in textile and thread industry [8]. A considerable proportion of the produced kola nuts in the south is transported to the Northern part of the country where there is high demand for the commodity. According to [9], kola is used for pharmaceutical purposes. It contains some active ingredients found in coffee and cocoa (caffeine, theobromine, kolatin) which prevents sleep, thirst and hunger and also acts as an anti-depressant [10].

Chlordane was the first cyclodiene insecticide to be used in agriculture and was the second most important organochlorine insecticide in the United States in 1976-77, behind toxaphene, with an estimated annual production of 9 million kilograms [11]. Chlordane has been used to control insects in vegetable crops, small grain, maize, potatoes, sugarcane, sugar beets, fruits, cotton and jute [12]. Food is the major source of exposure of the general population to chlordane, but the use of chlordane on food crops has decreased and residues in food of animal origin are low [13]. In experimental animals (rats and dogs), prolonged exposure to levels in the diet exceeding 3-5 mg/kg resulted in the induction of hepatic microsomal enzymes and, at a later stage, liver hypertrophy with histological changes. At higher levels (i.e., >15 mg/kg body weight per day), chlordane is hepatotoxic [14]. At dosages above 30 mg/kg diet, chlordane interferes with reproduction in rats and mice. There are no indications for teratogenicity in the rabbit at 15 mg/kg body weight per day [14]. Chlordane produces hepatocellular carcinomas in mice. Chlordane can interfere with cell to cell communication in vitro, a characteristic of many promoting agents [15].

Endosulfan is a pesticide belonging to the organochlorine group of pesticides, under the Cyclodiene subgroup. It is used in vegetables, fruits, paddy, cotton, cashew, tea, cocoa, coffee, tobacco and timber crops [16].

Residues of endosulfan were detected in tomatoes from Brazil [17], Spanish pepper samples from Finland [18], food and vegetables in Croatia and vegetables in Cyprus [18]. High levels of residues were found in red pepper and egg plants in Catania (Italy) [18]. Endosulfan has also been detected from human tissues. It has been detected from cord blood samples obtained at the time of delivery [19]. There is experimental evidence of adverse effects of
endosulfan on the male reproductive system, delaying sexual maturity and interfering with the sex-hormone synthesis [20]. Endosulfan is a proven endocrine disruptor [21]. It has potential to induce hypo thyroidism [22]. It competes for estradiol for binding to estrogen receptors, thereby inhibiting hormonal function [23]. It harms the reproductive system by affecting semen quality, sperm count, spermatogonial cells, sperm morphology and other defects in male sex hormones [24].

Alachlor is a herbicide used for weed control on corn, soybeans, sorghum, peanuts, and beans. There are liquid, dry flowable, microencapsulated, and granular formulations. The timing of applications is preplant, pre-emergent, at plant for corn and soybeans, post-transplant, postemergent, and at ground crack for peanuts only. Alachlor has been evaluated for carcinogenic activity in rats and mice. In accordance with the 1996 EPA proposed Guidelines for Carcinogen Risk Assessment, alachlor was classified as "likely" to be a human carcinogen at high doses, but "not likely" at low doses [25](EPA, 1998). People may be exposed to small amounts of alachlor through the consumption of food containing residues of alachlor. Tolerances are pesticide residue levels that should not be exceeded in or on a raw agricultural commodity in the channels of interstate commerce when the pesticide is applied according to label directions.

The commodity “kola nut” is not without its associated challenges. A major challenge associated with kola nuts storage is the attack by kola nuts weevil both prior to the harvest and during storage [10]. The kola nut weevils Balnogastris kolae and Sophorhinus spp. are the most destructive field-to-store pests of kola nuts in West Africa [26]. The weevils are capable of causing 30-70% damage on the stored nuts while 100% damage has been recorded in cases of late harvest [27].

In order to prevent stored kola nuts from the attack of store pests, (weevils), kola nuts farmers and traders use various types of pesticides including banned ones [28]. The applied pesticides in their characteristic nature have the ability to permeate plant cells and remain as residues. Several authors have reported the presence of pesticide residues in various foods, vegetables, soils, sediments and diverse environmental matrices. [29] reported the detection of cyprodinil and fluodioxonil in fifteen commercial white wine samples produced in Rías Baixas area in Galicia NW Spain. The detection of carbofuran in water samples was reported by [30]. Alpha and beta endosulfan were detected in water using solid-phase microextraction [31, 32]. Fourteen fungicides were detected in in white grapes using multiresidue HPLC method [33]. Fungicide residues were analyzed in white grapes for winemaking using GC-MS detection [34]. Procymidine was determined in wash off through a Ribeiro vineyard in Galicia NW Spain by [35]. A sensitive method for the simultaneous quantification of eight commonly used grapevine fungicides in vineyard soils was developed by [36]. Organochlorines pesticides were detected in stream sediments collected along stream flows close to an industrial area in NW Spain where hexachlorobenzene and DDT were detected at levels between 29 and 392 µg kg⁻¹ respectively [37]. Endosulfan was detected in soil samples extracted from column periodically and analyzed with GC with electron capture detector [38]. Detection of carbofuran and organophosphorus pesticides were reported by [39] in natural water using solvent drop microextraction (SDME) followed by GC-Nitrogen-phosphorus detector. Six organophosphate (azinphos-ethyl, chlorfenvinphos, chlorpyriphos, ethoprophos, fenamiphos and malathion) and two organonitrogen (alachlor and dltamethrin) were detected in drinking water using SPE followed by GC-NPD detector [40]. Determination of diquat and paraquat herbicides were made by [41] simultaneously with SPE and HPLC coupled with diode array UV detection. Two insecticides, nematicide, herbicide and a combination of two fungicides were reported...
present in potato obtained from northwestern Spain by [42]. Fungicide residues were measured in vineyard soil and river sediments obtained from Galicia, Spain by [43] using SPE followed by GC-MS. [44] reported paraquat sorbed to soil and the major factor governing the sorption was the solid state organic fraction with the clay mineral also making significant contribution. Twenty three fungicides and insecticides were detected in leafy vegetables collected from Ourense NW Spain SPE and determined by GC-ITMS [45]. [46] used batch equilibrium method to study the adsorption-desorption of quaternary ammonium herbicides in vineyard soils. The determination of tebuconazole residues in grapes, musts and wines in A.O.C. Valdeorras, Spain was reported by [47]. The determination of metalaxyl in Technical Ridomyl Gold plus and Ridomyl Gold MZ was reported by [48]. [49] reported the detection of polychlorinated biphenyls in both products and by-products of a mussel shell incinerator facility while [50] documented the surveillance of fungicidal dithiocarbamates residues in fruits and vegetables in which 6% of the samples exceeded the maximum residue limit set for dithiocarbamates in fruits and vegetables.

When plant and animal material having pesticide residues at concentrations inimical to human health are consumed, several health hazards could be created within the system which can lead to cancer, kidney failure and a host of other health problems.

In Nigeria, there are limited data on pesticide residues in kola nuts; and lack of sufficient empirical data showing the presence and/or levels of pesticides residues may hamper the creation of awareness and sensitization on the dangers associated with human consumption of kola nuts preserved with toxic synthetic pesticides.

The present study sets out to appraise the levels of some pesticides in kola nuts purchased from selected markets in the Southwestern, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh kola nuts were bought from kola nuts vendors in selected markets Oja Oba, Orita-merin, Beere and Orita-mefa within Ibadan metropolis in Oyo State, Nigeria. Some kola nuts were also purchased from kola nut vendors in Shagamu, Ilishan and Mamu in Ogun State while another set of kola nuts were bought in selected markets in Ekusa and Orafidina in Osun State, Nigeria (Fig. 1). In each location, five kola nuts were bought from each vendor and three vendors were selected in each locations. In all, thirty sub-samples were collected from each of the three States.

The purchased kola nuts according to the vendors have been preserved for a period of one and two years. Information received from the vendors showed that, the various kola nuts were treated with synthetic pesticides to increase the shelf life of the commodity and to protect them from the attack of weevils. Most of the vendors did not know the chemical names of the various pesticides they use in treating the nuts.
Fig. 1. Map of Nigeria showing locations where kola nuts were collected

2.2 Sample Preparation

The kola nuts were chopped with clean knife to facilitate sun-drying of the samples. The chopped kola nuts were sun-dried for three days under intense sun shine after which the samples were transferred into a Gallenham oven for proper drying at a temperature of 50°C for 72 hours. When a constant weight was attained, the samples were removed and ground with ceramic mortar and pestle in the laboratory.

2.3 Extraction of Pesticides in Kola Nuts

A slight modification of the method used by [17] was adopted in the extraction procedure. Three gram (3 g) of each sample was mixed with 10g sodium sulphate and then transferred to a 250ml separating funnel. 20ml of hexane was added followed by 100ml acetonitrile saturated with hexane. The mixture was shaken for about one minute. The acetonitrile was drained into a 1L separating funnel and the mixture further extracted three times with 50ml portion of acetonitrile. The extracts were combined, washed with 500ml distilled water followed by 40 ml saturated NaCl and 50 ml hexane. The mixture was then allowed to stand for 30 minutes and the aqueous layer drained into another 1L separating funnel and extracted with further 50 ml hexane. All the hexane extracts were combined and passed through a plug of 10g sodium sulphate into a flask. The solvent was evaporated using a rotary evaporator.

The residue was dissolved in 10ml methanol and diluted with 25ml of distilled water. A portion (a portion of this solution which is equivalent to 1g of kola sample was taken for pesticide residue analysis after clean up.
2.4 Gas chromatographic Analysis

The gas chromatographic analysis of pesticide residue in kola nuts samples was performed on an HP 5890 II Gas Chromatograph with a split/splitless injector port equipped with electron capture detector. The GC was equipped with a VF-17 capillary column (60m x 0.25mm i.d., 0.25µm film thickness) from Varian, Germany. Helium was used as carrier gas at a constant pressure of 27 psi. The injection temperature was 300°C and the injection volume was 1µL (splitless). All pesticides residue standards were purchased from USA. The chromatographic analysis was performed under the following conditions: the detector temperature was 320°C. The injector temperature was 220°C and the column temperature was 160°C (isothermal) the carrier gas was at a flow of 3 cm/sec. 1ml of sample was injected into the GC. In the GC analysis, peaks were identified by comparing their retention times with those of the standards under the same injection conditions. The peak areas of the various peaks whose retention times coincide with the standards were extrapolated on their corresponding calibration curves to obtain the concentration. The limit of quantification was 0.01mg kg\(^{-1}\). Under the experimental conditions, the retention times for Mecoprop, Atrazine, 2,4-D, Carbofuran, DBCP, Pentachlorophenol, Ferroprop, Alachlor, 2,4-DB, Metolachor, Lindane, Chlorpyrifus, DDT, Aldrin, Endrin, Chlordane, endosulfan and Dieldrin are: 6.70, 7.385, 8.51, 8.932, 9.67, 11.357, 12.82, 14.15, 15.396, 16.598, 17.664, 18.661, 19.677, 20.784, 21.902, 24.609, 26.007 and 27.427 minutes respectively. The linearity of the methods was evaluated using a concentration range for the pesticide residues analyzed by GC. In all the various cases, good linearity was achieved with correlation coefficients > 0.995.

3. RESULTS AND DISCUSSION

3.1 Chlordane Residue in Kola Nuts

An array of pesticides which includes herbicides and insecticides were determined in all the kola nuts samples obtained from selected markets in Ogun, Osun and Oyo States.

In samples obtained from selected markets in Oyo State, two of the eighteen pesticides assessed in the samples were detected. The two pesticides were insecticides. They were chlordane and endosulfan. Chlordane residue in the kola samples ranged from 0.114 – 0.123 µg kg\(^{-1}\) with a mean of 0.116 µgkg\(^{-1}\) (Table 1). Sample obtained from OY 6 had the lowest detectable chlordane while sample from OY 2 had the highest detectable chlordane. Results show that, 50% of the total number of kola nuts obtained from the selected markets in Oyo State had chlordane residue.

Chlordane (insecticide) residue was detected in all the kola nut samples obtained from Osun State at concentration levels which ranged from 0.102 – 0.115 µg kg\(^{-1}\) with an average value of 0.109 µg kg\(^{-1}\) (Table 2) while in samples obtained from Ogun State, it was detected in 70% of the kola nuts samples at levels ranging from 0.102 – 0.12 µg kg\(^{-1}\) with an average value of 0.11 µg kg\(^{-1}\). Sample obtained from OG 7 had the lowest detectable chlordane residue while sample from OG 6 had the highest value of chlordane.

The detection of chlordane in most of the kola nuts from the various locations of sample collection is an indication that, the use of chlordane in the preservation of kola nuts from the attack of weevils is common among kola nuts farmers and vendors within the study areas of Oyo, Osun and Ogun State. The detection in 100% of kola nuts samples from Osun State
suggests that, the use of chlordane in kola nuts preservation is very popular among kola nut processors in Osun State compared with other locations.

Chlordane is an organochlorine cyclodiene pesticide derived from hexachloropentane. Hexachlorocyclopentadiene forms an adduct with cyclopentadiene and chlorination of the adduct gives two isomers, alpha and beta. The mixture is called chlordane. It was used extensively as an insecticide from its introduction in 1947. The use pattern for chlordane in the USA in the mid-1970s followed a pattern of 35% by pest control mostly on termites; 28% on agricultural crops including maize and citrus; 30% for home lawn and garden use; and 7% on turf and ornamental plants [51]. The use of chlordane was banned in 1988 in the USA but can still be legally manufactured only to be sold or used by foreign countries. The decision to ban chlordane was borne out of concern that, people may be exposed to its hazard by eating food contaminated with chlordane. Laboratory mice that were fed with chlordane over long period of time had higher incidence of liver cancer than untreated mice [52]. Exposure to chlordane metabolites may be associated with testicular cancer. The incidence of seminoma in men with the highest blood level of chlordane was almost double of that of men with the lowest level [53]. Postrate cancer has been associated with transnonachlor levels, a component of chlordane [54]. Japanese workers who used chlordane over a long period of time had minor changes in liver function [55]. Despite the fact that, the concentration of chlordane in the various kola nut samples were low, its bioaccumulation in human body due to long term consumption of kola nuts may lead to chronic poisoning more so that, chlordane is lipophilic and gets bioaccumulated in fatty tissues of animals. The concentration of chlordane in the serum of Japanese pesticide spraymen who had been spraying chlordane formulation for < 3 and > 5 years were on average 2.4ng g⁻¹ and 5.1 ng g⁻¹ respectively [56]. Determination of chlordane of chlordane in meat, fish, birds and terrestrial animals ranged from 2 to 34 ng g⁻¹ wet weights [57]. Consumption of foods containing chlordane residue may result in measurable concentrations of its compound in human tissues [58]. The accumulation of chlordane in human system has been documented by some authors. In Canadian newborns in 1993-1995, the concentration of chlordane in cord blood from non-Inuit infants ranged from 0.01 – 0.07µgL⁻¹ [59]. The mean concentration of chlordane measured in 12 samples of breast milk from Arctic Canada in 1996 was 1.27 ng g⁻¹ of fat [60]. From the various evidences which show the bioaccumulation and residual effects of chlordane in human system, the possibility of chlordane being accumulated in the blood, fat and tissues of consumers of kola nuts sold in the study areas is therefore, suggestible.

### Table 1. Pesticide residues in kola nuts from selected markets in Ibadan

<table>
<thead>
<tr>
<th>Location</th>
<th>Chlordane</th>
<th>Endosulfan</th>
</tr>
</thead>
<tbody>
<tr>
<td>OY 1</td>
<td>nd</td>
<td>0.88±0.01</td>
</tr>
<tr>
<td>OY 2</td>
<td>0.123±0.01</td>
<td>1.87±0.03</td>
</tr>
<tr>
<td>OY 3</td>
<td>0.118±0.02</td>
<td>2.27±0.04</td>
</tr>
<tr>
<td>OY 4</td>
<td>nd</td>
<td>0.81±0.02</td>
</tr>
<tr>
<td>OY 5</td>
<td>0.114±0.01</td>
<td>2.20±0.03</td>
</tr>
<tr>
<td>OY 6</td>
<td>0.106±0.02</td>
<td>0.91±0.02</td>
</tr>
<tr>
<td>OY 7</td>
<td>0.120±0.01</td>
<td>0.85±0.02</td>
</tr>
<tr>
<td>OY 8</td>
<td>nd</td>
<td>1.73±0.04</td>
</tr>
<tr>
<td>OY 9</td>
<td>nd</td>
<td>1.63±0.03</td>
</tr>
<tr>
<td>OY 10</td>
<td>nd</td>
<td>0.98±0.01</td>
</tr>
</tbody>
</table>

*Key: nd = not detected*
Table 2. Pesticide residue in kola nuts from selected markets in Osun State

<table>
<thead>
<tr>
<th>Location</th>
<th>Alachlor ( \mu g \ kg^{-1} )</th>
<th>Endrin ( \mu g \ kg^{-1} )</th>
<th>Chlordane ( \mu g \ kg^{-1} )</th>
<th>Endosulfan ( \mu g \ kg^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS 1</td>
<td>nd</td>
<td>0.102±0.04</td>
<td>0.103±0.02</td>
<td>0.90±0.02</td>
</tr>
<tr>
<td>OS 2</td>
<td>nd</td>
<td>0.105±0.01</td>
<td>1.50±0.01</td>
<td></td>
</tr>
<tr>
<td>OS 3</td>
<td>0.111±0.01</td>
<td>nd</td>
<td>2.72±0.04</td>
<td></td>
</tr>
<tr>
<td>OS 4</td>
<td>nd</td>
<td>0.110±0.01</td>
<td>2.49±0.03</td>
<td></td>
</tr>
<tr>
<td>OS 5</td>
<td>nd</td>
<td>0.105±0.03</td>
<td>2.61±0.04</td>
<td></td>
</tr>
<tr>
<td>OS 6</td>
<td>0.102±0.02</td>
<td>nd</td>
<td>2.58±0.03</td>
<td></td>
</tr>
<tr>
<td>OS 7</td>
<td>0.104±0.03</td>
<td>nd</td>
<td>1.48±0.02</td>
<td></td>
</tr>
<tr>
<td>OS 8</td>
<td>nd</td>
<td>0.113±0.02</td>
<td>1.62±0.01</td>
<td></td>
</tr>
<tr>
<td>OS 9</td>
<td>nd</td>
<td>0.108±0.01</td>
<td>0.91±0.01</td>
<td></td>
</tr>
<tr>
<td>OS 10</td>
<td>nd</td>
<td>0.110±0.02</td>
<td>1.27±0.03</td>
<td></td>
</tr>
</tbody>
</table>

*Key: nd = not detected*

Table 3. Pesticide residues in kola nuts from selected markets in Ogun State

<table>
<thead>
<tr>
<th>Location</th>
<th>Alachlor ( \mu g \ kg^{-1} )</th>
<th>Chlordane ( \mu g \ kg^{-1} )</th>
<th>Endosulfan ( \mu g \ kg^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>OG 1</td>
<td>nd</td>
<td>nd</td>
<td>0.79±0.02</td>
</tr>
<tr>
<td>OG 2</td>
<td>0.135±0.02</td>
<td>0.119±0.02</td>
<td>2.42±0.08</td>
</tr>
<tr>
<td>OG 3</td>
<td>nd</td>
<td>0.112±0.01</td>
<td>2.02±0.04</td>
</tr>
<tr>
<td>OG 4</td>
<td>nd</td>
<td>0.104±0.02</td>
<td>0.90±0.02</td>
</tr>
<tr>
<td>OG 5</td>
<td>0.128±0.01</td>
<td>0.106±0.02</td>
<td>1.24±0.04</td>
</tr>
<tr>
<td>OG 6</td>
<td>nd</td>
<td>0.126±0.02</td>
<td>2.15±0.06</td>
</tr>
<tr>
<td>OG 7</td>
<td>nd</td>
<td>0.102±0.02</td>
<td>1.89±0.08</td>
</tr>
<tr>
<td>OG 8</td>
<td>nd</td>
<td>nd</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>OG 9</td>
<td>nd</td>
<td>0.112±0.01</td>
<td>0.88±0.02</td>
</tr>
<tr>
<td>OG 10</td>
<td>nd</td>
<td>nd</td>
<td>2.04±0.05</td>
</tr>
</tbody>
</table>

*Key: nd = not detected*

3.2 Endosulfan Residue in Kola Nuts

Endosulfan residue was detected in all kola nuts samples obtained from Oyo State at a range of 0.81 – 2.27 \( \mu g \ kg^{-1} \). Sample obtained from OY 4 had the lowest concentration of endosulfan while sample from OY 3 had the highest value of endosulfan residue in the kola nuts samples (Table 1). Endosulfan residues in samples from Osun State ranged from 0.904 – 2.72 \( \mu g \ kg^{-1} \) (Table 2) while its content in samples obtained from Ogun State ranged from 0.794 – 2.42 \( \mu g \ kg^{-1} \) with a mean value of 1.53 \( \mu g \ kg^{-1} \). Sample obtained from OG 1 had the lowest endosulfan residue while sample from OG 2 had the highest value (Table 3). The detection of endosulfan in all the samples from the three States is a strong indication that, endosulfan is one of the most popular insecticides used among kola processors and vendors in Nigeria. Despite the fact that, the levels of endosulfan detected in the various samples are lower than 0.1mg kg\(^{-1}\) set as maximum residue limit by the European Union for some common foods, the fact that it is present even at low concentration is of concern basically because kola nuts are eaten raw without heat treatment.

In a study carried out by [61], endosulfan residue was detected in 108 fresh and dried kola nuts at a concentration range of 2.0 – 99.00 mg kg\(^{-1}\). The concentration of endosulfan in the present study is far lower than these figures.
There are experimental evidences of adverse effects of endosulfan on the male reproductive system, delaying sexual maturity and interfering with the sex-hormone synthesis [62]. Endosulfan is a proven endocrine disruptor [22]. It has potential to induce hypothyroidism [63]. It competes for estradiol for binding to estrogen receptors, thereby inhibiting hormonal function [64]. The estrogenic potential of endosulfan increases in the presence of other estrogenic organochlorines [65]. It induces proliferation of human breast estrogen sensitive MCF7 cells (in vitro) thereby increasing breast cancer risk [66]. It harms the reproductive system by affecting semen quality, sperm count, spermatogonial cells, sperm morphology and other defects in male sex hormones [24]. Endosulfan has the capacity to alter the genetic material particularly chromosomes in mammalian cultures [67]. It is found to inhibit testicular androgen biosynthesis in laboratory animal experiments and exhibits significant risk in renal and testicular damage [68]. It may have adverse effects on central nervous system by inhibiting brain acetyl cholinesterase causing uncontrolled discharge of acetyl choline [69]. Endosulfan ingestion is known to affect the kidneys and liver [70]. It inhibits leucocyte and macrophage migration (this is the inhibition of the natural immune system by disrupting anti-body protection) causing adverse effects on humoral and cell-mediated immune system. It is also a potential tumor promoter [71].

Reports on endosulfan residue in food, soil, air, body tissues etc are available from all parts of the globe. Residues of endosulfan were detected in tomatoes from Brazil [17], Spanish pepper samples from Finland , food and vegetables in Croatia and vegetables in Cyprus; endosulfan residues were also detected from cow milk in Colombia far exceeding the WHO and FAO reference levels [18]. Endosulfan has also been detected from human tissues. It has been detected from cord blood samples obtained at the time of delivery [19] human sera [72,73], adipose tissue [74] and human milk samples obtained from healthy lactating women in Spain [75], human breast milk from Egypt, Colombia and Nicaragua [76]. High levels of endosulfan were detected in human breast milk in Sub Saharan Africa and also from India [77].

The detection of endosulfan in human serum, breast milk and body fat is an indication of its accumulative ability in human body. It then suggests that, consumers of kola nuts from the study may have some level of endosulfan accumulated in their body more especially that, endosulfan is lipophilic.

Linear regression was used to determine the relationship between endosulfan and chlordane residues in kola nuts obtained from the study areas. The $R^2$ obtained for kola nut samples from Osun (Fig. 3) and Ogun States (Fig. 4) is an indication that, kola nut traders within the selected area of Osun and Ogun States use chlordane and endosulfan simultaneously in the preservation of kola nuts. However, the rate was found higher in Osun State than Ogun State. On the other hand, the data obtained in samples from Oyo State (Fig. 2) showed no indication of simultaneous use of endosulfan and chlordane by kola processors or vendors.

### 3.3 Alachlor Residues in Kola Nuts

Analysis show that, 30% of the samples contained alachlor (herbicide) residue ranging from 0.102 – 0.111 µg kg$^{-1}$ while there was no detectable alachlor in 70% of the kola samples. Endrin residue was detected in 20% of the sample population obtained from selected markets in Osun State at levels ranging from 0.102- 0.105 µg kg$^{-1}$. Endosulfan residue was detected in all the kola nuts at concentrations ranging from 0.904 – 2.72 µg kg$^{-1}$ with a mean value of 0.912 µg kg$^{-1}$. Among the three pesticides detected in the
samples, endosulfan had the highest concentration in the kola nut samples. Sample obtained from OS 3 had the highest concentration of alachlor, chlordane and endosulfan residues.

Results of pesticides residue analysis in kola nut samples obtained from the selected markets in Ogun State is presented in Table 3. Results show that, 20% of the analyzed samples contained alachlor (herbicide) residues which ranged between 0.135 and 0.128 µg kg\(^{-1}\). The residue was detected in samples from OG 2 and OG 5

![Graph 2](image1.png)

**Fig. 2. Relationship between endosulfan and chlordane residues in kola nuts obtained from Oyo State**

![Graph 3](image2.png)

**Fig. 3. Relationship between endosulfan and chlordane residues in kola nuts obtained from Osun State**
Fig. 4. Relationship between endosulfan and chlordane residues in kola nuts obtained from Ogun State

The linear regression between chlordane and endosulfan residues in kola nuts obtained from selected markets within Oyo, Osun and Ogun States is presented in Figs. 2, 3 and 4 respectively. Results show linear regression $R^2 = 0.106$ for samples from Oyo State, $R^2 = 0.629$ for samples from Osun State and $R^2 = 0.542$ for samples from Ogun State.

4. CONCLUSION

The study has shown that, kola nuts obtained from the selected markets in the south western Nigeria contained some levels of chlordane and endosulfan as residues while few samples among kola nuts from Ogun and Osun States had herbicide (alachlor) residues in them.

Despite the fact that, the levels of endosulfan and chlordane residues detected in the various kola samples were below the maximum residue set by the European Union, their presence in kola nuts is a concern due to the fact that, the consumers of these contaminated kola nuts may develop or suffer from the chronic effects of chlordane and endosulfan which are known endocrine disruptors.

There was indication of simultaneous treatment of kola nuts with endosulfan and chlordane by kola nut processors. Based on the present findings, it is therefore recommended that, alternative insecticides which are safe, biodegradable and environmental friendly should be sought for the purpose of kola preservation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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