Original Research Article

Organochlorine Pesticide Residues Levels in Kola Nuts (*Cola nitida* Schott & Endl.) and Estimation of Risk Exposition in Côte d'Ivoire

ABSTRACT

Background: The Kola nut represents a significant economic interest for this country as well as many African households and public authorities. Despite its obvious importance, the kolanut sector of the kola nut is facing a delicate sanitary quality of the marketed product. About The majority of the production (90%) of produced kolanut is consumed daily fresh by people and posescould cause a serious organochlorine pesticide toxicity health problem for consumers if the toxicity due to organochlorine pesticide residues were proven.
Aims: This study aimed to detrmineddetermine the organochlorine pesticide residues levels in kola nuts and assessestimate the risks of kola nuts consumption on population health in Côte d'Ivoire.
Study Design: Samples were collected from Farmers, rural Collectors, urban Stores in Districts (Mountains, Comoe, Lagoons, Down-Sassandra) and big storage Centers of Anyama and Bouake for three separate periods of kola nuts harvesting (2016-2017 ; 2917-2018 and 2018-2019).
Methodology: Concentrations of 24 organochlorine pesticide (OCPs) residues were measured using a gas chromatograph equipped with an electron capture detector.
Results: The OCPs concentrations ranging from 5.19 ± 0.96 µg/kg to 92.93 ± 51.85 µg/kg for Aldrin and Lindane. The results indicate that Methoxychlor, DDE (op'), Endrin ketone, Hexachlorobenzene, Chlorfenapyr, Chlorthal dimethyl and Quitozene concentrations are below the quantification limit (LOQ).
Based on the concentrations and the daily consumption of kola nuts estimated at 0.6 g/person in Côte d'Ivoire, the intakes values estimated of OCPs vary from $5.4.10^{-5} \pm 3.55.10^{-5}$ µg/kg/day to $7.96.10^{-4} \pm 4.44.10^{-4}$ µg/kg/day for Aldrin and Lindane, respectively. The Exposure Daily Doses (EDD) are all lower than the toxicological reference values. Thus, the occurrence of a toxic effect from OCPs after kola nuts consumption is very unlikely since the hazard quotient HQ sum is less than 1 ($\sum HQ = 0.13 < 1$). Consumption of kola nuts from Côte d'Ivoire is nodoes not pose a health risk to the consumer's health.
Conclusion: The risk of adverse effects from consuming kola nuts contaminated with residues of organochlorine pesticide is very unlikely.

Keywords: *Cola nitida, organochlorine pesticide, health risk, consumption, Côte d'Ivoire*

1. INTRODUCTION

Pesticides are substances used to kill, repel, or control certain forms of plant or animal life that are considered to be pests. Nowadays, more thanover 1100 pesticides chemicals are used in various combinations and at different stages of cultivation and during postharvest storage to protect crops against a range of pests [1, 2]. Pesticides belong to different chemical classes but the major ones are organophosphates, carbamates, pyrethroids and organochlorines [3].
Organochlorine pesticides (OCPs) are an important group of persistent organic pollutants, their chemical stability allows them to remain active in the environment for decades [4]. Such lipophilic compounds are persistent in the environment and are readily conveyed over long distances or bioaccumulated through the food chain. Being persistent in the environment, their accumulations in the food chain, their sub-acute and chronic toxicity are detrimental to human and animal health [5]. Moreover, they tend to accumulate in living organisms and are known to be responsible for carcinogenic, mutagenic and teratogenic effects. They also have toxic effects on the nervous, immune, reproductive, renal, hepatic and hematopoietic systems [6].
This proven toxicity is a real public health problem for many governments and a hindrance to the export of some agricultural products that are widely prized by Western industries such as kola nuts.

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Indeed, Kola nuts have an increasing interest for industries, mainly because of their richness in bioactive and functional compounds such as polyphenols, caffeine and theobromine [7, 8]. They constitute an important raw material in the formulation of pharmaceutical, food, cosmetic and textile products [9, 10].
Despite its obvious economic importance, the sector of kolanut is concerned with several troubles regarding the final quality of the marketed product. According to Deigna [9], one of the major constraints for the kola stakeholders is the post-harvest preservation of the raw crops. Indeed, kola nuts are generally consumed fresh [7, 8,10]. Yet, the fresh crops state easily allows proliferation of microbes, ants and other parasites. In order to control th~~e~~is crop’s post-harvest enemies and to keep the fruits fresh, the farmers and traders generally soak the raw kola nuts in organic pesticides solutions [11, 9].
The use of pesticides in the kola sector is observed during the crop’s carriage and processing. Indeed, the kola nut~~s~~ distribution channel is generally from farmers to the big storage, processing and export centres, with temporary stay ~~from~~ at rural collectors and small urban stores [12]. During their processing, carriage and sale, organochlorine pesticides could be laid on the kola nuts stock. Among organochlorines such as Dichloro Diphenyl Trichloroethane (DDT) and Hexachlorocyclohexane (HCH), though prohibited, are still used by some Ivorian farmers [6, 11]. However, current bibliographic data available in Côte d’Ivoire concerning organochlorine pesticides are mostly about products such as coffee, cocoa, cotton, fish, milk, milk products and kola nuts [13, 14, 15]. Concerning kola nuts, A few data show~~ed~~s the presence of organochlorine pesticides during kola nut~~s~~ post-harvest processing. Furthermore, the works of Biego et al. [11], Aikpokpodion et al. [16] and Deigna [17] show~~ed~~s the presence of organochlorine pesticides in kola nuts at concentrations over the Codex Alimentarius maxim~~al~~um permisisble limits~~values admitted by the *Codex Alimentarius*~~.
About ~~The majority of the production (~~90%~~)~~ of kola nut~~s~~ is consumed daily and fresh ~~by people~~ [10]. This ~~and could~~ potentially poses ~~cause~~ a serious organochlorine pesticide toxicity health ~~problem~~ risk for consumers~~ if the toxicity due to organochlorine pesticide were proven~~. The presence of organochlorine pesticides could also slow down the export of this raw material to new markets, which would constitute a significant shortfall for all actors in the kola sector.
Therefore, the aim of this study was to determine pesticide levels in kola nuts produced in Côte d'Ivoire in order to assess~~estimate~~ the health risks for the consumers.

2. MATERIAL AND METHODS
2.1 Investigation Site

The study was conducted in the main areas of kola nut~~s~~ production, big storage and distribution centers in Côte d'Ivoire. The investigated regions are located between 2°30' and 8°30' of West longitude and between 4°30' and 10°30' of North latitude. Thus, the mountain district (pole 1), the Districts of Comoe and Lagoons (pole 2) and the District of Bas-Sassandra (pole 3) were selected as production areas while the cities of Anyama and Bouake represent the storage and distribution centers (Fig.1~~.)~~).).

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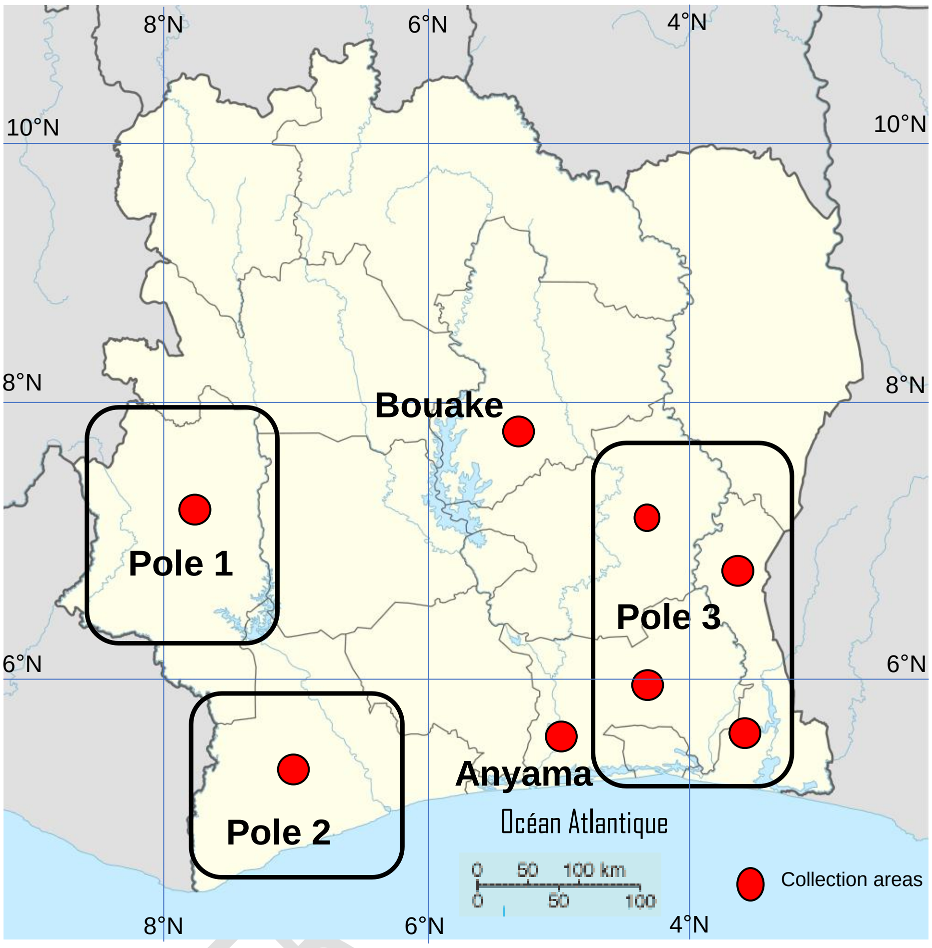

Fig.1. Map showing kolanut samples collection sites

2.2 Plant Materials and Sampling

The biological materials of th~~is~~e current study consist~~s~~ed of fresh *Cola nitida* Vent. (Schott & Endl) nuts collected from ~~F~~farmers, rural ~~Collectors~~collectors, urban ~~Stores~~ stores and big storage c~~C~~enters for three separate periods of Kola nut~~s~~ harvesting (2016-2017 ; ~~2917~~2017-2018 and 2018-2019), in accordance with ~~the~~ Regulation No 333/2007 of the European Commission [18]. A total of~~So,~~ 81 ~~Samples~~ samples were collected ~~by~~ from storage c~~C~~enters (Anyama and Bouake cities) and by production pole namely 27 samples per type of actors. In total, 810 fresh kola nut~~s~~ samples, weighing 2 kg each, were used for this study. The Kola nut~~s~~ was authenticated by N'Guessan botanist in the National Floristic Center (CNF) in Abidjan, Côte d'Ivoire, Training and Research Unit of Biosciences, Felix HOUPHOUËT-BOIGNY University where a voucher specimen was documented.

2.3 Extraction of Organochlorine Pesticide~~s~~ Residues according to the QuEChERS procedure

An initial monophasic extraction of 10 or 15 g of sample by acetonitrile, at a rate of 1 mL of actetonitrile per 1 g of sample was carried out. The addition of salts (NaCl, 1 g) and buffers (1.5 g of sodium citrate

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Comment [CB10]: is this the name of the botanist? Perhaps rephrase the whole sentence and break it up into two so that we can see who authenticated and where...

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or sodium acetate) promotes liquid-liquid separation [19]. After centrifugation, the acetonitrile phase containing the pesticide is recovered. The matrix can be further purified, and the excess water removed during a solid phase extraction step and in dispersive mode with anhydrous magnesium sulfate ($MgSO_4$). An aliquot of 1 µL of the final extract is injected into the analytical system.

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2.4 Reagent and Solvents

Analytical grade reagents and solvents were used. They were High Performance Liquid Chromatography (HPLC) grade: Hexane and dichloromethane from Sigma Aldrich; deionized water from SDS and a mixed standard solution of 24 organochlorine pesticides (EPA 608 Supelco) concentrated at 20 µg/L. These standard organochlorine pesticides were , Hexachlorobenzene Chlorfenapyr, Chlorthal dimethyl, Quitozene, Aldrin, Endrin, Dieldrin, Heptachlor, α-Endosulfan, β-Endosulfan, Endosulfan sulfate, Endrin ketone, Cis heptachlor epoxyde, Trans heptachlor epoxyde, Hexachlorocyclohexane (α-HCH, β-HCH, δ-HCH and γ-HCH), The Dichloro Diphenyl Trichloroethane (DDT) family: dichlorodiphenyltrichloroethane and its metabolites (p,p'-DDT, o,p'-DDT, p,p'DDE, o,p'-DDE, p,p'-DDD) and Methoxychlor.

2.5 Estimation of the Risk of Exposure to Pesticides Residues from Kolanut~~s~~ Consumption

The risks considered in this study derived solely from the consumer exposure through ingestion of kola nuts contaminated with organochlorine pesticides. The assessment methodology was conducted according to the risk assessment model of the Codex Alimentarius ~~about risk assessment~~ [20]. This procedure follows four main steps including the hazard identification, ~~the~~ hazard characterization, ~~the~~ exposure assessment and risk characterization [21].
Assessment risk organochlorine pesticides to the calculation of the Exposure Daily Dose (EDD) from the average amount of 0.6 g per day of kola nuts consumed by an Ivorian adult [22,10, 15]. The exposure scenarios where the individual is the most exposed ~~have been~~were used (maximalist assumption). EDD of organochlorine pesticides linked to the consumption of kola nuts were determined as follows:

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$$EDD = C \times Q \times F/P$$

Where : EDD is the exposure daily dose (µg/kg/d) ; C the Concentration of organochlorine pesticides in kolanut (µg/kg) ; Q the Daily consumption kola nut (kg/d) ; F the Frequency of exposure (F = 1) and P the body weight of an Ivorian adult.

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* The average body weight of an adult is conventionally equal to 70 kg according to the American Environmental Protection Agency (US EPA) [23].

The risk characterization for threshold effects was expressed by the hazard quotient (HQ). It was calculated for the oral route of exposure.

$$HQ = EDD/TRV$$

HQ is the hazard quotient ; EDD the exposure daily dose (µg/kg/d) ; TRV the Toxicity Reference Value fixed by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) [24].

If HQ <1, the occurrence of a toxic effect is very unlikely.

If HQ ≥ 1, the appearance of a toxic effect cannot be excluded.

2.5 Statistical Analysis

Data was ~~has been~~ captured with Excel Spreadsheet and ~~were~~ statistically treated using Statistical Program for Social Sciences (SPSS 20.0, SPSS for windows, USA) at 5% significance. The statistical test consisted in a one-way analysis of variance (ANOVA) with the origin of kola nuts. The statistical differences were~~have been~~ highlighted by the test of Duncan test at the 5% level of significance.

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3. RESULTS

3.1 Organochlorine Pesticides Residues Contents Extracted from Kola nuts Collected

The concentrations of dichlorodiphenylethane, cyclodienes, benzene hexachloride, and other organochlorine pesticides (Chlorfenapyr, Chlorthal dimethyl, Quitozene and, Hexachlorobenzene) in kola nuts samples are presented in Tables 1, 2, 3 and 4. The results indicate that Methoxychlor, DDE (op'), Endrin ketone, Hexachlorobenzene, Chlorfenapyr, Chlorthal dimethyl and Quitozene concentrations are below the limit of quantification (LOQ) for all samples analyzed. The concentrations of Dieldrin, Heptachlor, Cis heptachlor epoxyde, Trans heptachlor epoxyde, a-Hexachlorocyclohexane, b-Hexachlorocyclohexane and d-Hexachlorocyclohexane from the farmers are below the limit of quantification (LOQ). Statistical analysis revealed significant differences between different organochlorine pesticide~~s~~ levels determined in the kola nuts regardless of~~whatever the~~ origins ($p<0.05$).

Table 5 presents the average values of organochlorine pesticide~~s~~ contents in kola nuts collected from actors.

The concentration of dichlorodiphenylethane ranged from 11.11 ± 7.88 µg/kg to 17.59 ± 8.13 µg/kg, 12.22 ± 6.84 µg/kg to 66.48 ± 35.29 µg/kg, 12.96 ± 15.70 µg/kg to 95.74 ± 46.11 µg/kg and 13.89 ± 11.14 µg/kg to 94.54 ± 42.72 µg/kg for farmers, rural hawkers, communal storage sites and wholesale stores, respectively. The cumulative mean concentrations of farmers (73.89 ± 27.37 µg/kg) was less than rural hawkers (171.67 ± 88.57 µg/kg), communal storage sites (235 ± 112.38 µg/kg) and wholesale stores (239.81 ±118.03 µg/kg).

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The concentration of cyclodienes range from 7.59 ± 4.88 µg/kg to 15.56 ± 7.64 µg/kg, 5.19 ± 0.96 µg/kg to 86.85 ± 39.10 µg/kg, 8.70 ± 7.15 µg/kg to 107.78 ± 33.84 µg/kg and 23.06 ± 11.87 µg/kg to 125.09 ± 54.33 µg/kg for farmers, rural hawkers, communal storage sites and wholesale stores, respectively. The total cyclodienes levels were 49.26 ± 3.82 µg/kg for farmers, 395.21 ± 172.66 µg/kg for rural hawkers, 469.66 ± 262.46 µg/kg for communal storage sites and 465.08± 201.22 µg/kg for wholesale stores.

The contents of HCHs had the respective range of not detected (ND) to 22.22 ± 10.86 µg/kg, 65.74 ± 26.88 µg/kg to 101.85 ± 38.66 µg/kg, 92.41 ± 54.41 µg/kg to 136.11 ± 27.01 µg/kg and 71.76 ± 43.62 µg/kg to 112.13 ± 46.35 µg/kg for farmers, rural hawkers, communal storage sites and wholesale stores, respectively. Also, the cumulative levels estimated in these actors varied from 22.22 ± 10.86 µg/kg, 329.07± 129.26 µg/kg, 387.03 ± 173.85 µg/kg and 438.7 ± 161.76 µg/kg for farmers, rural hawkers, wholesale stores and communal storage sites, respectively.

The mean concentration of organochlorine pesticides residues in kola nuts samples are presented in Table 6.

Results showed the mean contents of the organochlorine pesticides residues in kola nuts samples collected ranging from 6.30 ± 4.14 µg/kg to 92.93 ± 51.85 µg/kg for Aldrin and Lindane, respectively. The total concentration of DDT, Cyclodienes and HCH were 192.04 ± 29.58 µg/kg, 374.88 ± 31.90 µg/kg and 315.82 ± 13.12 µg/kg, respectively.

Comment [CB17]: there is a lot of data that you have shared in the paragraphs above, a lot of it and it sort of hides the take home message. Perhaps share with us the major highlights of the results and then we will see tghe rest in the tables. This is not a dissertation or thesis so readers are mainly interested in the highlights of the study

Based on the ~~Regulation Food and Agricultural Organisation/World Health Organisation (~~FAO/WHO~~)~~ [25] regulations for ~~setting maximum~~ levels ~~for~~of dichlorodiphenylethane and other organochlorine pesticides (Chlorfenapyr, Chlorthal dimethyl, Quitozene and, Hexachlorobenzene) in foodstuffs for human consumption, the samples analyzed revealed lower levels than the maximum permissible values.

The levels of benzene hexachloride (HCHs) in kola nut~~s~~ samples were higher than the FAO/WHO [25] maximum permissible limits~~ set by FAO/WHO [25]~~. The ~~We noted that the percentage of~~ analyzed samples that were above the Maximum Residue~~s~~ Levels (MRLs) var~~y~~ied from 80 to 98.52 %.

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Among the Cyclodienes detected in kola nuts, the mean~~s~~ levels of Aldrin, Dieldrin, α-Endosulfan, β-Endosulfan and Endosulfan sulfate ~~in kola nuts samples are~~were below the maximum residue~~'s~~ levels.

Heptachlor, Cis heptachlor epoxyde and Trans heptachlor epoxyde mean concentrations were higher than the maximum permisible limits. Thus, 79.26 to 80 % of the determined concentrations ~~are~~were above the FAO Maximum Residue~~s~~ Levels (MRLs).

Table 1. Concentrations of dichlorodiphenylethane in kola nuts samples according to the collected area

Origin of the kola samples	Methoxychlor	DDD (op')	DDD (pp')	DDE (op')	DDE (pp')	DDT (op')	DDT(pp')
F1	<LD	9.44 ± 5.27^{a}	9.44 ± 5.83^{cd}	<LD	15.56 ± 3.91^{b}	16.67 ± 7.07^{d}	16.11 ± 6.01^{c}
F2	<LD	13.89 ± 7.41^{a}	18. 89 ± 7.41^{b}	<LD	14.44 ± 9.50^{b}	14.44 ± 9.82^{d}	17.22 ± 6.67^{c}
F3	<LD	11.67 ± 6.12^{a}	<LD	<LD	21.67 ± 8.66^{ab}	21.67 ± 6.12^{de}	15.56 ± 8.46^{c}
C1	<LD	13.33 ± 12.50^{a}	15.00 ± 9.01^{bc}	<LD	14.44 ± 7.26^{b}	48.89 ± 22.83^{cd}	61.67 ± 48.35^{b}
C2	<LD	20.00 ± 13.46^{a}	10.56 ± 5.27^{bcd}	<LD	13.33 ± 8.29^{b}	43.89 ± 17.10^{cd}	72.78 ± 25.26^{ab}
C3	<LD	14.44 ± 8.08^{a}	11.11 ± 5.46^{bcd}	<LD	18.89 ± 5.46^{ab}	91.67 ± 47.37^{b}	65.00 ± 31.62^{ab}
S1	<LD	20.00 ± 26.10^{a}	12.78 ± 5.07^{bcd}	<LD	20.56 ± 7.63^{ab}	59.44 ± 38.60^{cb}	73.89 ± 47.55^{ab}
S2	<LD	9.44 ± 3.91^{a}	10.56 ± 5.27^{bcd}	<LD	18.89 ± 5.46^{ab}	95.00 ± 39.29^{b}	92.22 ± 36.84^{ab}
S3	<LD	9.44 ± 4.64^{a}	28.89 ± 9.61^{a}	<LD	27.78 ± 10.63^{a}	132.78 ± 29.17^{a}	93.33 ± 40.00^{ab}
Center 1	<LD	11.48 ± 8.06^{a}	18.89 ± 12.81^{b}	<LD	27.41 ± 13.04^{a}	97.96 ± 50.52^{ab}	93.70 ± 41.24^{ab}
Center 2	<LD	16.30 ± 13.27^{a}	16.85 ± 9.82^{bc}	<LD	26.48 ± 15.49^{a}	75.19 ± 44.71^{cb}	95.37 ± 44.91^{a}

*Concentrations in µg/kg. **LD :** Limits of detection = 5 µg/kg*

Table 2. Concentrations of cyclodienes in kola nuts samples according to the collected area

Origin of the kola samples	Aldrin	Dieldrin	α-Endosulfan	β-Endofulfan	Endosulfan sulfate	Endrin ketone	Heptachlor	Cis heptachlor epoxyde	Trans heptachlor epoxyde
F1	<LD	<LD	13.33 ± 5.59^{b}	13.33 ± 8.29^{a}	12.78 ± 5.65^{d}	<LD	<LD	<LD	<LD
F2	8.33 ± 5.59 ^{bc}	<LD	<LD	16.67 ± 7.50^{a}	12.22 ± 5.07^{d}	<LD	<LD	<LD	<LD
F3	9.44 ± 5.83^{b}	<LD	21.67 ± 11.46^{b}	16.67 ± 7.50^{a}	13.33 ± 8.66^{d}	<LD	<LD	<LD	<LD
C1	<LD	23.89 ± 11.67^{b}	19.44 ± 10.14^{b}	18.89 ± 10.83^{a}	35.56 ± 20.53^{cd}	<LD	93.33 ± 43.23^{bc}	90.56 ± 28.88^{ab}	95.56 ± 35.22^{ab}
C2	5.56 ± 1.67 ^{c}	42.22 ± 16.79^{a}	17.22 ± 7.12^{b}	16.67 ± 9.68^{a}	83.89 ± 37.81^{a}	<LD	65.00 ± 32.79^{c}	68.33 ± 35.35^{b}	84.44 ± 35.22^{ab}
C3	<LD	24.44 ± 9.50^{b}	18.89 ± 11.12^{b}	15.56 ± 10.14^{a}	76.11 ± 28.81^{a}	<LD	101.67 ± 26.57^{abc}	101.67 ± 47.30^{a}	76.67 ± 16.20^{b}
S1	<LD	26.11 ± 11.93^{b}	32.22 ± 25.51^{b}	18.33 ± 14.14^{a}	67.22 ± 21.38^{ab}	<LD	72.22 ± 41.99^{c}	67.22 ± 20.33^{b}	103.89 ± 17.10^{ab}
S2	<LD	43.89 ± 5.46^{a}	73.89 ± 122.77^{a}	24.44 ± 17.70^{a}	44.44 ± 6.35^{bc}	<LD	124.78 ± 2.36^{ab}	105.00 ± 29.37^{a}	102.78 ± 31.93^{ab}
S3	16.11 ± 5.58 ^{a}	35.00 ± 9.35^{a}	20.00± 7.91^{b}	23.33 ± 10.31 ^{a}	82.78 ± 45.35^{a}	<LD	115.56 ± 50.96^{ab}	83.33 ± 33.35^{ab}	116.67 ± 47.70^{a}
Center 1	<LD	25.56 ± 11.87^{b}	25.56± 10.95^{b}	23.15 ± 18.04^{a}	48.70 ± 28.61^{bc}	<LD	134.81 ± 66.32^{a}	91.11 ± 20.72^{ab}	102.22 ± 39.72^{ab}
Center 2	<LD	24.26 ± 11.24^{b}	20.56 ± 12.43^{b}	25.74 ± 20.93^{a}	90.56 ± 43.20^{a}	<LD	115.37 ± 37.70^{ab}	97.19 ± 36.68^{a}	105.37 ± 45.82^{ab}

Means with the same letters exponentiating in the same column are not different at 5% according to Duncan test.

*Concentrations in µg/kg. .**LD :** Limits of detection = 5 µg/kg; **F1, C1, S1**: Farmers, Collectors, Stores of Bas-Sassandra; **F2, C2, S2**: Planters, Collectors, Stores of districts of Comoe and lagoons; **F3, C3, S3**: Planters, Collectors, Stores of district of mountain district; **Center:** big storage and distribution centers (1: Anyama ; 2: Bouake)*

Table 3. Concentrations of benzene hexachloride in kola nuts samples according to the collected area

Origin of the kola samples	α-HCH	β-HCH	δ-HCH	Ɣ-HCH
F1	<LD	<LD	<LD	13.89 ± 7.82^{e}
F2	<LD	<LD	<LD	27.22 ± 12.28^{e}
F3	<LD	<LD	<LD	25.56 ± 7.26^{e}
C1	56.11 ± 12.69^{c}	99.44 ± 46.40^{bc}	55 ± 15.41^{d}	81.67 ± 33.17^{d}
C2	61.67 ± 15.61^{c}	92.78 ± 44.73^{bc}	77.22 ± 40.93^{bcd}	92.78 ± 44.66^{cd}
C3	100.00 ± 31.22^{ab}	113.33 ± 21.36^{ab}	65.00± 12.50^{cd}	92.22 ± 28.63^{cd}
S1	85.00 ± 31.72^{bc}	76.67 ± 54.60^{c}	49.44 ± 40.35^{d}	126.11 ± 34.53^{abc}
S2	120.56 ± 24.68^{a}	136.67 ± 30.10^{a}	124.44 ± 199.11^{a}	132.78 ± 16.60^{ab}
S3	121.67 ± 31.52^{a}	90.00 ± 33.07^{bc}	103.33 ± 65.29^{ab}	149.44 ± 24.04^{a}
Center 1	113.52 ± 34.92^{ab}	89.07 ± 29.09^{bc}	88.70 ± 31.61^{bc}	116.11 ± 48.56^{abc}
Center 2	110.74 ± 54.89^{ab}	99.80 ± 49.75^{bc}	54.81 ± 47.79^{d}	101.30 ± 40.56^{bcd}

*Concentrations in µg/kg. **LD :** Limits of detection = 5 µg/kg*

Table 4. Concentrations of other pesticides in kola nuts samples according to the collected area

Origin of the kola samples	Hexachlorobenzene	Chlorfenapyr	Chlorthal dimethyl	Quitozene
F1	<LD	<LD	<LD	<LD
F2	<LD	<LD	<LD	<LD
F3	<LD	<LD	<LD	<LD
C1	<LD	<LD	<LD	<LD
C2	<LD	<LD	<LD	<LD
C3	<LD	<LD	<LD	<LD
S1	<LD	<LD	<LD	<LD
S2	<LD	<LD	<LD	<LD
S3	<LD	<LD	<LD	<LD
Center 1	<LD	<LD	<LD	<LD
Center 2	<LD	<LD	<LD	<LD

*Concentrations in µg/kg. **LD :** Limits of detection = 5 µg/kg*

*Means with the same letters exponentiating in the same column are not different at 5% according to Duncan test. **LD :** Limits of detection; **F1, C1, S1**: Farmers, Collectors, Stores of Bas-Sassandra; **F2, C2, S2**: Planters, Collectors, Stores of districts of Comoe and lagoons; **F3, C3, S3**: Planters, Collectors, Stores of district of mountain district; **Center:** big storage and distribution centers (1: Anyama ; 2: Bouake)*

Table 5. Concentrations of Organochlorine Pesticides in kola nuts according to the actors

Organochlorine Pesticides	Farmers	Collectors	Stores	Centers
Methoxychlor	<LD	<LD	<LD	<LD
DDD (op')	11.67 ± 6.35^{a}	15.93 ± 11.52^{a}	12.96 ± 15.70^{a}	13.89 ± 11.14^{a}
DDD (pp')	11.11 ± 7.88^{b}	12.22 ± 6.84^{b}	17.41 ± 10.68^{a}	17.87 ± 11.35^{a}
DDE (op')	<LD	<LD	<LD	<LD
DDE (pp')	17.22 ± 8.13^{b}	15.56 ± 7.25^{b}	22.41 ± 8.81^{a}	26.94 ± 14.19^{a}
DDT (op')	17.59 ± 8.13^{c}	61.48 ± 37.67^{b}	95.74 ± 46.11^{a}	86.57 ± 48.63^{a}
DDT(pp')	16.30 ± 6.88^{c}	66.48 ± 35.29^{b}	86.48 ± 41.08^{a}	94.54 ± 42.72^{a}
ΣDDTs	**73.89 ± 27.37**	**171.67 ± 88.57**	**235 ± 112.38**	**239.81 ±118.03**
Aldrin	7.59 ± 4.88^{a}	5.19 ± 0.96^{b}	8.70 ± 7.15^{a}	<LD
Dieldrin	<LD	30.19 ± 15.22^{a}	35.00 ± 11.60^{a}	24.91 ± 11.47^{b}
α-Endosulfan	13.33 ± 9.90^{b}	18.52 ± 9.28^{b}	42.04 ± 73.55^{a}	23.06 ± 11.87^{b}
Endofulfan beta	15.56 ± 7.64^{b}	17.04 ± 9.93^{ab}	22.04 ± 32.00^{ab}	24.44 ± 19.39^{a}
Endosulfan sulfate	12.78 ± 6.40^{b}	65.19 ± 35.93^{a}	64.81 ± 32.30^{a}	69.63 ± 41.99^{a}
Endrin ketone	<LD	<LD	<LD	<LD
Heptachlor	<LD	86.67 ± 37.13^{b}	104.10 ± 45.66^{b}	125.09 ± 54.33^{a}
Cis heptachlor epoxyde	<LD	86.85 ± 39.10^{a}	85.19 ± 31.36^{a}	94.15 ± 29.67^{a}
Trans heptachlor epoxyde	<LD	85.56 ± 30.11^{b}	107.78 ± 33.84^{a}	103.80 ± 42.50^{a}
ΣCyclodienes	**49.26 ± 3.82**	**395.21 ± 172.66**	**469.66 ± 262.46**	**465.08± 201.22**
α-HCH	<LD	72.59 ± 28.63^{b}	109.07 ± 33.22^{a}	112.13 ± 46.35^{a}
β-HCH	<LD	101.85 ± 38.66^{a}	101.11 ± 47.12^{a}	94.44 ± 38.94^{a}
δ-HCH	<LD	65.74 ± 26.88^{b}	92.41 ± 54.41^{a}	71.76 ± 43.62^{b}
Ɣ-HCH	22.22 ± 10.86^{d}	88.89 ± 35.09^{c}	136.11 ± 27.01^{a}	108.70 ± 44.94^{b}
ΣHCH	**22.22 ± 10.86**	**329.07± 129.26**	**438.7 ± 161.76**	**387.03 ± 173.85**
Hexachlorobenzene	<LD	<LD	<LD	<LD
Chlorfenapyr	<LD	<LD	<LD	<LD
Chlorthal dimethyl	<LD	<LD	<LD	<LD
Quitozene	<LD	<LD	<LD	<LD
∑ Other pesticides	**-**	**-**	-	-

Concentrations in µg/kg. ***LD :*** *Limits of detection = 5 µg/kg*

Means with the same letters exponentiating in the same line are not different at 5% according to Duncan test.
Concentrations in µg/kg. ***LD :*** *Limits of detection = 5 µg/kg*

Table 6. Mean concentrations of Organochlorine Pesticides in kola nuts samples

Organochlorine Pesticides	Minimun ($\mu g.kg^{-1}$)	Average ($\mu g.kg^{-1}$)	Maximum ($\mu g.kg^{-1}$)	EU-FML* ($\mu g.kg^{-1}$)	(%) ≥ FML
Methoxychlor	-	<LD	-	100	0
DDD (op')	<LD	13.67 ± 11.51	85	500	0
DDD (pp')	<LD	15.30 ± 10.16	45	500	0
DDE (op')	-	<LD	-	500	0
DDE (pp')	<LD	21.81 ± 11.85	50	500	0
DDT (op')	<LD	69.59 ± 49.48	185	500	0
DDT(pp')	<LD	71.67 ± 46.67	180	500	0
ΣDDTs		**192.04 ± 29.58**			
Aldrin	<LD	6.30 ± 4.14	35	50	0
Dieldrin	<LD	24.00 ± 15.11	75	50	2.96
α-Endosulfan	<LD	24.00 ± 35.16	400	100	0
β-Endofulfan	<LD	20.70 ± 15.09	85	100	0
Endosulfan sulfate	<LD	56.41 ± 40.52	180	100	16,30
Endrin ketone	-	<LD	-	10	0
Heptachlor	<LD	89.21 ± 61.92	280	20	79.26
Cis heptachlor epoxyde	<LD	73.07 ± 44.90	189	20	80
Trans heptachlor epoxyde	<LD	81.19 ± 51.33	180	20	80
ΣCyclodienes		**374.88 ± 31.90**			
α-HCH	<LD	82.19 ± 54.31	185	10	80
β-HCH	<LD	79.37 ± 52.19	175	10	80
δ-HCH	<LD	61.33 ± 48.47	180	10	76.30
Ƴ-HCH	<LD	92.93 ± 51.85	190	10	98.52
ΣHCH		**315.82 ± 13.12**			
Hexachlorobenzene	-	<LD	-	10	0
Chlorfenapyr	-	<LD	-	50	0
Chlorthal dimethyl	-	<LD	-	50	0
Quitozene	-	<LD	-	100	0
∑ Other pesticides		-			

***LD :** Limits of detection = 5 µg/kg*

3.2 Human Health Risk Assessment

The regularly exposed populations are those adults who consume kola nuts on a daily basis ~~consume kola nuts~~. Table 7 presents the data of the model of quantitative evaluation of the risks related to the consumption of kola nuts. The estimated daily intake and health risk index (HI) were calculated for each chemical contaminant. The Exposure Daily Doses (EDD) are all lower than the Toxicity Reference Value (TRV) fixed by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) [24]. In fact, mean EDD values ranged from $5.4.10^{-5}$ ~~± 3.55.10^-5 µg/kg/d~~ to $7.96.10^{-4}$ ~~± 4.44.10^-4~~ µg/kg/d for Aldrin and Lindane, respectively. Therefore, the average risks of oral exposure to Organochlorine Pesticides Residues from the consumption of the kolanut are all less than 1. Also, total HQ values for DDT, cyclodienes and HCH were less than 1 (Σ HQ = 0.13 < 1).

Table 7. Quantitative evaluation of the exposure of Organochlorine Pesticides Residues

Measured parameters		Mean concentrations of OCPs (µg/kg)	EDD (µg/kg/d)	TRV (µg/kg/d)	HQ = R
Dichlorodiphenylethane	**Methoxychlor**	<LD	$<4.28.10^{-5} \pm 0.00$	5	$<8.57.10^{-6}$
	DDD (op')	13.67 ± 11.51	$1.17.10^{-4} \pm 9.86.10^{-5}$	10	$1.17.10^{-5}$
	DDD (pp')	15.30 ± 10.16	$1.31.10^{-4} \pm 8.71.10^{-5}$	10	$1.31.10^{-5}$
	DDE (op')	<LD	$<4.28.10^{-5} \pm 0.00$	10	$<4.28.10^{-6}$
	DDE (pp')	21.81 ± 11.85	$1.87.10^{-4} \pm 1.01.10^{-4}$	10	$1.87.10^{-5}$
	DDT (op')	69.59 ± 49.48	$5.96.10^{-4} \pm 4.24.10^{-4}$	10	$5.96.10^{-5}$
	DDT(pp')	71.67 ± 46.67	$6.14.10^{-4} \pm 4.00.10^{-4}$	10	$6.14.10^{-5}$
Cyclodienes	**Aldrin**	6.30 ± 4.14	$5.4.10^{-5} \pm 3.55.10^{-5}$	0.1	$5.4.10^{-4}$
	Dieldrin	24.00 ± 15.11	$2.06.10^{-4} \pm 1.29.10^{-4}$	10	$2.06.10^{-5}$
	α-Endosulfan	24.00 ± 35.16	$2.06.10^{-4} \pm 3.01.10^{-4}$	6	$3.43.10^{-5}$
	β-Endofulfan	20.70 ± 15.09	$1.77.10^{-4} \pm 1.29.10^{-4}$	6	$2.96.10^{-5}$
	Endosulfan sulfate	56.41 ± 40.52	$4.83.10^{-4} \pm 3.47.10^{-4}$	6	$8.06.10^{-5}$
	Endrin ketone	<LD	$<4.28.10^{-5} \pm 0.00$	0.2	$<2.14.10^{-4}$
	Heptachlor	89.21 ± 61.92	$7.65.10^{-4} \pm 5.31.10^{-4}$	0.1	$7.65.10^{-3}$
	Cis heptachlor epoxyde	73.07 ± 44.90	$6.26.10^{-4} \pm 3.85.10^{-4}$	0.1	$6.26.10^{-3}$
	Trans heptachlor epoxyde	81.19 ± 51.33	$6.96.10^{-4} \pm 4.40.10^{-4}$	0.1	$6.96.10^{-3}$
Benzene hexachloride	α-HCH	82.19 ± 54.31	$7.04.10^{-4} \pm 4.65.10^{-4}$	0.06	$1.17.10^{-2}$
	β-HCH	79.37 ± 52.19	$6.80.10^{-4} \pm 4.47.10^{-4}$	0.06	$1.13.10^{-2}$
	δ-HCH	61.33 ± 48.47	$5.26.10^{-4} \pm 4.15.10^{-4}$	0.06	$8.76.10^{-3}$
	Ɣ-HCH	92.93 ± 51.85	$7.96.10^{-4} \pm 4.44.10^{-4}$	0.01	$7.96.10^{-2}$
Other pesticides	**Hexachlorobenzene**	<LD	$<4.28.10^{-5} \pm 0.00$	0.07	$<6.12.10^{-4}$
	Chlorfenapyr	<LD	$<4.28.10^{-5} \pm 0.00$	0.1	$<4.28.10^{-4}$
	Chlorthal dimethyl	<LD	$<4.28.10^{-5} \pm 0.00$	20	$<2.14.10^{-6}$
	Quitozene	<LD	$<4.28.10^{-5} \pm 0.00$	70	$<6.12.10^{-7}$
Σ HQ			**0.13**		

LD : *Limits of detection = 5 µg/kg*

EDD : Exposure Daily Dose ; **TRV** : Toxicity Reference Value ; **HQ** : Hazard Quotient ; **R** : Risk ; **d** : day.

4. DISCUSSION

Quantitative analysis of the different samples revealed the presence of these organochlorine pesticide~~s~~ residues (OCPs) in the kola nuts with variable levels according to the collected area and the type of pesticide~~s~~. Thus, all the sub-groups of organochlorine pesticides, DDT, cyclodienes and HCH ~~are~~were present in the samples collected from farmers, rural hawkers, communal storage sites and wholesale stores. These results show~~ed~~ that kola nut~~s~~ actors use various types of pesticides to ward off pests from kola nuts and to overcome the insect pest problems ~~of the nuts~~ during storage [9, 11].
Many of the OCP~~s~~ species evaluated are breakdown products of the parent pesticide. For example, breakdown products of DDT include DDD and DDE [26]. From their mean values, it was obvious that the most predominant dichlorodiphenylethane in kolanuts was DDT (pp'), while the least occurring was DDD (op'). The mean level of DDT (pp') detected in kola nut~~s~~ samples (71.67 ± 46.67 µg/kg) was higher than the 50 ± 12 µg/kg mean value reported by Sosan [27] in kola nut~~s~~ samples from selected markets in Osun State, Southwestern Nigeria. Factors responsible for the observed difference might include difference in the systemic abilities to store the pesticides in kola nuts. Amount of pesticides applied and mode of contamination are ~~some of the~~ other factors that could account for the differences ~~of~~in pesticides found in kola nuts [26].
The results from th~~is~~e prresent study ~~have shown~~ reveal that the cyclodiene~~s~~ subgroup ~~being~~is the most frequently detected. Obviously, heptachlor was the most predominant cyclodiene~~s~~ detected in the kola nuts, while the least occurring was aldrin. The predominance of Heptachlor could probably be as a result of biochemical transformations of parent OCPs to this metabolite. Also, the level of aldrin in the kola nuts was lower than those of its metabolite, dieldrin. This might imply that there might have~~had~~ been in vivo metabolism of the original aldrin into dieldrin [26].
All the HCH isomers were detected in the nuts with lindane as the most abundant organochlorine pesticide~~s~~ compound~~s~~ in the kolanuts. According to Nuapia *et al*. [28] lindane is a reasonably stable compound and is ~~considered as~~ one of the less persistent organochlorine pesticides. The mean level of lindane detected in kola nut~~s~~ samples (92.93 ± 51.85 µg/kg) was higher than the 31.0 ± 36.3 µg/kg mean value reported by Biego *et al*. [11] in similar samples obtained from~~ a~~ big storage centers of Anyama in Côte d'Ivoire. Lindane residues were detected in kolanut samples~~ kola nuts~~ from farmers. Indeed, the use of pesticides in kola plantations constitute a contamination source ~~of kola nuts~~ from farm~~ers in lindane~~. According to Kouadio *et al*. [10], ~~the majority of~~most producers use phytosanitary products essentially consisting of Callifan super 40 EC, Rund up 360 SL, Thiosulfan 60 EC, Thiametoxam, and Durexa~~,~~ among others ~~etc~~. for the maintenance of the plots.
Data obtained indicated that the content of organochlorine pesticide~~s~~ residues in the kola nuts among planters is lower than the levels recorded from other actors (collectors, stores and storage centers). Indeed, the increase in organochlorine pesticides concentrations during the distribution channel~~circuit~~, from planters to big storage centers~~,~~ would be due to ~~the~~ post-harvest treatment. The conservation of fresh kola nuts for long time and against pests requires several soaking cycles in chemical pesticides often composed of prohibited pesticides such as DDT (dichlorodiphenyltrichloroethane) [11, 9, 10].
It can be seen that most of the OCPs detected had their levels below the recommended FAO/WHO values [25]. The average contents of Dichlorodiphenylethanes had values lower than the ~~accepted~~ permissible maximum limits. A few of the cyclodienes had their value within the given FAO/ WHO-MRL range, whereas a majority of the cyclodienes fell below th~~e~~at range. All the chlorinated benzenes had values that were higher than the recommended FAO/WHO values [25]. It was noted from th~~is~~e present study that the kola nuts investigated contained chlorinated benzenes and cyclodienes at a much higher level than dichlorodiphenylethanes.
The exposure daily doses (EDD) obtained are all lower the Toxicity Reference Value (TRV) fixed by French Agency for Food, Environmental and Occupational Health and Safety (ANSES) [24]. Thus, Hazard Quotient (HQ) calculated from EDD and TRV are less than 1. Indeed, the risk estimates revealed that total HQ values for DDT, cyclodienes and HCH were less than 1 (Σ HQ < 1~~).This~~). This situation indicates that kola nuts would not represent a health risk for humans and would be safe for ~~people~~ comsumption. On the other hand, the regular consumption of a quantity of kola nuts leading to an EDD higher than the TRV would represent a danger for the consumer's health. However, the findings from study of Sosan *et al*. [27] showed that the kola nuts from their study were highly contaminated with the investigated pesticides with unacceptable exposure risk. Contaminated kola nuts in Organochlorine Pesticides Residues represent a health risk for prostate cancer [29,30], liver cancer [31], diabetes [32], reproductive and developmental defects [33, 34] and act as endocrine disruption [35] with acute immunotoxicity [36] and neurotoxicity [37]. Thus, there is high need to give urgent attention to kolanut actors on the use of chemicals for protection against ~~storage~~ insects at storage. Indeed, kola nut is largely consumed fresh, it is therefore important to intensify efforts to

Comment [CB20]: why is it obvious?

Comment [CB21]: Is this not 'Round up'?

Comment [CB22]: Do you mean preservation?

Comment [CB23]: if these pestcides are prohibited, how do farmers get hold of them and where do they get them. Is it legal to use the pestcides? what are the penalties involved?

Comment [CB24]: how much does one need to conusme daily to reach toxicity levels? keep in mind that heavy metals normally linger in the body after consumption reduce the presence of these Organochlorine Pesticides Residues by raising awareness among actors on good practices for the production and conservation of kola nuts. These good practices pass by the restriction of the use of pesticides in the production and storage of kola nuts.

Comment [CB25]: what are the alternative means of preventing against these pestcides...organic methods that you would advise the farmers to use?

5. CONCLUSION

This study revealed the presence of Organochlorine Pesticides Residues at varying levels in kola nuts. Most~~The most~~ of the OCPs detected had their levels below the recommended FAO/WHO values. These chemical contaminants in kola nuts come from the use of chemical pesticides for storage of the kola nuts. Also, the use of chemical pesticides in kola plantations constitute a contamination source of kola nuts in Organochlorine Pesticides Residues. Estimated daily doses in Organochlorine Pesticides Residues from kola nuts, always remains below the different toxicological reference values. Total HQ values for DDT, cyclodienes and HCH were less than 1. Consequently, ~~the occurrence of a~~ toxicity ~~effect~~ is very unlikely for the consumer. Kola nuts from Côte d'Ivoire would be safe for consumption. However, this satisfaction must not forget the bad practices of the actors in production and conservation of kola nuts. Thus, the implementation of efficient technical during production and conservation will be able to guarantee better sanitary quality for kola nuts.

Comment [CB26]: always or remained?

Comment [CB27]: technical what?

Comment [CB28]: preservation or storage?

Comment [CB29]: what are the alternatives for pest reduction during production and storage that you can recommend

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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