Original Research Article

Temporal evolution of organochlorine pesticides residues in kola nuts (*Cola nitida* vent nuts. Schott & Endl.) processing in Eastern of Côte d'Ivoire

. ABSTRACT

Aims: The objective of this study is to monitor the evolution of organochlorine residues levels in kola nuts collected from various actors in the eastern producing region of Côte d'Ivoire.
Study design: Kola nuts samples were collected from farmers, collectors and urban stores in the Eastern of Côte d'Ivoire.
Place and Duration of Study: Health Department of Hydrology Health and Toxicology, Training and Research Unit of Pharmaceutical and Biological Sciences, Abidjan, Côte d'Ivoire, running 2016-2019.
Methodology: A total of 225 samples were analyzed using Gas chromatography–tandem mass spectrometry (Agilent 7010B Triple Quadrupole GC/MS System)
Results: Data showed that all 21 pesticides analyzed were detected in kola nuts samples. Statistical analysis indicated no significant difference in OCPs sub-group used by actors during the three-crop studied. The mean levels of HCHs, DDTs and cyclodienes were ranged from 5±1– 136.67±77.3 µg/kg FW, 5±1 – 116.67±63.2 µg/kg FW and 5±1 - 145±63.2 µg/kg FW, respectively. Otherwise, farmer's levels of OCPs were lower than those detected in kola nuts from collectors and urban stores samples. Thus, compared to the MRL set by the World Health Organization/Food and Agricultural Organization, the farmer's samples are lower than limits fixed, unlike the contents registered with collectors and urban stores. In the latter, nearly 80% to 100% of kola nuts collected are contaminated with hexachlorinated residues (HCHs) and heptachlor.
Conclusion:.There is the need to keep monitoring ecotoxicological chemical substances in kola nuts produced in Côte d'Ivoire and take steps that ensure health safety of end users. Care should be taken since residues could pose chronic health risk for adults and children

Keywords: Côte d'Ivoire, kola nuts, organochlorine, pesticides residues

1. INTRODUCTION

The economy of Côte d'Ivoire, as for most countries in the sub-Saharan zone, is mainly based on agriculture, which contributes 28% to PIB [1]. In this sector, the cultivation of kola nuts occupies a prominent place with an annual production of 260,000 tons of fresh nuts, making Côte d'Ivoire the world's leading producer and exporter of kola nuts [2,3]. In addition, the richness of bioactive and functional compounds (polyphenols, caffeine, theobromine, etc.) of translated kola nuts is attracting increasing interest from Western industries [4,5,6]. Thus, they are used as ingredients in the formulation of certain pharmaceuticals and energy drinks [7,8].

In addition, the kola nuts plays an important social role in African societies where it symbolizes the sacred. Indeed, it is used in socio-cultural rites such as weddings, baptisms, manifestations of friendships, funerals, rituals and sacrifices [9,10,11]. The kolanut is also used as a stimulant, promoting the physical and psychic endurance of manual workers [12,13].

Despite its nutritional and socio-economic importance, the kola sector in Côte d'Ivoire faces many difficulties in terms of production, conservation, marketing and the quality of the product sold. According to [14], one of the major constraints of kola cultivation lies in soil infertility. To improve their yields, some producers use chemical fertilizers and pesticides [15], which in the long term can influence the sanitary quality of nuts. Also, the post-harvest conservation of the kola nut poses a serious problem for the actors of the sector. Indeed, the kola nut is attacked by pests such as weevils, fungi that can cause 30 to 70% losses during storage [16,17]. To remedy these harms, the various actors use traditional conservation techniques involving the use of pesticides [18,19,20] throughout the post-harvest process including, among other things, de-stressing, soaking in water, pulping, washing, storage and handling of kola nuts [21,22]. Most of these pesticides found are essentially organochlorines, organophosphate, pyrethroids whose active molecules are known to have short-, medium- and long-term toxicity [23,24]. A previous study revealed the presence of organochlorine residues in three producing regions of Côte d'Ivoire [25]. The objective of this study is to estimate the temporal evolution of organochlorine residues in kola nuts marketed in Côte d'Ivoire during three successive annual marketing years.

2. MATERIAL AND METHODS

2.1 Sample collection and preparation

This study was conducted in the main areas of kola nuts production in Côte d'Ivoire. Thus, samples were randomly purchased from Eastern regions (**Image 1**.).

The plant material consists of fresh nuts of *Kola nitida* Vent. (Schott & Endl) collected from farmers, rural collectors, and urban stores in accordance with the Regulation N^o 333/2007 of the European Commission [26] during three crops (2016-2017, 2017-2018, 2018-2019) from August 2016 to May 2019. The purchased kola nuts according to the traders have been preserved with synthetic insecticides to protect the nuts from attack of weevils and to increase the shelf life of the product. A total of 27 samples were collected from each actor (farmer, collector and urban store) by region. In total, 225 fresh kola nuts samples (10 kg/sample), were used for this study. Kola nuts were authenticated by a 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.

The samples were wrapped in aluminum foils before they were packed in black polyethylene bags, labelled and taken to the laboratory. In the laboratory, samples were treated according to the method described by Sosan and Oyekunle [20] and then stored in a refrigerator at 4°C prior to further analysis.

2.2 Extraction of pesticides residues according to 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 or sodium acetate) promotes liquid-liquid separation [27]. 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.

2.3 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 21 organochlorine pesticides (EPA 608 Supelco) concentrated at 20 µg/L. These standard organochlorine pesticides were Aldrin, Endrin, Dieldrin, Heptachlor, α-Endosulfan, β-Endosulfan, Endosulfan sulfate, Hexachlorocyclohexane (α-HCH, β-HCH, δ-HCH and γ-HCH), The DDT family: dichlorodiphenyltrichloroethane and its metabolites (p,p'-DDT, o,p'-DDT, p,p'DDE, o,p'-DDE, p,p'-DDD) and Methoxychlor.

2.4 Instrumental analysis

Gas chromatography–tandem mass spectrometry (Agilent 7010B Triple Quadrupole GC/MS System) was employed. A Agilent VF-1701 ms, 30 m x 0,25 mm, 0,25µm (p/n CP9151) column was employed for the separation. The injection mode was pulsed splitless, its volume was 2.0 µL, and its temperature was 280°C. The temperature program of the oven was set at 80°C for 2 min, ramped 20°/min to 200°C, ramped 10°C /min to 300 C, hold at 300 C for 5 min, ramped 25°C/min to 325 C, and held at 325°C for 11 min. Further, electron ionization (EI) and multiple reaction monitoring (MRM) modes were employed for quantitation. The EI voltage was 70 eV, and the MRM conditions are given in Table 1. MRM is a combination of the precursor and productions. The compound was quantified by MRM1, employing the peak area value. The ratio of the peak area value of MRM2 to that of MRM1 was qualitatively compared to the standard solution.

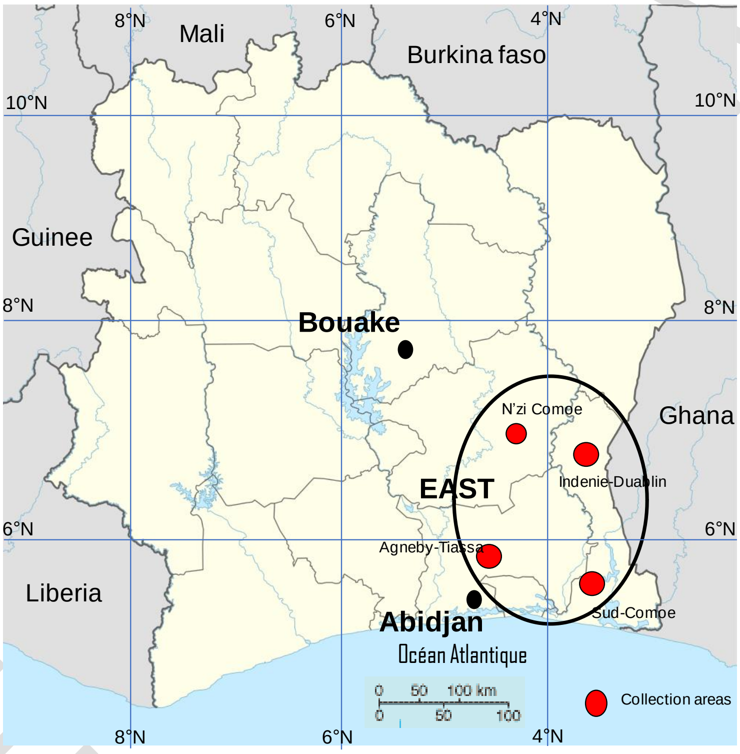

Image.1. Map showing kolanuts samples collection areas

2.5 Statistical analysis

The results obtained from the chromatographic analysis were classified using descriptive statistics (mean, range, minimum, maximum and standard deviations) of SPSS statistics 22 software. The total concentration was obtained by summing the average concentrations of detected pesticides. The variability of total levels of organochlorine sub-group was studied by one-way analysis of variance (ANOVA) using Statistica 7.1 software. The obtained results were compared with the Maximum Residue Limits (MRLs). The total average was performed by the method of the least significant difference (P=.05).

3. RESULTS AND DISCUSSION

3.1 Results

Organochlorine pesticides (OCPs) are widely used in agricultural production for the control of various insects. Most of them have been banned, yet their residues still appear as pollutants in food as well as in the environment. OCPs investigated in this study belong to three broad classes, namely: dichlorodiphenylethanes (DDT), cyclodienes and hexachlorocyclohexanes (HCH). The results from

this study have shown that all the kola nut samples were contaminated by all the 21 organochlorine pesticides analyzed with HCH and DDT subgroup being the most frequently detected especially at storekeepers and collector's samples.
Table 1 shows the pesticides residues levels of kola nuts collected from farmers, collectors and urban stores during 3 crops (2016-2017, 2017-2018, 2018-2019).
In general, the levels of aldrin, endrin ketone, DDE (op'), metoxychlor and hexachlorobenzene are identical in all samples of kolanuts collected (5±1 µg/kg FW) regardless of the kola crop and the actor considered.
Thus, cyclodiene levels range from 5±1 µg/kg FW to 91.67±52.1 µg/kg FW, 5±1 µg/kg FW and 118.33±54.33 µg/kg FW and 5±1 µg/kg FW and 145±63.2 µg/kg FW respectively, for the 2016-2017, 2017-2018 and 2018-2019 seasons. These data indicate that except for heptaclore and these derivatives (Cis and Trans), where 80% of the samples analysed have levels above the maximum reference limits set for these molecules (20 µg/kg), all kola nuts samples analysed have a cyclodiene residues contamination rate below the MRLs (Table 1).
As for DDT (and its derivatives), the average levels recorded range from 5±1 µg/kg FW to 58.33±17.6 µg/kg FW (2016-2017), from 5±1 µg/kg FW to 80±33.7 µg/kg FW (2017-2018) and from 5±1 µg/kg FW to 116.67±63.2 µg/kg FW (2018-2019). These values are all below the limit values of 100 µg/kg and 500 µg/kg for methoxychlor and DDT (and its derivatives) respectively.
For lindane and its derivatives, the concentrations obtained during these successive crops ranged from 5±1 µg/kg FW to 108.33± 63.2 µg/kg FW, from 5±1 µg/kg FW to 133.33± 66.2 µg/kg FW and 5±1 µg/kg FW to 136.67±77.3 µg/kg FW. The results obtained indicate that 80% to 100% of the concentrations are above the MRLs (10 µg/kg) set for these hexachlorinated derivatives.
The evolution of total pesticide levels during the 3 crops is shown in Fig. 1. The cumulative average concentrations observed in farmers vary from one subgroup to another. These values range from 63.33±26.3 µg/kg FW to 75.67±42.3 µg/kg FW, 71.67± 33.2 µg/kg FW to 88.33± 28.4 µg/kg FW and 26.67±11.3 µg/kg FW to 40±22.7 µg/kg FW, respectively for cyclodienes, DDT (and its derivatives) and HCH (isomers).

At the collector level, observed concentrations range from 296.67±88.6 µg/kg FW to 490±74.6 µg/kg FW (cyclodienes), 140±77.8 µg/kg FW to 196.67±83.7 µg/kg FW (DDTs) and from 261.67±95.3 µg/kg FW to 351.67±87.4 µg/kg FW (HCHs).

As for the samples collected in the different stores, the observed levels of cyclodienes, DDTs and HCHs ranged from 350±87.6 µg/kg FW to 436.67±102.4 µg/kg FW, 141.67±98.3 µg/kg FW to 225±124.6 µg/kg FW and 303.33±105.2 µg/kg FW to 420±201.3 µg/kg FW, respectively.
n general, statistical analysis indicate no significant difference between pesticide concentrations recorded from one crop to another, regardless of the actor or subgroup of residues considered (P= .05).

Table. 1. Pesticides residues levels (µg/kg FW) in kola nuts samples

	F1	**C1**	**S1**	**F2**	**C2**	**S2**	**F3**	**C3**	**S3**	EU-MRL* (**($\mu g.kg^{-1}$)**)	% above MRL
Aldrin	5.0±1	5.0±2	5.0±1	5.0±1	5.0±2	5.0±1	5.0±1	5.0±1	5.0±2	50	0
Dieldrin	5.0±1	18.33±6.8	16.67±4.7	5.0±2	20.0±11.3	26.67±12.4	5.0±2	33.33±11.8	35.0±14.6	50	0
α-Endosulfan	10.0±2	13.33±9.2	16.67±8.6	15.0±2.3	23.33±9.3	30.0±11.3	15.0±7.1	21.67±12.3	50.00±33.2	100	0
β-Endofulfan	13.33±6.5	13.33±7.2	13.33±6.5	13.33±5.6	23.33±8.6	21.67±9.6	13.33±6.3	20.0±8.6	20.00±11.4	100	0
Endosulfan sulfate	10.0±5.2	28.33±14.3	66.67±32.1	16.67±8.4	41.67±36.2	71.67±33.2	11.67±5.3	36.67±14.2	63.33±23.5	100	0
Endrin ketone	5.0±1	5.0±2	5.0±2	5.0±1	5.0±2	5.0±2	5.0±1	5.0±1	5.0±1	10	0
Heptachlore	5.0±1	71.67±26.8	71.67±33.4	5.0±2	63.33±18.6	61.67±21.4	5.0±2	145.0±63.2	83.33±32.7	20	80
Cis heptachlor epoxyde	5.0±2	73.33±32.1	63.33±36.2	5.0±1	95.0±62.3	65.0±33.2	5.0±1	103.33±44.2	73.33±28.4	20	80
Trans heptachlor epoxyde	5.0±1	68.33±32.6	91.67±52.1	5.0±3	98.33±54.2	118.33±54.3	5.0±1	120.0±33.5	101.67±39.2	20	80
DDD (op')	6.67±3.2	6.67±2.5	6.67±2.4	8.33±5.2	20.0±11.3	40.0±22.1	13.33±6.7	13.33±6.3	13.33±6.7	500	0
DDD (pp')	8.33±7.2	8.33±6.1	8.33±6.2	11.67±7.4	23.33±9.3	15.0±11.1	8.33±3.3	13.33±4.5	15.0±5.3	500	0
DDE (op')	5.0±1	5.0±1	5.0±2	5.0±2	5.0±2	5.0±2	5.0±2	5.0±1	5.0±2	500	0
DDE (pp')	15.0±6.5	10.0±5.8	16.67±8.4	16.67±9.5	15.0±4.6	23.33±12.1	15.0±7.2	18.33±9.6	21.67±11.4	500	0
DDT (op')	16.67±8.6	46.67±21.4	50.0±25.3	11.67±6.3	40.0±17.2	80.0±33.7	21.67±11.4	60.0±22.5	48.33±22.4	500	0
DDT(pp')	15.0±7.4	58.33±17.6	50.0±21.3	13.33±7.2	45.0±22.4	55.0±25.3	20.0±5.6	81.67±63.2	116.67±63.2	500	0
Methoxychlor	5.0±2	5.0±2	5.0±2	5.0±2	5.0±1	5.0±2	5.0±1	5.0±1	5.0±1	100	0
Hexachlorobenzene	5.0±2	5.00±1	5.0±1	5.0±2	5.0±1	5.0±1	5.0±1	5.0±1	5.0±1	10	0
α-HCH	5.0±1	50.0±33.2	70.0±36.2	5.0±1	51.67±33.2	95.0±56.2	5.0±2	66.67±24.1	90.0±32.6	10	85
β-HCH	5.0±1	78.33±41.2	73.33±44.1	5.0±2	76.67±42.1	36.67±14.2	5.0±1	143.33±78.3	120.0±44.1	10	82
δ-HCH	5.0±1	43.33±21.7	46.67±33.2	5.0±1	61.67±33.5	33.33±15.2	5.0±1	60.0±32.1	68.33±24.6	10	85
ϒ-HCH	15.00±5.6	85.0±63	108.33±63.2	6.67±3.2	83.33±45.3	133.33±66.2	20.0±8.3	76.67±27.3	136.67±77.3	10	100

F : Farmers ; S : Urban Stores ; C : *Collectors*; *1 : First harvest crop; 2: Second harvest crop ; 3 : Third harvest crop*
**EU LMR : European Union pesticides database (2021)*

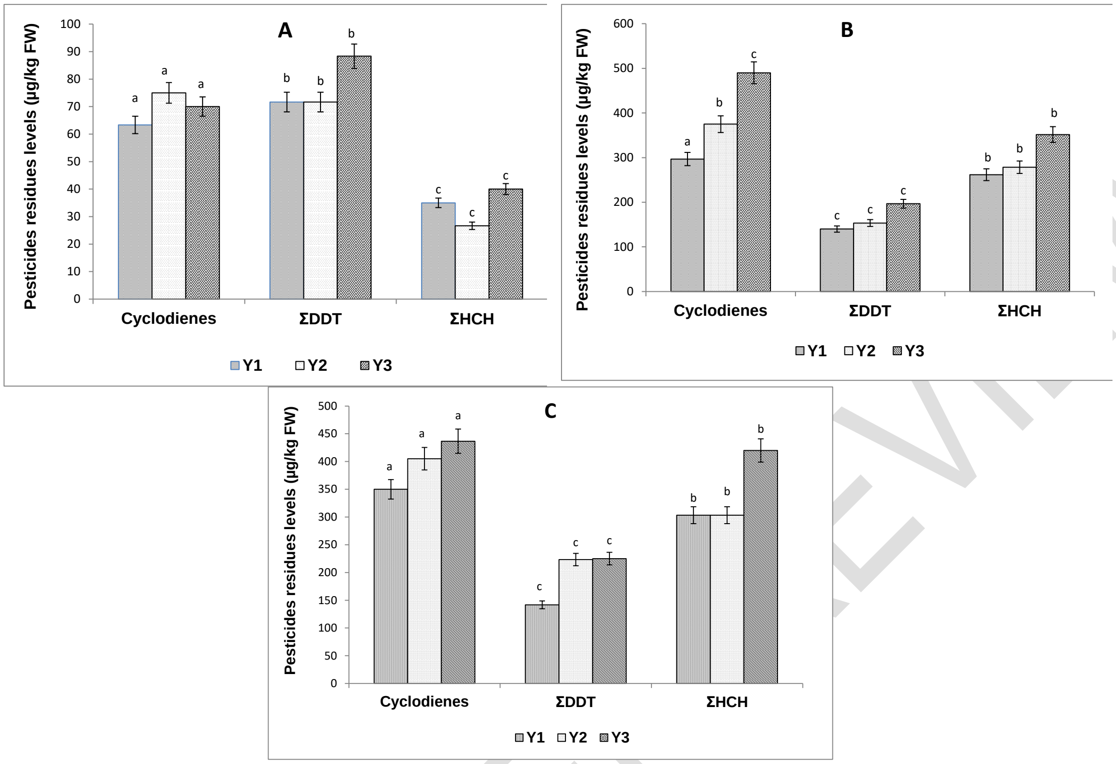

Fig. 1. Evolution of pesticides residues in kola nuts

A : Farmers ; B : Collectors ;C : Stores

Y1 : first crop year ; Y2 : Second crop year ; Y3 : Third crop year

Fig. 2 shows the cumulative average concentrations of the different subgroups of organochlorine pesticides analyzed. Cumulative levels range from 69.44±36.4 µg/kg FW to 397.22±125.3 µg/kg FW, 77.22±47.3 µg/kg FW to 196.67±88.4 µg/kg FW and from 33.89±17.8 µg/kg FW to 342.22±105.7 µg/kg FW, respectively in cyclodienes, DDTs and hexachlorinated isomers (HCHs). In general, the lowest levels were observed in samples collected from farmers, while samples from stores had the highest levels. Thus, the proportion of pesticide residues used annually among the various actors varies from 5% to 18% among producers, from 37% to 45% among warehouse workers and from 45% to 51% among rural collectors.

In addition, statistical analysis indicates that there is no significant difference between the pesticide contents recorded in the samples of collectors and stores (Fig. 2).

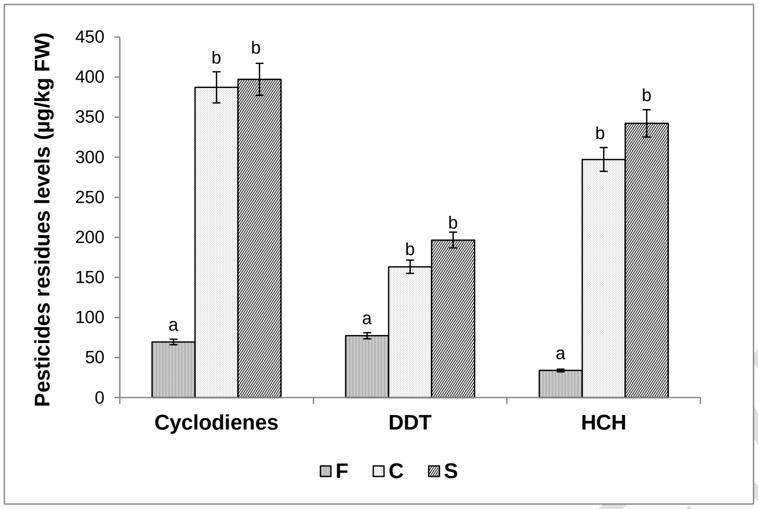

Fig. 2. Difference in pesticides residues levels from selected actors

The cumulative levels of organochlorine residues shown in Fig. 3 indicate a variation in the contamination of kolanuts during the three marketing years carried out. Indeed, the results obtained indicate an increasing evolution of the quantities of pesticides used from the first crop (2016-2017) to the third crop (2018-2019).

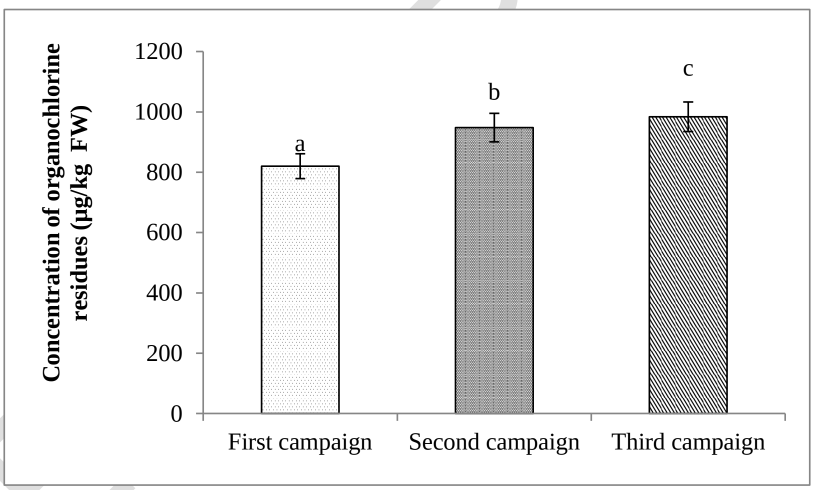

Fig. 3. Evolution of the cumulative concentration of organochlorines during the 3 marketing years

3.2 Discussion

The organochlorine pesticides identified during this study can be grouped into different subgroups including cyclodienes (aldrin, dieldrin, endosulfane and heptachlor), DDTs (methoxychlor, DDT and its derivatives) and HCHs (lindane and its isomers) [20,28]. The presence of these families in kola nuts has also been reported during the work of some authors [20,29,30]**.** This finding implies the use of the same active substances in the treatment of kolanuts in sub-Saharan Africa. In addition, numerous studies have highlighted their presence in many fruits and vegetables [28,31,32]**.**

The results obtained indicate the presence of pesticide residues in all the actors considered (farmers, collectors and urban stores). The presence of these contaminants at the level of farmers is justified using phytosanitary products in the kola sector in Côte d'Ivoire. Indeed, more than 3/4 of the kola trees are grown in the system of association of crops with the cocoa or coffee tree as a basic crop [30,33]. As a result, the use of agricultural inputs for the maintenance of these plants is a source of contamination of kolanuts with pesticide residues [10,34,35]. In addition, as access to labour is increasingly difficult, most producers use phytosanitary products for the maintenance of plots [36,37].

In addition, the concentrations of pesticides determined in kolanuts collected from farmers are statistically lower than those of other actors (collectors and urban stores). This concentration phenomenon is different from the dilution phenomenon observed by Biego *et al.* [18] and Kouadio [37] in their studies on the content of organochlorine pesticides in cocoa beans and the contamination of toxic metals and PAHs in kola nuts. The difference in pesticide levels during the distribution channel from producers to big storage centers, through rural collectors and urban stores is due to post-harvest treatments and the method of packaging kola nuts. Thus, the use of organic inputs during post-harvest treatments of kolanuts could justify the presence of different pesticide residues in the samples analyzed. Indeed, the processing of kola nuts includes harvesting, depilculing, sorting, packaging and storage [21,38,39]. Some of these steps require soaking in storage solutions consisting of water and organic inputs [37,39]. Thus, according to Nimaga [30], N'guessan *et al.* [22] and Kouadio [37], adding these inputs during these steps to avoid the development of fungi and weevils would result in contamination with pesticide residues and other organic pollutants. This could be due to the misuse and persistence of organochlorine compounds in the environment. Indeed, according to Adjagodo *et al.* [40] and Sosan and Oyekunle [20], so-called "first generation" organochlorine insecticides such as aldrin, endrin, DDT, dieldrin, heptachlor and lindane have been massively used in the chemical control of coffee, cocoa and cotton pests in sub-Saharan Africa. However, most active substances are now unregulated or have been banned from use in sub-Saharan Africa by the Sahelian Pesticide Committee in accordance with the Stockolm Convention [41]. As a result, their presence in kolanuts could raise a health risk to populations if their effects were confirmed. In addition, the results indicate an increasing evolution of the quantities of pesticides used from one crop to another. This situation could reflect an increasing use of plant protection products in the kola sector. This would correspond to the growing evolution of pure kola nuts tree crops in Côte d'Ivoire as stipulated by Deigna *et al.* [33] and Dibi [42].

Particular attention given their involvement in the contamination of many agricultural products of high consumption. Indeed, the analysis of the various samples of kola nuts indicates the presence of hexachlorinated residues (HCHs) with average concentrations (5±1 µg/kg FW – 143.33± µg/kg FW) higher than the MRL established for the kola nut (10 µg/kg) in 80 to 100% of the samples. These average values are higher than those recorded by Biego *et al.* [18] and Sosan and Oyekunle [20] during work on kola nuts collected in Anyama, Côte d'Ivoire (93.1±99.8 µg/kg PS) and north of the Delta in Nigeria (44 ± 41 µg/kg PS). Authors have also highlighted the presence of HCH residues in fruits and vegetables [28,43]. According to Nimaga [30] and N'guessan et *al.* [22], the levels of HCH contamination recorded during this study could imply the use of organochlorine pesticides (notably lindane) in post-harvest treatments of kolanuts.

The kolanut samples analysed also contain dichorodiphenyltrichloroethane (DDTs) with a predominance for the metabolites DDT (pp') and DDT (op'). The values obtained ranged from 5±1 µg/kg FW and 116.67±66.58 µg/kg FW. Those are superior to those obtained by Biego et *al.* [18] and Sosan and Oyekunle [20] during their work on pesticides in kola nuts. The average levels observed in the latter were 16±23.6 µg/kg DW and 108±98 µg/kg DW, respectively. Also, Oyekunle *et al.* [44] revealed the presence of DDT residues in cocoa beans at concentrations of 57.76 ± 81.48 µg/kg DW (Ile-Ife) and 82.17±54.53 µg/kg DW

(Ondo) in Nigeria. The presence of DDT in samples suggests recent use of these active substances in production areas or their persistence in the environment [20]**.** Indeed, according to some authors, the persistence of DDT in soils would be 50% after 10 to 15 years [45,46,47]. As for their metabolites (DDE and DDD), the concentration obtained vary from 5±1 µg/kg to 21.81± 8.74 µg/kg FW. Their presence in kolanuts reflects a probable degradation of DDT during the different stages of nuts processing. According to Mahugija *et al.* [48] and Adeleye *et al.* [49], DDT undergoes slow degradation to DDD by reducing dechlorination under anaerobic conditions and to DDE under aerobic conditions by dehydrochlorination.

The cyclodienes residues detected in this study were α-Endosulfan, β-Endosulfan, Endosulfan sulfate, Heptachlor, Aldrin, Dieldrin and Endrin. It shows that kola nut actors in Côte d'Ivoire use cyclodienes in kolanuts processing. The total cyclodienes levels determined from kolanuts in this study (284.63 µg/kg FW) was lower than the mean values of 1.46 mg/kg DW reported by Sosan and Oyekunle [20]. The levels of aldrin (5±1 µg/kg FW) in this study were lower than those of its metabolite, dieldrin (range (5±1 – 35 µg/kg FW). This might imply that there had been metabolism of the original aldrin into dieldrin. These different compounds have also been identified in vegetables from sub-saharan areas [29,47].

The presence of residues above MRLs indicated that high concentrations of the pesticides were still being used and detected in our environment with the possibility of causing systemic toxicity for the regular consumers.

4. CONCLUSION

This study has showed the presence of organochlorine pesticide residues in kola nuts collected during three successive crops from various actors in Eastern Côte d'Ivoire.
The contents of the samples collected from the farmers are lower than MRLs established. However, during the post-harvesting process, kolanuts farmers and traders use various types of pesticides including banned ones for various functions. This results in an increase in the contaminant rate in rural collectors and urban stores. Thus, 80% to 100% of kola samples were contaminated with residues of cyclodienes (heptachlor and its derivatives) and hexachlorinated (lindane and its derivatives).

In addition, statistical analyses revealed that there is no significant difference between the quantities of pesticides used during the three crops among the different actors considered. However, the total amounts of pesticides applied per season evolve positively over time. Thus, care should be taken since residues could raise chronic health risk for adults and children. The actors in the kola sector should therefore find ways and means to limit the use of pesticides during the storage and conservation of kola nuts to also ensure better marketability of the raw material.

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