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 play 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, organophosphorus, pyrethrinoids 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 collected in Côte d'Ivoire by Gas chromatography–tandem mass spectrometry (GC-MS/MS). The most universal extraction technique to isolate a wide range of pesticides is the QuEChERS method. This method was used in this study to ensure excellent extract, clean-up and high analytes recover in application to different food matrices such as fruits and vegetables, fruit juices, raisings, cereals and fish tissue [26,27].

2. MATERIAL AND METHODS

2.1 Study area and sampling

This study was conducted in the Eastern regions of Côte d'Ivoire which are among the major kola producing baskets (**Fig. 1**.).
The plant material consists of fresh nuts of *Cola nitida* Vent. (Schott & Endl) collected from farmers, rural collectors, and urban stores in accordance with the Regulation N° 333/2007 of the European Commission [28] during three crops (2016-2017, 2017-2018, 2018-2019) from August 2016 to May 2019. The collected kola nuts according to the merchants 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 28 samples were collected from each actor (farmer, collector and urban store) by region. In total, 252 fresh kola nuts samples (10 kg/sample), were collected for this study (Table 1). 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 using QuEChERS method

The pesticides residues were extracted according to the method described by Anastassiades *et al.,* **[29].** The method utilizes acetonitrile (MeCN) for extraction (1 mL MeCN/1 g sample) using Vortex mixing followed by addition of 4 + 1 (w/w) anhydrous MgSO4:NaCl (0.5 g salts per g sample) to induce partitioning of the MeCN extract from the water in the sample. After centrifugation, 1 mL of the extract is mixed with 25 mg primary secondary amine (PSA) sorbent + 150 mg anhydrous MgSO4 in a simple approach that is termed dispersive solid-phase extraction (dispersive SPE) cleanup. The extract is centrifuged again and transferred to an autosampler vial for analysis by gas chromatography/mass spectrometry (GC-MS/MS).

2.3 Reagent and solvents

Analytical grade reagents and solvents were used. They were High Performance Liquid Chromatography (HPLC) grade: acetonitrile (99.9%), hexane (99%), toluene (99.5%) and dichloromethane (99.5%) from Sigma Aldrich Co, Germany; deionized water from SDS and a 21 organochlorine mixed standard solution of certified reference material, 20 µg/mL of each component in hexane: toluene (1:1) (EPA 608 Supelco). These standard organochlorine pesticides include 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

The determination of pesticides residues content in kola nuts was conducted according to the method decribed by Hirokazu *et al*. [30] with slight modification. 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, 0,25 µm (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 2**. 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.

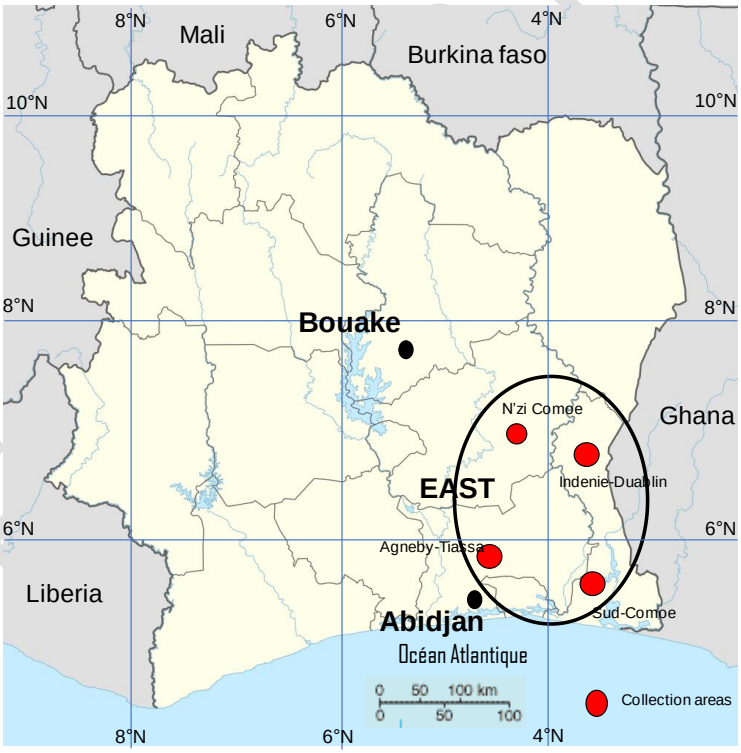

Fig.1. Map showing kola nuts samples collection areas

Table 1. Sampling method

Collection area	N'zi Comoe	Indenie Duablin	Agneby-Tiassa	Sud-Comoe	Total
Farmers	7	7	7	7	28
Collectors	7	7	7	7	28
Urban stores	7	7	7	7	28
Total*	84				

**: samples collected during one corp.*

Table 2. Standard values and MRM conditions of some selected pesticides

Coumpounds	Analytical concentration (µg/mL)	MRM conditions			
		MRM1	CE[a]	MRM2	CE[a]
Aldrin	0.05	293.0→220.0	24	293.0258.0	12
Dieldrin	0.05	263.0→193.0	28	277.0→241.0	8
Chlorpyriphos-ethyl	0.2	314.0→258.0	14	286.0→258.0	6
Chlorpyriphos-methyl	0.1	286.0→93.0	24	288.0→93.0	24
Chlorthal-dimethyl	0.01	301.0→223.0	26	301.0→273.0	16
Cyfluthrin	0.1	226.0→206.0	14	226.0→199.0	6
Cyhalothrin	1	208.0→181.0	6	197.0→141.0	14
Cypermethrin	1	181.0→152.0	26	209.0→116.0	14
O,p'-DDE	1	246.0→176	28	318.0→248.0	22
p,p'-DDE	1	246.0→176.0	28	318.0→248.0	22
o,p'-DDD	1	235.0→165.0	24	237.0→165.0	24
p,p'-DDD	1	235.0→165.0	24	237.0→165.0	24
o,p'-DDT	1	235.0→165.0	24	237.0165.0	24
p,p'-DDT	1	235.0→165.0	24	237.0→165.0	24
Deltamethrin	0.5	253.0→93.0	18	253.0→174.0	6
α-Endosulfan	3	265.0→194.0	8	339.0→267.0	4
β-Endosulfan	3	265.0→194.0	10	339.0→267.0	4
Endosulfan-sulfate	3	272.0→237.0	14	387.0→253.0	10
Endrin	0.05	281.0→245.0	10	281.0→209.0	22
Fenpropathrin	0.03	265.0→210.0	10	265.0→89.0	28
Fenthion	0.05	278.0→109.0	202	278.0→125.0	20
Fenthion oxon	0.05	262.0→247.0	16	262.0 217.0	16
Fenthion-oxon-sulfone	0.05	215.0→109.0	12	294.0→230.0	6
Fenthion-oxon-sulfoxide	0.05	263.0→109.0	6	278.0→263.0	16
Fenthion-sulfone	0.05	310.0→246.0	4	310.0→136.0	18
Fenthion sulfoxide	0.05	294.0→279.0	4	279.0→169.0	14
Fenvalerate	1.5	225.0→119.0	18	225.0→147.0	8
Flucythrinate	0.05	451.0→199.0	10	225.0→147.0	8
Fluvalinate	0.05	250.0 → 55.0	16	250.0→200.0	22
Heptachlor	0.05	272.0→237.0	16	337.0→266.0	16
cis-Heptachlorepoxide	0.05	353.0→263.0	14	355.0→265.0	16
trans-Heptachlorepoxide	0.05	353.0→253.0	16	355.0→291.0	8
Hexachlorobenzene	0.1	284.0→249.0	22	249.0→214.0	16
α-Hexachlorcyclohexane	0.3	219.0→183.0	6	181.0→145.0	14
β-Hexachlorcyclohexane	0.3	219.0→183.0	8	181.0→145.0	16
δ-Hexachlorcyclohexane	0.3	219.0→183.0	8	181.0→145.0	16
ε-Hexachlorcyclohexane	0.3	219.0→183.0	8	181.0→145.0	14
γ-Hexachlorcyclohexane	0.6	219.0→183.0	6	181.0→145.0	14
Malathion	1	173.0 → 99.0	14	173.0→127.0	4
Methoxychlor	0.05	227.0→169.0	28	227.0→212.0	16
Mirex	0.01	272.0→237.0	16	332.0→297.0	22
Parathion-ethyl	0.5	291.0→109.0	12	291.0 → 81.0	28
Paraoxon-ethyl	0.5	275.0 → 99.0	16	275.0→149.0	4
Parathion-methyl	0.2	263.0→109.0	10	246.0→216.0	4
Paraoxon-methyl	0.2	230.0→200.0	6	230.0→136.0	10
cis-Permethrin	1	183.0→168.0	14	163.0→127.0	6
trans-Permethrin	1	183.0→168.0	14	163.0→127.0	6
Phosalone	0.1	182.0→111.0	16	367.0→182.0	6
Pirimiphos-ethyl	0.05	318.0→166.0	14	333.0→180.0	6
Pirimiphos-methyl	4	290.0→233.0	10	305.0→290.0	8
Procymidone	0.1	283.0 →96.0	10	283.→255.0	10
Prothiophos	0.05	267.0→239.0	8	309.0→239.0	16

**CE: Collision energy*

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 3 shows the pesticides residues levels of kola nuts collected from farmers, collectors and urban stores during 3 campaigns (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 kola nuts collected (5±1 µg/kg FW) regardless of the kola crop and the actor considered.

Thus, cyclodiene levels expressed in Fresh Weight (FW) are ranged 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 heptaclor and these derivatives (Cis and Trans), where 80% of the samples analysed have levels above the Maximum Residues Limits (MRLs) set for these molecules (20 µg/kg), all kola nuts samples analysed have a cyclodiene residues contamination rate below the MRLs (Table 3).

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 MRLs of 100 µg/kg and 500 µg/kg set respectively for methoxychlor and DDT (and its metabolites).

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 hexachlorocyclohexane metabolites.

The evolution of total pesticide levels during the 3 campaigns is shown in Fig. 2. 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. 3. Pesticides residues levels (µg/kg FW) in kola nuts samples

	F1	C1	S1	F2	C2	S2	F3	C3	S3	EU-MRL* ((µg.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
Y-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)*
Limit of detection = 1.4 – 1.8 µg/kg; Limit of Quantification = 10 µg/kg

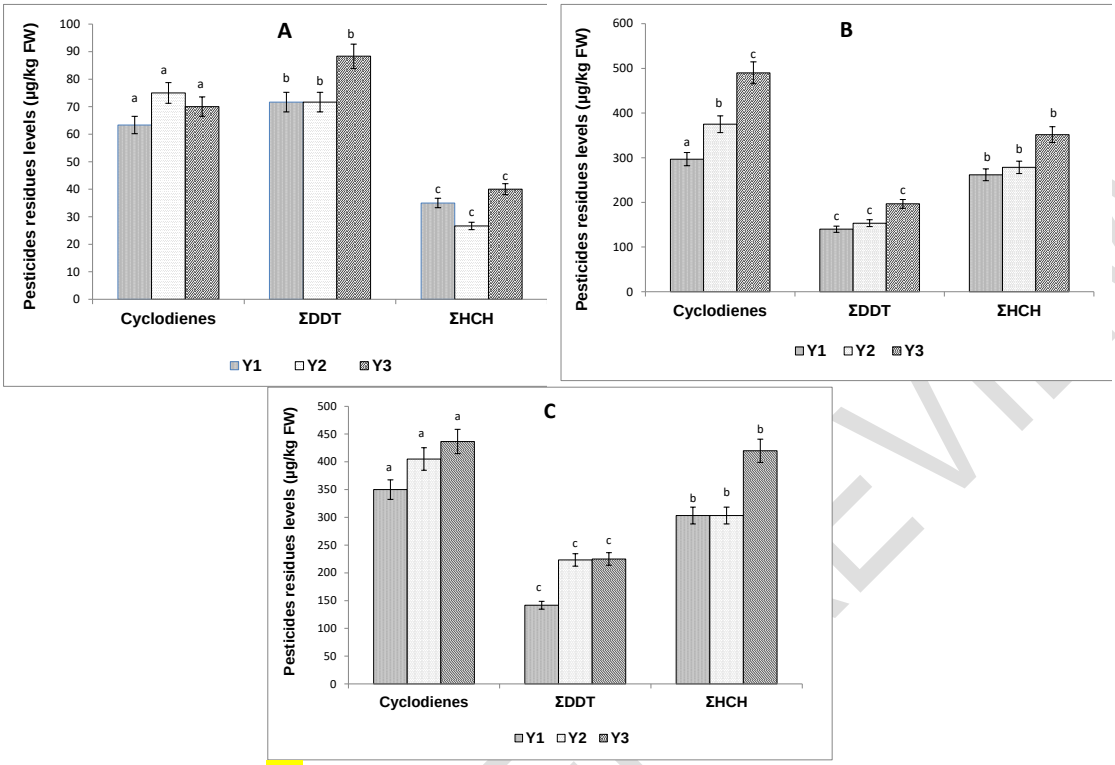

Fig. 2. Evolution of pesticides residues in kola nuts

A : Farmers ; B : Collectors ;C : Stores

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

Fig. 3 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. 3**).

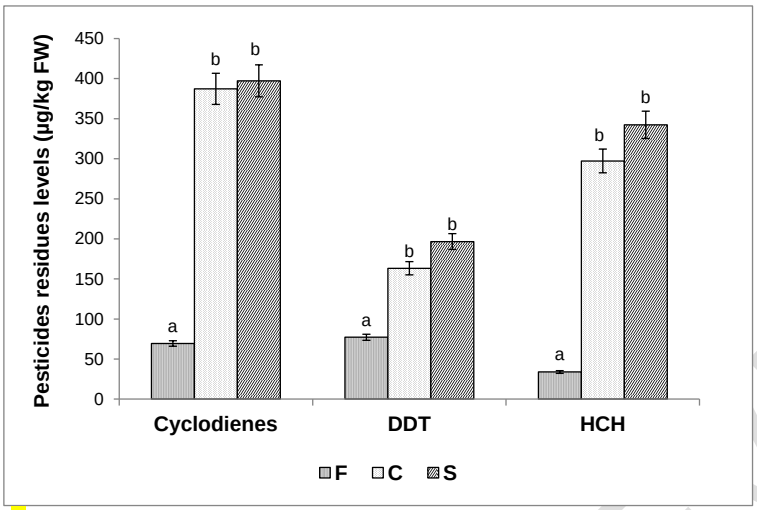

Fig. 3 Difference in pesticides residues levels from selected actors

The cumulative levels of organochlorine residues shown in Fig. 4 indicate a variation in the contamination of kola nuts 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).

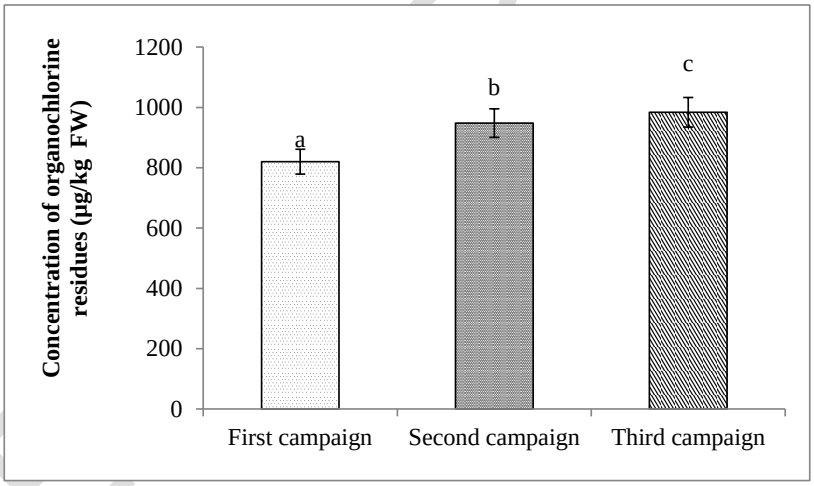

Fig. 4. Evolution of the cumulative concentration of organochlorines during the 3 marketing years

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). The presence of these families in kola nuts has also been reported during the work of some authors [20,31,32]**.** 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 [35,39]. As a result, the use of agricultural inputs for the maintenance of these plants is a source of contamination of kola nuts with pesticide residues [10,37,38]. As the access to labour is increasingly difficult, most producers use phytosanitary products for the maintenance of plots [39,40].

In addition, the concentrations of pesticides determined in kola nuts 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 [40] 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 kola nuts 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 [21,41,42]. Some of these steps require soaking in storage solutions consisting of water and organic inputs [40,42]. Thus, according to Nimaga [33], N'guessan *et al.* [22] and Kouadio [40], 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.* [43] 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 [44]. 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.* [36] and Dibi [45].

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 nuts (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 [28,46]. 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 kola nuts.

The kola nuts 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.* [47] 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 [48,49,50]. 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.* [51] and Adeleye *et al.* [52], 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 kola nuts processing. The total cyclodienes levels determined from kola nuts 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 [29,50].

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, kola nuts 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 hexachlocyclohexane (lindane and its metabolites).

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.

REFERENCES

Reference to a journal:
1. PTAAO (PROGRAMME DE TRANSFORMATION DE L'AGRICULTURE EN AFRIQUE DE L'OUEST). PEST MANAGEMENT PLAN. Rapport final, Ministry of Agriculture and Rural Development. Abidjan, Côte d'Ivoire, 2018; 93p. French

2. Sery M, Bonsson B, Gnogbo R, Gbedie N, Ouattara Y, Legnate H, & Keli J,. Influence of genotype and number of leaves on nursery growth of kola cuttings (Kola nitida [Vent.] Schott et Endlicher.). *International Journal of Biological Chemistry*. 2019; 13(7): 3144-3156.

3. POGCI (Official Portal of the Government of Côte d'Ivoire). Kola sector: Côte d'Ivoire, 1st producer and exporter in the world. 2019. Availabble at www.gouv.ci.

4. Niemenak N, Onomo P, Lieberei R & Ndoumou D. Purine alkaloids and phenolic compounds in three *Kola species* and *Garcinia kola* grown in Cameroon. South African Journal of Botany. 2008; 74: 629-638.

5. Onomo P, Niemenak N, Ndoumou O, & Lieberei R. Change in amino acids content during germination and seedling growth of Kola sp. African Journal of Biotechnology. 2010; 9(35) : 5632-5642

6. Nyamien Y, Coulibaly A, Belleville M, Petit E, Adima A, & Biego H. Simultaneous Determination of Caffeine, Catechin, Epicatechin, Chlorogenic and Caffeic Acid in Kola nitida Dried Nuts from Côte d'Ivoire Using HPLC. *Asian Journal of Biotechnology and Bioresource Technology*. 2017; 1(2): 1-7.

7. Atanda O, Olutayo A, Mokwunye F, Oyebanji A, & Adegunwa M. The quality of Nigerian kola nuts. *African Journal of food Science*. 2011; 5(17): 904-909.

8. Dah-Nouvlessounon D, Adoukonou-Sagbadja H, Diarrassouba N, Adjanohoun A, Baba-Moussa F, Sezan A, & Baba-Moussa L. Indigenous knowledge and socio-economic values of three kola species (*Kola nitida, Kola acuminata and Garicnia kola*) used in southern Benin. *European Scientific Journal*. 2015; 11: 206-227.

9. Ojo G, Nwoha P, Ofusori D, Ajayi S, Odukoya S, Ukwenya V, Bamidele O, Ojo O, & Oluwayinka O. Microanatomical effects of ethanolic extract of Kola nitida on the stomach mucosa of adult wistar rats. *African Journal of Traditional, Complementary and Alternative Medicines*. 2009; 7: 47-52.

10. Adeosun A, Adejobi B, Famaye A, Idrisu M., Ugioro O, & Nduka A. Combined effect of kola testa based organic manure and NPK fertilizer on soil, leaf chemical composition and growth performance of kola (*Kola nitida*). *Research Journal of Agriculture and Environmental Management*. 2013; 2: 183-189.

11. Eze I, Hemkens G, Bucher HC, Hoffmann B, Schindler C, Kunzli N, Schikowski T, & Probst-Hensch M. Association between ambient air pollution and diabetes mellitus in Europe and North America: a systematic review and meta-analysis. *Regard sur la santé de l'environnemen.* 2015; 123(5): 381-389. French

12. Odebode A. Phenolic compounds in the kola nut (*Kola nitida* and *Kola aculinata*) (Sterculiaceae) in Africa. *Revista de Biologia Tropical*. 1996; 44(2): 513-515.

13. Atawodi S, Fundstein B, Haubner R, Spiegelhalder B, Bartsch H, & Owen R. Content of polyphenolic compounds in the Nigerian stimulants Kola nitida ssp alba, Kola nitida ssp. Rubra A. Chev, and Kola acuminata Schott and ENdl. And their antioxidant capacity. Journal of Agriculture and Foods Chemistry. 2007; 55 : 9824-9828

14. Asogwa E, Oluyole K, Ndubuaku T, & Uwagboe E. Kola nut production, processing and marketting in the southeastern states of Nigeria. *American-Eurasian Journal and Environmental Sciences*. 2012; 12: 463-468.

15. Adebiyi S, Uwagboe O, Agbongiarhuoyi E, Ndagi I, & Aigbekaen E. Assessment of agronomic practices among kola farmers in Osun State, Nigeria. World Journal of

Agricultural Sciences. 2011; 7(4) :400-403

16. Daramola A, & Taylor T. Studies on the ré-infestation of kola in store by kola weevil in Nigeria, Niger. *Journal stored Production Ressource*. 1980; 11: 61-63.

17. Dembélé A, Oumarou B, Traore K, Mamadou K, Coulibaly D, & Abba T. The chemical control of the pests in the truck farming and the quality of vegetables in african urban cities: the health hazards and security of consumers. *European Journal of Scientific Research*. 2008; 20(4): 836-843.

18. Biego H, Yao D, Ezoua P, Chatigre O, & Kouadio L. Levels of organochlorine pesticide contamination of *nitid kola nuts*. International *Journal of Biological and Chemical Sciences*. 2009; 3(6): 1238-1245. French

19. Aikpokpodion E, Oduwole O, & Adebiyi S. Appraisal of Pesticide Residues in Kola Nuts Obtained from Selected Markets in Southwestern, Nigeria. *Journal of Scientific Research & Reports.* 2013; 2(2): 582-597.

20. Sosan B and Oyekunle A. Organochlorine Pesticide Residue Levels and Potential Human Health Risks in Kola nuts from Selected Markets in Osun State, Southwestern Nigeria. *Asian Journal of Chemical Sciences*. 2017; 2(4): 11 p.

21. Biego H, and Bonsson B. Good practices in the cultivation of kolatier. Farm Advisor's Manual. Fonds Interprofessionnel pour la Recherche et le Conseil Agricole (FIRCA). 2019 ; 50p. French

22. N'Guessan J, Nimaga D, Akpo A, & Amani N. Analysis of post-harvest treatment practices for kola (Kola nitida) using the haccp system in three cities of Côte d'Ivoire. *GSC Biological and Pharmaceutical Sciences*. 2019; 8(1): 51-63.

23. Mansour A. Pesticide exposure – Egyptian scene. *Toxicology*, 198: 91-115.
OMS/FAO, 2010. International Code of Conduct for the: Distribution and Use of Pesticides: Guidelines for Pesticide Registration. OMS et FAO. 2004; 43 p. French

24. Ishwar Y, and Ningombam D. Pesticides classification and its impact on human and environment. *Environmental Science and Engineering*. 2017; 6: 140-158.

25. Deigna-Mockey V, Biego H, Nyamien Y, Konan Y, Coulibaly A., Ake A, & Sidibe D. Organochlorine Residues Levels in Some Selected Kolanuts (Cola nitida Schott & Endl.) in Côte d'Ivoire. *Asian Journal of Advances in Agricultural Research*. 2020; **13(4)**: 24-35.

26. Hernández, F.,; Cervera, M. I..; Portolés, T.; Beltrán, J.; Pitarch, E. Emotional eating and food intake after sadness and joy. *Analytical methods,2013 ; 5*, p. 5875-5894

27. Margarita S. Pesticides -Strategies for Pesticides Analysis. Edited by Margarita Stoytcheva, Intech open access, Croatia, 2011.

28. European Commission Regulation (ECR) No 333/2007 of 28 March 2007 laying down the sampling methods of samples and methods of analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo (a) pyrene in foodstuffs. Official Journal of the European Union. 2007; 10p. French.

29. Anastassiades M, Lehotay J., Stajnbaher D, & Schenck F. Fast and easy multiresidue

method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, Journal of AOAC. 2003.

30. Hirokazu. Development and validation of an analysis method for pesticide residues by gas chromatography–tandem mass spectrometry in Daikenchuto. *Journal of Natural Medicines*. 2021 ; 75 : 344-360.

31. Olisah C, Okoh O, & Okoh A. Occurrence of organochlorine pesticide residues in biological and environmental matrices in Africa: A two-decade review. *Heliyon*. 2020; 6:e03518.

32. Adeleye S, Bratte G, & Obetta E. Effects of Variety and Different Storage Structures on the Quality of *Kola acuminata* and *Kola nitida* in Storage. *Journal of Environmental Science, Toxicology and Food Technology.* 2015; 9(6): 57-61.

33. Nimaga D. Influence of post-harvest treatments on biochemical and organoleptic quality of kola nuts (*Cola nitida*) during storage. Phd, Nangui Abrogoua University, Abidjan, Côte d'Ivoire. 2015; 162p. French

34. Jawich D. Study of the toxicity of pesticides vis-à-vis two types of yeasts: kinetic and molecular approach. Doctoral Thesis, University of Toulouse, France. 2006; 134 p. French

35. Adeleye O, Sosan B, & Oyekunle O. Dietary exposure assessment of organochlorine pesticides in two commonly grown leafy vegetables in South-western Nigeria. *Heliyon*. 2019; 5(6): e01895.

36. Deigna-Mockey V, Kouadio KR, Konan NY, & Biego HM. Diagnosis in Production and Post-harvest Processing of Nuts of *Kola nitida* (Malvaceae) in Côte d'Ivoire. *Journal of Agriculture and Ecology Research International*. 2016; 9(2): 1-11.

37. Kamilou O, Hodabalo ., Kissao G, Komlan M, & Esso J. Evaluation and health risks of bioaccumulation of heavy metals in fish species in the Togolese lagoon system. *VertigO Energy & Environmental Science*. 2014; 14(2). French.

38. Siapo YM, Tahiri A, and Ano EJ. Evaluation of peasant phytosanitary practices in cocoa orchards in the department of Daloa, Côte d'Ivoire. *European Scientific Journal*. 2018; 14(33): 267-280. French

39. N'zi C. Proposal of pesticides for the preservation of kola nuts. Project « Kola nut conservation test ». FIRCA. 2012; 7 p. French

40. Kouadio R. Sanitary quality of Kola nitida nuts (Wind.) Schott & Endl. From Côte d'Ivoire: Risk of exposure to metal micropollutants and polycyclic aromatic hydrocarbons. These of Unique Doctorate. Félix HOUPHOUËT-BOIGNY University. Abidjan, Côte d'Ivoire. 2021; 199p. French

41. Legnate H, Yapo A, Aidara S, Konan A, Kébé I, Bonson B, Camara M, & Kéli Z. Report of the National Center for Agronmic Research (CNRA), Cultivating kola nut plant well in Côte d'Ivoire. 2010; 4 p. French

42. Adou M, Kouadio O, & Kouadio C. Technical sheet for improving post-harvest storage and conservation of kola nuts. *Journal of Pharmaceutical and Scientific Innovation*. 2018; 7(3): 88-90.

43. Adjagodo A, Tchibozo MD, Kelome NC, and Lawani R. Flows of pollutants related to anthropogenic activities, risks to surface water resources and the food chain around the world: a bibliographic synthesis. *Int. J. Biol. Chem. Sci.* 2016; 10(3): 1459-1472. French

44. CSP (Conseil Sahélien des Pesticides). Global list of pesticides authorized by the CSP. 2018; 48 p. French

45. Dibi D. Constraints of cooperatives in the kola nut trade in Bouaké (Côte d'Ivoire). *European Scientific Journal.* 2018; 68-80. French.

46. Rusibamayila CS, Ak'habuhaya JL, and Lodenius M. Determination of pesticide residues in some major food crops of Northern Tanzania. *Journal of Environmental Science and Health.* 1998; 33(4): 399-409.

47. Oyekunle AO, Akindolani OA., Sosan MB, & Adekunle AS. Organochlorine pesticide residues in dried cocoa beans obtained fromcocoa stores at Ondo and Ile-Ife, Southwestern NigeriaJ. *Toxicology Reports*. 2017; **4**: 151-159.

48. ATDSR. Toxicological profile of aldrin/dieldrin. Agency for toxic substances and desease registry. U.S. Department of Health and Human Services. 2002.

49. OMS. Elementary mercury and inorganic mercury compounds: human health aspects. Concise International Chemical Assessment Document 50. Geneva. 2003.

50. Ayad-Mokhtari N. Identification and dosing of pesticides in agriculture and related environmental problems. Magister's Thesis, University of Ortan, Algeria. 2012; 86 p.

51. Mahugija J, Khamis F, Lugwisha E. Determination of levels of organochlorine, organophosphorus, and pyrethroid pesticide residues in vegetables from markets in Dar-Es-Salam by GC-MS. *International Journal of Analytical Chemistry*. 2017; 9.

52. Adeleye AS, Bratte A.G, and Obetta SE. Effects of Variety and Different Storage Structures on the Quality of *Kola acuminata* and *Kola nitida* in Storage. *Journal of Environmental Science, Toxicology and Food Technology.* 2015; **9(6)**: 57-61.