Original Research Article

Comment [A1]:

ASSESSMENT OF THE RISK OF EXPOSURE TO PYRETHRINOIDS AND ORAGNOPHOSPHORUS PESTICIDES FOUND IN CÔTE D'IVOIRE KOLA NUTS (COLA NITIDA Schott & Endl.)

Comment [A2]: Re-cast as : Assessment of the risk of exposure of Cote D'ivore kola nuts (Cola nitida ……)to pyrethroids and organophoshate pesticides.

ABSTRACT

Aims: The aim of this study was to assess the risk of exposure to pyrethrinoids and oragnophosphorus pesticides found in Côte d'Ivoire kola nuts

Study design: Kolanuts samples were collected from farmers, collectors and urban stores in 3 regions (western, southwestern and eastern) and 2 big storage centers of Côte d'Ivoire.

Comment [A3]: kolanut

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 2017-2020.

Methodology: Pyrethrinoids and organophosphorus residues were qualitatively and quantitatively determined using Liquid Chromatography-Tandem mass spectrometry Agilent 1290 Infinity II LC (LC-MS/MS) and their potential health risks assessed in kola nuts samples from selected regions of Côte d'Ivoire. Health risk estimates were analyzed using Estimated Average Daily Intake (EADI) and Hazard Index (HI) with risk categorized for non-carcinogenic health effects.

Results: A total of 12 PYR and 3 Ops were identified and their residues were detected in all the kola nuts samples analyzed. The results showed that the mean concentration of Ops in all kola nuts was 5±1 µg/kg FW. This level is the same observed for acrinathrin, delatmethrin, fenpropathrin, fenvelerate, flucythrinate, tau-fluvalinate, cyfluthrin et tefluthrin. As for the other pyrethroids, the values obtained are 17.2±6.2 µg/kg FW, 25±14.6 µg/kg FW, 30±11.3 µg/kg FW and 39.4±21.2 µg/kg FW, respectively for bifenthrin, cypermethrin, permethrin and cyhalothrin. The average contents determined for each active molecule are all lower than the MRLs fixed for these residues in the kolanut. As a result, all the Estimate Average Daily Intake (EADIs) determined in adult Ivorians are lower than the corresponding Toxicological References Values (TRVs). Thus, the Hazard Quotient (HQ) calculated for each substance is less than 1.

Comment [A4]: PYR and OPS should be stated in full here

Conclusion: The risk of adverse effects from consuming kola nuts contaminated with residues of pyrethrinoids or organophosphorus is very unlikely.

Keywords *: pesticide residues, organophosphorus, pyrethrinoids, kola nuts, exposure risk, Côte d'Ivoire*

INTRODUCTION

Advances in plant protection have largely contributed to increased yields and regular production [1,2]. Thus, synthetic phytosanitary products have proved to be very effective and reliable on large surfaces. African agriculture in general has more than others, developed production systems based on the use of these products in large quantities more or less controlled. But today, the systematic use of these products is called into question, with the growing awareness of the risks they can generate for the environment, and even for human health [3,4].

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Carcinogenic, neurotoxic or endocrine disrupting effects of pesticides have been demonstrated in animals [5,6,7,8]. The question of the risks for humans (pesticide applicators and their families, exposed non-agricultural rural people, consumers) is therefore raised. It is the subject of lively controversy, but it is listed as a priority in all Health-Environment reports and plans, which require epidemiological studies on this point [1].

Recently, studies have revealed the presence of pesticide residues and other contaminants in fruits and vegetables [9,10,11]. The concentrations recorded in most cases are below the Maximum Residues Limits (MRLs) established by the applicable international organizations within the framework of food safety quality control. However, some authors have recorded levels higher than the preestablished values. For example, according to Mahugija *et al.* [12] the concentrations of chlorpyrifos detected in cabbage samples significantly exceeded the MRL and the concentrations were 2–2.4 times greater than its MRL of 1.0 mg/kg. Also, lindane (Ɣ-HCH) and methoxychlor were detected in onion samples with the levels above above the permissible limits, with values ranging from 40–160 µg/kg [13]. The work of Sosan and Oyekunle [14] on kola nuts also highlighted the presence of organochlorine residues with a risk to the health of consumers.

Kola nuts are economic crops that play important roles in socio-economic and traditional life of African people [15,16]. The nuts are consumed for their stimulant properties and their taste and are traditionally used in Western and Central Africa during weddings, funerals and ritual sacrifices [3,17]. They are also used in the pharmaceutical and food industries to produce cardiac stimulants, laxatives, sedatives and sodas [18].The bulk of Kola nuts produced in West Africa, in particular in Côte d'Ivoire is consumed locally and exported to Nigeria, Mali and Burkina Faso as fresh nuts [19]. So, to keep the kola nuts in this fresh state and reduce the rate of loss (30% - 70%) during the post-harvest process, the various actors use traditional conservation techniques involving the use of pesticides [14,20,21] throughout the post-harvest process including, among other things, de-stressing, soaking in water, pulping, washing, storage and handling of kola nuts [22,23]. Most of these pesticides found are essentially organochlorines, organophosphorus, pyrethroids whose active molecules are known to have short-, medium- and long-term toxicity [4,24,25]. This constitutes a risk to the health of the consumer if the toxicity of these compounds were proven.

The objectives of the study were to determine the concentrations of pesticide residues in kola nuts collected from produced regions of Côte d'Ivoire, compare the detected levels with the maximum residue limits (MRLs) of pesticides as well as the Acceptable Daily Intakes (ADIs) and also estimate the potential health risks associated by calculating the Hazard Quotient (HQ) which allows discussion about health risk due to kola nuts consumption.

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Comment [A7]: organophosphates

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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 three regions: region of Montains, Down Sassandra and Comoe-Lagoon which represent Western, Southwestern and Eastern regions, respectively (Fig 1.).

The plant material consists of fresh nuts of *Cola nitida* Vent. (Schott & Endl) collected from farmers, rural collectors, urban stores and big storage centers in accordance with the Regulation N° 333/2007 of the European Commission [26] during 2018-2019 crop from May to August. 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. The samples were wrapped in aluminum foils before they were packed in black polyethylene bags, labeled and taken to the laboratory. In the laboratory, samples were treated according to the method described by Sosan and Oyekunle [14] and then stored in a refrigerator at 4°C prior to further analysis.

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Comment [A10]: make this clearer. 27 samples from farmer, collector and urban store in three regions should be 243 samples and not 225 ?

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 12 pyrethrinoids and 3 organophosphorus pesticides (EPA 608 Supelco) concentrated at 20 µg/L. These standard pesticides were acrinathrin, deltamethrin, fenpropathrin, fenvelerate, flucythrinate, tau-fluvalinate, cyfluthrin, tefluthrin, bifenthrin, cyhalothrin, cypermethrin, permethrin (pyrethroids) and chorpyrifos ethyl, chlorpyrifos methyl and phosalone (organophosphorus).

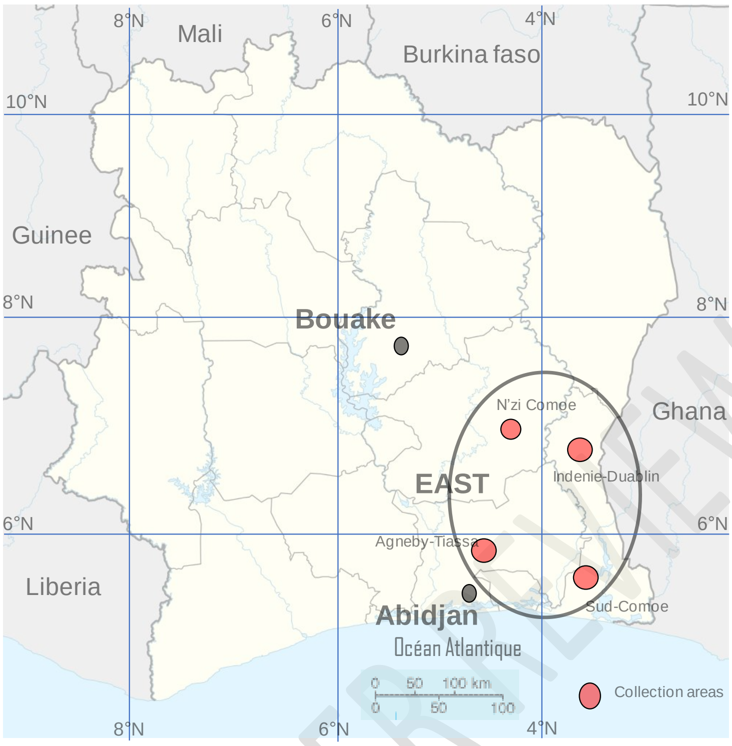

Fig. 1. Map showing kola nuts samples collection areas

2.4 Determination of pesticides concentrations in kola nuts samples

Analysis were performed according to Agilent technologies procedure with some modification described by Chua *et al*. [28]. The filtered solution (1 µL) was inserted into HPLC vial to be injected into LC-MS/MS system. Chromatographic separation was done by using an Agilent 1290 Infinity II LC and Agilent ZORBAX RRHD Eclipse Plus C18 3.0 × 150 mm, 1.8 µm (p/n 959759-302). The detection was done via mass spectrometry 4000 QTRAP (Applied Biosystems-AB Sciex, Life Technologies Corporation, Carlsbad, CA). The analysis of the fraction was performed using the Analyst software (version 1.4.2). Gradient system was used to elute the sample before entering the mass spectrometry system. The mobile phase A consists of 0.1% formic acid in water/methanol (98/2, v/v) and 0.1% formic acid in methanol for mobile phase B. The mobile phase was developed using gradient system at 0-0.25 minutes, 10% B; 0.25-7.75 minutes, 10-100% B; 7.75-8.5 minutes, 100%B; 8.5-8.51 minutes, 100%-10% B for the final wash and column equilibration before the next injection. The injection volume was 5 µL. The mobile phase was filtered using 0.2 µm nylon membrane filter. The flow rate was set at 0.45 mL/min and system was run at 10 minutes.
The mass spectrometry (MS/MS) was done by using Electrospray Ionization (ESI) in negative and positive ion mode. The mass spectra were acquired with the ranges molecular weight (m/z) of 100-1500 with 20 mass ion accumulations. The capillary and voltage of the ESI source were maintained at 400 °C and 1 kV, respectively (both negative and negative mode). The ESI parameters used were as follows: nitrogen was used as ion source gas for nebulization at 40 psi; solvent drying at 40 psi; nitrogen gas was set at 10 psi; collision gas was set at high; declustering potential of 30-50 V, and collision exit energy, 10-20 V. The scan rate was 100 amu/s. A low energy collision dissociation (CID) was used in quantitative by Multiple Reactions Monitoring (MRM) mode. The MRM mode was used to generate the standard curve from a standard compound of aglycone. To develop the mass spectrum from targeted compounds, the compounds were directly delivered into ESI source. This procedure was called as the direct infusion where the sample was directly injected to the mass detector. Manual injection was done by using syringe pump and the injection flow rate was 0.5 mL/min at 1 µg/mL.

2.5 Human health risk assessment

Pesticide residues levels were compared with MRLs recommended by European Union [29]. Maximum residue limit of a pesticide is the maximum concentration of its residue that is legally permitted to remain in food after it has been treated with the pesticide [30]. Health risk indices of the residues detected were computed using the data obtained and food consumption assumptions since the consumption data play a major role in assessing the dietary risk of residues in food. The health risk estimates for the pesticide residues in kola nut was computed using two basic standard indices: the Estimated Average Daily Intake (EADI) and the Hazard Quotient (HQ) (Ribera and Taberly, 2011). Estimated Average Daily Intakes (EADIs) of a pesticide residue and food consumption assumption were used to determine long term health risks to consumers. The food consumption rate for kola nuts in Côte d'Ivoire is quoted to be 0.6 g d^{-1} [11,19]. For each type of exposure, the EADI was obtained by multiplying the mean residual pesticide concentration ($\mu g.kg^{-1}$) in the food of interest and the food consumption rate (kg d^{-1}) [14]. The EADIs expressed as g/kg/day was compared with already established Acceptable Daily Intake (ADI) to assess the long-term risk from exposure to the pesticide residues through kola nuts consumption since the ADI is based on exposure over a lifetime [31]. The HQ was used to assess the risks associated with the non-carcinogenic and carcinogenic health effects. In this study, the non-carcinogenic health effects were evaluated by dividing the EADI by their corresponding values of Toxicity Reference Values (TRVs), assuming average adult's body weight of 70 kg [32]. When the health risk index >1, the food involved is considered a risk to the consumers; when the index <1, the food involved is considered acceptable [25,33].
In addition, an estimate of the quantity of kola nuts likely to induce adverse effects for the consumer was determined according to the following equation:

$$\boldsymbol{Q} = \frac{(\mathbf{TRV\ x\ N})}{(\mathbf{EADI\ x\ m})}$$

With, *Q*, amount of kola nuts to consume; *TRV*, Toxicity Reference Values ; *N*, amount of kola nuts consumed per day (0.6 g) in Côte d'Ivoire ; *EADI*, Estimated Average Daily Intakes ; *m*, estimated mass of kola nut (43.7 g)

2.6 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 pyrethroids and organophosphorus 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) in force in both Côte d'Ivoire and the European Union [29]. The total average was performed by the method of the least significant difference ($p<0.05$). The different EADIs determined were compared with the Toxicity Reference Values (TRV) to estimate the risk of consumer exposure.

3. RESULTS AND DISCUSSION

3.1 Annual average content of organophosphorus and pyrethroid residues in kola nuts

Table 1 shows the average concentrations of pyrethroids and organophosphates in kola nuts collected from different actors (farmers, rural collectors, urban stores and big storage centers) in the different study regions. In general, the levels of organophosphorus residues are identical (5±1 µg/kg FW) regardless of the actor or the collection area considered. The same is true for certain pyrethroid derivatives: acrinathrin, delatmethrin, fenpropathrin, fenvelerate, flucythrinate, tau-fluvalinate, cyfluthrin and tefluthrin.
Regarding the other pyrethroid residues, the average levels obtained vary from one actor to another regardless of the region considered. The recorded values are between 16.67±6.5 µg/kg FW and 131.67±52.7 µg/kg FW, 20±6.8 µg/kg FW and 103.33±26.5 µg/kg FW, 15 ± 5.6 µg/kg FW and 136.67±56.2 µg/kg FW and 36.67±16.3 µg/kg FW and 118.89±62.3 µg/kg FW, respectively for the samples collected in the East, South-West, West and in the big storage centers (Anyama and Bouake).
Furthermore, statistical analyzes indicate that the levels observed at the level of producers in each study region are lower than those of urban collectors and stores. As for the consolidation centers, the concentrations recorded are statistically identical at p <5%.

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3.2 Kola nuts average content of organophophorus and pyrethrinoid residues according to the different actors

The average cumulative contents in the different actors are presented in Table 2. In general, most of the compounds analyzed have a maximum concentration lower than 10 µg/kg FW in the different samples. However, for bifenthrin, cyhalothrin, cypermethrin and permethrin, the mean concentrations recorded are between 10.3 µg/kg FW and 98.7 µg/kg FW. This reflects the overall satisfaction of the different samples analyzed with observed concentrations lower than the MRLs set for each residue concerning kola nuts (Table 2).

Figure 2 shows the average cumulative concentrations of pyrethrinoids and organophosphorus according to the different actors studied. The average values of pyrethroid residues vary between 12.64 ± 10.18 µg / kg FW and 30.28 ± 33.7 µg / kg FW, respectively among producers and urban stores. However, statistical analyzes indicate that there is no significant difference between the values determined at $p < 5\%$. As for organophosphorus, the levels recorded are all identical regardless of the actor (5±1 µg/kg FW).

3.3 Assessment of exposure and characterization of the risk associated with the consumption of kola nuts

Table 3 presents the risk of exposure of the population following the consumption of kola nuts contaminated with residues of pyrethroids and organophosphates. The risk of adverse effects occurring in exposed consumers is the likelihood of adverse effects occurring. The characterization of this risk was carried out on the basis of the average exposure levels described above. Hazard Quotient (HQ) were determined for each pesticide residue analyzed in this study.

In general, the determined EADIs vary from 4.28 x 10^{-6} µg.kg^{-1}bw.d^{-1} to 2.91 x 10^{-4} µg.kg^{-1}bw.d^{-1}. These EADIs determined by the quantity of nuts consumed are lower than the toxicological reference values (TRV) set for each active substance. These values vary between 0.5 µg.kg^{-1}bw.d^{-1} and 50 µg.kg^{-1}bw.d^{-1}. These different exposure values to determined pesticide residues make it possible to characterize the risk of exposure.

Thus, all the Hazard Quotient (HQ) determined for all the active substances are less than 1. In fact, the various indices determined vary between 8.57 x 10^{-7} and 1.33 x 10^{-3}. This corresponds to HQ <1 for each active molecule encountered. Also, the combined index corresponding to the sum of all the determined indices is less than 1 (1.46 x 10^{-3}). Therefore, under the above exposure scenarios, the occurrence of a toxic effect from pesticides in kola nuts is unlikely.

However, the regular consumption of a quantity of kolanuts leading to an EADI greater than the TRVs would represent a health risk for the consumer, because of bioaccumulative and toxic characteristics of pesticides. Table 4 shows the different amounts of kola nuts for each active molecule that may induce harmful effects on the health of consumers. The quantities of kola nuts determined vary between 10 (cyhalothrin) and 16018 (Flucythrinate) nuts in pyrethroids. While in organophosphorus, the quantities observed vary from 320 to 3204 nuts, respectively for chlorpyrifos ethyl and phosalon.

Table 1. Mean concentrations of pyrethrinoids and organophosphorus residues (µg/kg FW) in selected kola nuts

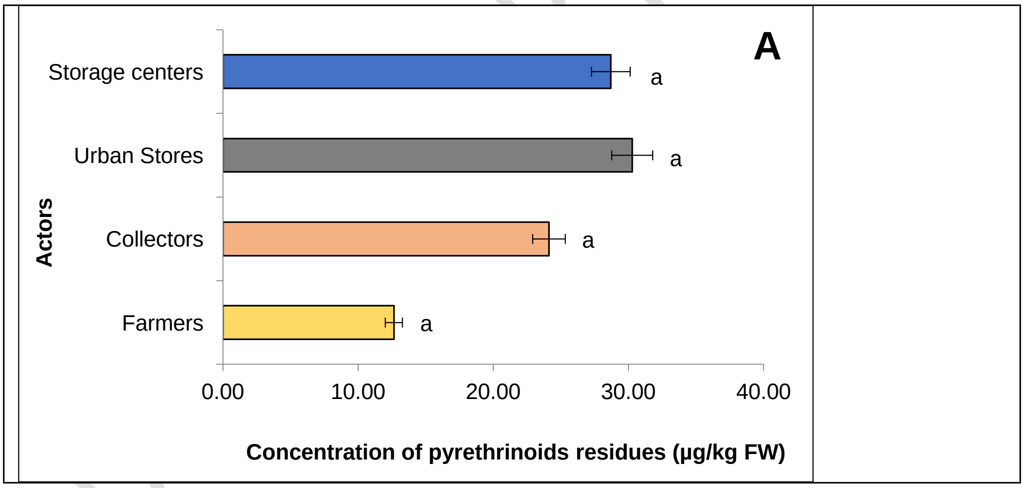
	EAST			SOUTH-WEST			WEST			SC	
	F	C	US	F	C	US	F	C	US	SC1	SC2
Acrinathrin	5.00±1	5.00±1	5.00±1	5.00±1	5.00±1	5.00±2	5.00±2	5.00±1	5.00±2	5.00±1	5.00±1
Deltamethrin	5.00±2	5.00±2	5.00±2	5.00±2	5.00±1	5.00±1	5.00±1	5.00±1	5.00±2	5.00±1	5.00±2
Fenpropathrin	5.00±2	5.00±1	5.00±2	5.00±2	5.00±2	5.00±1	5.00±1	5.00±1	5.00±1	5.00±2	5.00±2
Fenvalerate	5.00±1	5.00±1	5.00±1	5.00±1	5.00±1	5.00±2	5.00±1	5.00±2	5.00±1	5.00±2	5.00±2
Flucythrinate	5.00±1	5.00±2	5.00±2	5.00±1	5.00±1	5.00±1	5.00±2	5.00±1	5.00±1	5.00±2	5.00±3
Tau-fluvalinate	5.00±2	5.00±1	5.00±1	5.00±1	5.00±1	5.00±1	5.00±2	5.00±1	5.00±2	5.00±2	5.00±1
Cyfluthrin	5.00±2	5.00±1	5.00±1	5.00±1	5.00±1	5.00±2	5.00±1	5.00±1	5.00±1	5.00±3	5.00±1
Tefluthrin	5.00±1	5.00±1	5.00±1	5.00±1	5.00±2	5.00±1	5.00±1	5.00±2	5.00±1	5.00±2	5.00±1
Bifenthrin	16.67±6.5	23.33±	111.67±62.3	20.00±6.8	33.33±11.4	25.00±10.2	15.00±5.6	26.67±14.2	26.67±14.1	36.67±16.3	38.89±11.8
Cyhalothrin	30.00±12.3	68.33±	58.33±25.3	38.33±21.3	103.33±26.5	85.00±26.3	50.00±24.3	111.67±63.1	136.67±56.2	80.00±35.8	89.17±36.2
Cypermethrin	20.00±11.2	45.00±	66.67±15.2	25.00±12.4	78.33±33.2	61.67±25.4	30.00±10.4	81.67±24.5	45.00±24.1	82.22±22.4	66.11±22.8
Permethrin	28.33±9.8	41.67±	131.67±52.7	30.00±9.5	83.33±24.8	101.67±63.2	31.67±9.6	51.67±23.8	120.00±63.4	118.89±62.3	96.67±51.3
Mean levels±SD	**11.25±8.3**[a]	**18.19±17.6**[b]	**34.03±38.7**[b]	**12.78±10.4**[a]	**28.19±31**[b]	**26.11±28**[b]	**13.89±12**[a]	**25.97±28**[b]	**30.69±25**[b]	**29.81±33**[a]	**27.57±30**[a]
Chlorpyriphos ethyl	5.00±1	5.00±	5.00±1	5.0±1	5.00±1	5.00±2	5.00±2	5.0±1	5.00±2	5.00±2	5.00±1
Chlorpyriphos methyl	5.00±2	5.00±	5.00±1	5.00±2	5.00±1	5.00±2	5.00±1	5.00±1	5.00±1	5.00±2	5.00±2
Phosalon	5.00±1	5.00±	5.00±2	5.00±1	5.00±2	5.00±1	5.00±1	5.00±2	5.00±1	5.00±1	5.00±2
Mean levels	**5±1**										

Mean values ±SD of pyrethrinoids and organophosphorus followed by the same alphabet letters in the same region are not signicantly different (P=.05)
F : Farmer, C : Collector, US : Urban store, SC : Storage center (Anyama and Bouake)
Limit of detection = 1.4 – 1.8 µg/kg; Limit of Quantification = 10 µg/kg;
SD: Standard Deviation

Table 2. Proportions of pyrethrinoids and organophosphorus detected above MRL (µg/kg FW)

	F	C	US	SC	[Min-Max]	EU-MRL*	% above MRL
Acrinathrin	5.0±1	5.0±2	5.0±1	5.0±2	<LD-10	50	0
Deltamethrin	5.0±1	5.0±2	5.0±2	5.0±1		100	0
Fenpropathrin	5.0±2	5.0±1	5.0±2	5.0±3		20	0
Fenvalerate	5.0±2	5.0±1	5.0±1	5.0±1		100	0
Flucythrinate	5.0±1	5.0±1	5.0±2	5.0±1		50	0
Tau-fluvalinate	5.0±1	5.0±2	5.0±2	5.0±1		10	0
Cyfluthrin	5.0±2	5.0±1	5.0±1	5.0±2		100	0
Tefluthrin	5.0±3	5.0±1	5.0±1	5.0±1		50	0
Bifenthrin	17.2±6.2	27.8±14.3	44.4±22.1	37.8±21.4	[10.3-48.6]	50	0
Cyhalothrin	39.4±21.2	94.4±35.2	93.3±36.2	84.8±41.7	[25.6-98.7]	100	0
Cypermethrin	25.0±14.6	68.3±25.6	57.8±41.2	74.2±32.4	[15.6-85.6]	100	0
Permethrin	30.0±11.3	58.9±33.2	97.8±36.8	7.8±2.6	[28.6-98.5]	100	0
Chlorpyriphos ethyl	5.0±2	5.0±2	5.0±2	5.0±2	<LD-10	10	0
Chlorpyriphos methyl	5.0±2	5.0±3	5.0±2	5.0±2		10	0
Phosalon	5.0±3	5.0±2	5.0±1	5.0±1		50	0

F : Farmer, C : Collector, US : Urban store, SC : Storage center;
** : European Union pesticides database (2021); LD: Limit of detection = 1.4 – 1.8 µg/kg;*
LQ: Limit of quantification = 10 µg/kg



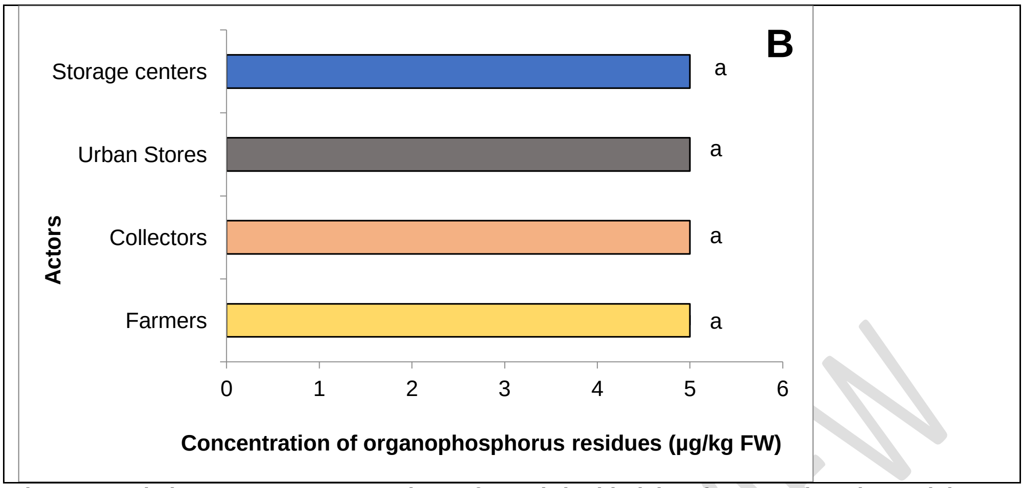


Fig. 2. Cumulative average concentrations of pyrethrinoids (A) and organophosphorus (B) depending on the actors selected.

Table 3. Quantitative assessment of exposure to organophosphorus and pyrethrinoid pesticides

	Mean Value	EADI (µg/kg bw/d)	TRV (µg/kg bw/d)	Hazard Quotient	Health Risk
Acrinathrin	5.0±1	4.28×10^{-5}	10	4.28×10^{-6}	No
Deltamethrin	5.0±2		10	4.28×10^{-6}	No
Fenpropathrin	50±2		30	1.43×10^{-6}	No
Fenvalerate	5.0±1		20	2.14×10^{-6}	No
Flucythrinate	5.0±1		50	8.57×10^{-7}	No
Tau-fluvalinate	5.0±1		5	8.57×10^{-6}	No
Cyfluthrin	5.0±2		3	1.43×10^{-5}	No
Tefluthrin	5.0±1		5	8.57×10^{-6}	No
Bifenthrin	33.9±15.5	2.91×10^{-4}	15	1.94×10^{-5}	No
Cyhalothrin	77.4±25.7	6.63×10^{-4}	0.5	1.33×10^{-3}	No
Cypermethrin	54.7±19.7	4.68×10^{-4}	50	9.38×10^{-6}	No
Permethrin	76±35.7	6.51×10^{-4}	50	1.30×10^{-5}	No
Chlorpyriphos ethyl	5.0±1	4.28×10^{-5}	1	4.28×10^{-5}	No
Chlorpyriphos methyl	5.0±2		10	4.28×10^{-6}	No
Phosalone	5.0±1		10	4.28×10^{-6}	No
			Hazard Index (HI)	1.46×10^{-3}	No

EADI : Estimate Average Daily Intake ; TRV : Toxicological References Values

Table. 4 Quantity of kola nuts representing a risk for the consumer

	EADI (µg/kg bw/d)	TRV (µg/kg bw/d)	Q (g)
PYRETHROIDS			
Acrinathrin	4.28×10^{-5}	10	3204
Deltamethrin		10	3204
Fenpropathrin		30	9611
Fenvalerate		20	6407
Flucythrinate		50	16018
Tau-fluvalinate		5	1602
Cyfluthrin		3	961
Tefluthrin		5	1602
Bifenthrin	2.91×10^{-4}	15	707
Cyhalothrin	6.63×10^{-4}	0.5	10
Cypermethrin	4.68×10^{-4}	50	1464
Permethrin	6.51×10^{-4}	50	1054
ORGANOPHOSPHORUS			
Chlorpyriphos ethyl	4.28×10^{-5}	1	320
Chlorpyriphos methyl		10	3204
Phosalone		10	3204

EADI : Estimate Average Daily Intake ; TRV : Toxicological References Values ; Q : Quantity of kola nuts

DISCUSSION

The results obtained indicate the presence of pesticide residues in all the actors studied (farmers, collectors, stores and big storage center). The main compounds analyzed come from two main families of pesticides used in agriculture: organophosphorus and pyrethrinoids [2].

The presence of these contaminants at farmer level is justified by the use of phytosanitary products in the kola sector in Côte d'Ivoire. In fact, more than 3/4 of the kola trees are cultivated in the crop association system with cocoa or coffee as the staple crop [34,35]. As a result, the use of agricultural inputs to maintain cocoa and coffee trees constitutes a source of contamination of kola nuts with pesticide residues [36,37,38]. In Côte d'Ivoire, the lack of adequate protective equipment for pesticide users during spraying sessions and non-compliance with instructions for use expose them to health problems [39].

In general, the concentrations of pesticides determined in the kola nuts collected from farmers are statistically lower than those of other actors (collectors, urban stores, consolidation center). Indeed, the kola nuts treatment process includes harvesting, defolliculating, skinning, sorting, packaging and storage [22,40,41]. Some of these steps require soaking in preservation solutions made up of water and organic inputs [41,42].

Thus, according to some authors, the addition of these inputs during these stages to avoid the development of fungi and weevils would result in contamination by residues of pesticides and other organic pollutants [23,34,42].

Among the organophosphorus residues detected are chorpyrifos (-ethyl and -methyl) and phosalon. The average concentrations obtained are 5±1 µg/kg FW for all the substances detected. These values are lower than the maximum reference limits established for these compounds in kolanuts, which are 10 µg/kg and 50 µg/kg, respectively for the chlorpyrifos derivatives and phosalon. Few data exist on the presence of organophosphorus derivatives in kola nuts. However, some authors have highlighted their presence in cocoa beans, coffee beans and certain fruits and vegetables [10]. Thus, higher levels were reported during the work of Zakia [43] and Okoffo *et al.* [44] in cocoa beans collected in Takoradi, Tema and Dormaa in Ghana.

The exposure dose determined for these substances is equal to $4.28.10^{-5}$ µg/kg/d and is lower than the Toxicological Reference Values set at 1 µg/kg/d for ethyl chlorpyrifos and 10 µg/kg/d for methyl chlorpyrifos and phosalone. Therefore, the Hazard Quotients are all less than 1. Thus, the occurrence of adverse effects due to the presence of organophosphorus detected in kolanuts would be unlikely.

Among the pyrethroid residues detected, the molecules with the highest concentrations are permethrin, cypermethrin, cyhalothrin and bifenthrin. The respective average concentrations of these substances determined in the different samples are 76±33.5 µg/kg FW ; 54.7±19.7 µg/kg F ; 77.4±25.7 µg/kg FW and 33.9±15.5 µg/kg FW. These values are lower than the MRLs (100 µg / kg) set for permethrin, cypermethrin and cyhalomethrin and bifenthrin (50 µg/kg). Few data exist on the presence of pyrethroid derivatives in kola nuts. However, some authors have highlighted their presence in cocoa beans, coffee beans and certain fruits and vegetables [10]. Thus, Frimpong *et al*. [45] and Okoffo *et al*. [44] recorded low levels of permethrin (ND - 0.02 µg/kg DW), cypermethrin (0.01-0.03 µg/kg DW) and cyhalothrin (0.01-0.03 µg/kg DW) in cocoa beans in Ghana. The presence of pyrethroid derivatives in kolanuts could have serious consequences for human health, because it should be remembered that, according to the Pesticides Properties Database (PPDB), they are molecules harmful to ingestion, inhalation and in contact with the skin and cause respiratory tract irritation upon inhalation. Cyfluthrin is recognized as a toxic molecule for the liver and kidneys while cypermethrin is qualified as a molecule with carcinogenic potential [46]. As for cyhalothin, it is toxic to the thyroid glands and the nervous system [47]. These residues would therefore be the basis of the hematological and biochemical dysfunctions observed within the market gardeners population.

In addition, the highest exposure doses equivalent to $2.91.10^{-4}$ µg/kg bw/d (bifenthrin), $6.51.10^{-4}$ µg/kg bw/d (permethrin), $4.68.10^{-4}$ µg/kg bw/d (cypermethrin) and $6.63.10^{-4}$ µg/kg bw/d (cyhalothrin), are all lower than the TRVs set for these residues in kola nuts. As a result, all the Hazard Quotient are less than 1 and reflect a low probability of occurrence of adverse effects on the health of consumers linked to the presence of these substances.

The results obtained show that the estimated Intakes increase in an increasing way with the concentrations of pesticides present in the kola nuts. These results corroborate those of Ake *et al.* [48] and Kouadio [42] which state that the level of consumer exposure increases with the concentration of the contaminant in the foodstuff. The Daily Exposure Doses (EADI) obtained for all the families of pesticides analyzed are all lower than the Toxicological Reference Values (TRVs) which have been set by the National Agency for Health, Food, Environment and Work Safety [49]. Thus, the Hazard Quotient (HQ) calculated from the TRVs and EADIs are all less than 1 regardless of the active substance considered. This situation indicates that the consumption of cola nuts would not represent any danger to human health.

However, the regular consumption of a quantity of kola nuts leading to an EADI greater than the TRVs would represent a real health risk for the consumer, because of the bioaccumulative and toxic characteristics of pesticides. Thus, for a consumption of between 320 and 3204 kola nuts, the risk of adverse effects on the consumer could be possible for organophosphorus residues. Therefore, an inhibition of cholinesterase resulting in an accumulation of acetylcholine at the level of the synapse, thus preventing the transmission of nerve impulses could be observed. This mode of action would explain their high toxicity to humans and warm-blooded animals [50].

With regard to pyrethrinoids, the consumption of 10 kola nuts could cause the appearance of adverse effects on the health of consumers. The presence of pyrethroid pesticides has been associated with neurotoxic disorders because they interfere with the propagation of neuronal signals [51]. Precisely, they act on the sodium channels located along the cell membrane of the tail of neurons (axons). By keeping these channels open, pyrethrinoids trigger a series of electrical impulses in neurons that cause them to depolarize, resulting in various symptoms such as tremors, involuntary movements and salivation [51]. Oral exposure to pyrethroids can cause sore throat, nausea, vomiting and abdominal cramps, mouth ulcers, increased secretions and difficult swallowing [50].

Regarding simultaneous exposure to different pesticide residues by the Hazard Index method (HI), the results show that depending on the exposure scenario considered, the risk is unlikely according to the concentration addition model. However, it is known that the simultaneous ingestion of a toxicant could lead to synergistic, potentiating or, on the contrary, antagonist type interactions [33,52].

CONCLUSION

The results of this study made it possible to highlight the presence of organophophorus and pyrethrinoids pesticide residues in kola nuts samples collected from farmers, rural collectors, urban stores and big storage centers in Côte d'Ivoire. The average contents determined for each active molecule are all lower than the MRLs fixed for these residues in the kola nuts. As a result, all the EADIs determined in adult Ivorians are lower than the corresponding TRVs. Therefore, the calculated Hazard Quotient for each substance is less than 1. The risk of adverse effects from consuming kolanuts contaminated with residues of pyrethroids or organophosphates is very unlikely.

However, the risk of developing a pathology by exposed people would be real if they regularly consume kolanuts with high levels of pesticides. Indeed, according to the Sahelian pesticides committee, most of the residues found in kola samples are unapproved and prohibited for use in sub-Saharan Africa.

In general, contamination of kola nuts by pesticides can occur all along the value chain to the consolidation center. As a result, the risk of contamination of the population remains high. It is therefore necessary to control the use of phytosanitary products by the various actors in order to ensure greater sanitary quality of the kola nuts.

Comment [A13]: this section is contrary to your findings in the conclusion, where you opined that the cola are safe

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