Residual Content of Persistent Insecticides in the Fresh Nuts of *Cola Acuminata* Cohac from Kumbotso Local Government Area of Kano State and Implication for Public Health

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Abstract

The pesticide residues in kola nut, *Cola acuminata* obtained from Mariri Market, Kumbotso Local Government Area, Kano state of Nigeria was investigated. The study consisted of a survey and laboratory analysis of kola nut samples. One hundred structured questionnaires were administered through an open market format in the major kola nut market in the state. In addition, 1kg sample of kola nut was collected randomly from ten major kola nut sellers in the market for analysis. A 100g sample of the kola nut was pounded into paste in a blender of which 50g was soaked in 150mL acetone for 24h. The extracted sample was filtered through anhydrous sodium sulfate while the extract was evaporated to near dryness using a rotary evaporator at 28°C in the Laboratory. Residue was re-dissolved in 2 mL acetone and kept in the refrigerator for GCMS Analysis. Ten pesticidal compounds were detected from a comparison of data with reference to mass spectrometry data (NIST05.LIB) and significant occurrence in kola nut sample which had a corresponding molecules affiliated to some pesticides standards were detected in the sample

Keywords: *Cola acuminata*, Pesticide residue, Persistent insecticide.

INTRODUCTION

Fresh Kola nut is mainly chewed because of its stimulant characteristics. The proximate content of Kola nut includes about 1% protein, 1.35% fats and 45% starch. While its secondary metabolite constituents include caffeine, theobromine, tannins, phenolics,

kolatin and kolanin (Quarco, 1973). The presence of alkaloids and other phytochemicals makes kola nut suitable as raw materials for the manufacture of pharmaceutical products and beverages while the tannin content is a good source of dyes in textiles and thread (Petu-Ibikunle *et al.*, 2010).

Nigeria produces about 70% of world's kola nut with an annual estimated production

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of 200,000 metric tonnes of fresh nuts, mostly from South west, Nigeria (CBN, 2002). The South west accounts for about 88% of the total production; while only about 10% of the produce is exported, and the rest are consumed locally (Oluokun and Oladokun, 1999). Kola nut also has industrial usage for the production of wines, candles, beverages, drugs, soft drinks, animal feed formulation, liquid soap and dyes (Daramola, 1973). It is believed to dispel sleep, hunger and thirst and act as an anti-depressant. Tachie-Obeng and Browns (2006) observed that beverage could be made by boiling the powdered seed in water.

In Nigeria, kola nut is regarded in high esteem and as such often offered as a gift to traditional leaders and visitors. In northern Nigeria, where the bulk of kola nut is chewed, it is greatly relished as a stimulant in substitute for alcoholic drinks. As a result, there is a heavy trade of kola from the humid southern regions to the northern arid parts of West Africa. However, the insect pests of kola nut are the weevils, *Balanogastriis kolae* and *Sophrorhinus* spp. They are field-to-store pests which can cause up to 100% loss if left uncontrolled in storage (Asogwa *et al.*, 2008).

Consequently, kola nuts storage is usually laborious because the freshness of the nuts needs to be maintained for several months. Petu-Ibikunle *et al.* (2010) observed that the major obstacle encountered in kola nut business is basically storage facilities with insect pest causing 53.33% loss which causes reduction in the value and invariably the price of stored kola nuts.

Chlorpyrifos and diclorvos are directly applied to the kola nut for purpose of storage. However, this leaves Pesticides such as Lambdacylohathrin, Cypermethrin, Dimethoate and Chlorpyrifos are often recommended against insect pests of food crops because, they displayed a broad spectrum of

insecticidal activity coupled with low mammalian toxicity and low application rates for insect. This attribute makes them environmentally more acceptable than the older, more persistent and toxic organochlorine and organophosphorus insecticides. However they leave residues on food product (Cameron and Walter, 2000). Evaluation of pesticide residues in food is of public health importance and would help to ensure that residues are kept within safe limits (Ogah *et al.*, 2011).

Dietary intake constitutes the most common and principal pathway of exposure to pesticides and human exposure to pesticides through contaminated food intake can cause chronic and acute toxicities (Liu *et al.*, 2016). Thus, regular surveys and monitoring programmes of pesticide residues coupled with risk assessments have been carried out and reported in many developed countries worldwide, and this is done in order to protect consumer health, improve the management of agricultural resources and prevent economic losses in West Africa, many studies have reported contamination levels of pesticide residues in fruits and vegetables and in maize and cowpea (Akoto *et al.*, 2013).

In Nigeria, several studies have also reported high levels of pesticide residues in fruits, vegetables and other food produce, on cocoa and on kola nut as well as seafood products (Aikpokpodion *et al.*, 2013). The present study was conducted to determine the pesticide residues content in kola nut, *Cola acuminata* from Mariri Market, Kumbotso Local Government Area of Kano State, Nigeria.

MATERIAL AND METHODS

Experimental Site

The behavioural survey was conducted at Mariri market (11°54'2.27"N and 8°30'

58.79'E), Kumbotso Local Government Area of Kano State while laboratory analysis was conducted at the Multi-user Laboratory, Chemistry Department, Ahmadu Bello University, Zaria, Kaduna State and National Research Institute for Chemical Technology (NARICT), in Zaria, Kaduna State, Nigeria.

Collection of Primary Data

A total of 100 structured questionnaires was administered in the market to conduct oral interviews to kola nut sellers on the types of pesticide used for preservation, method of application, reasons for choosing the pesticide and length of storage of the kola nut after pesticide application.

Collection of Kola nut samples and Laboratory Analysis

Kola nut samples (1g) were collected randomly from ten (10) different sellers within the market. The collected sample was the representative of the entire kola nut present in the market. The sample collected (1 kg) was put in a polythene bag, labeled and transferred to the laboratory for further analysis following the method of Islam *et al.* (2009).

Sample Extraction and Gas Chromatographic Analysis

A 100 g sample of the kola nut was ground into paste, out of which 50 g was chopped and macerated in a blender. The ground paste was dipped in 150 mL acetone and kept overnight (Singh *et al.*, 2007). The extracted sample was filtered through anhydrous sodium sulfate and the resulting extract was evaporated to near dryness using a rotary evaporator at a temperature ranging between 26 to 28°C at the Multi User Laboratory, Ahmadu Bello University, Zaria (Barakat *et al.*, 1994). A 50 ml sample of the 0.2 mg/ml of the pesticides stock solution was added to

150 ml of the extracted kola nut sample. A stock solution of triphenylmethane (TPM) in acetonitrile at concentration of 1 mg/mL was used as internal standard. An aliquot of 20 µL of TPM solution in acetonitrile (1000 mg/L) was added to the spiked sample as internal standard.

Furthermore, 1 µl of the kola nut sample was injected into the GC (Shimadzu GCMS (QP 2010 plus with universal detector)) column RTX, 30 m x 0.25 mm/d, with 0.25 µm film thickness (5% diphenyl, 95% dimethyl polysiloxane), and observed for 5 minutes, within which the peaks and the areas under them were recorded per retention time

RESULTS

Table 1 showed the level of knowledge and usage of pesticides for post harvest operations. A total of 94% of the respondents confirmed knowledge and usage of pesticides for storing kola nut produce. while 6% indicated that they do not use pesticide for storing kola nut. In all, a total of five synthetic insecticides namely: chlorpyrifos, cypemetrin, dichlorvos, dimethoate, profenofos, and dieldrin, were encountered in the study as major residues of kola nut in Kumbotso Local Government of Kano state. Dimethoate was not detected in the samples studied. The result revealed that Chlorpyrifos 26.82% and Dichlorvos 25.12% were the main pesticides used in storing kola nut in the study area followed by Dieldrin with 16.81%. The choice of the various pesticides was largely based on availability according to 58% of the respondents. Respondent's choice of pesticides based on custom was 22%, while those choosing on the basis of ease of application were 17%.

Five major insecticides listed in Table 2 from three groups: organochlorines, organophosphate and pyrethroids were found while

dimethoate was not detected. Of these, chlorpyrifos was the most abundant accounting for 40% of total residues detected.

Pesticide residues in the Kola nut, *Cola acuminata*

Ten pesticidal compounds were detected from a comparison of data with reference to

mass spectrometry data (NIST05.LIB) and significant occurrence in kola nut sample which had a corresponding molecules affiliated to some pesticides standards were detected in the sample as shown in Table 3.

Table 1: Knowledge of Pesticide and usage in Mariri Market

Variables	Frequency	Percentage
Heard of Pesticides	269	93.73
Not heard of Pesticides	18	6.27
Total	287	100.00
Types of Pesticides Used		
Dichlorvos (DD Force).	98	25.82
Phostoxin	59	14.11
Lambdacyhalothrin	27	6.44
Cypermethrin	16	3.29
Dimethoate	23	4.72
Chlorpyrifos	116	26.12
Dieldrin	77	16.81
Cold storage/packaging materials	3	0.62
Reasons for Selecting the Pesticides		
Custom	62	21.60
Availability	168	58.53
Ease of usage/use	48	16.72
Others	6	2.62

Table 2: Shows the summary of significant occurrence of pesticide in the kola nut sample in comparison with reference to mass spectrometry data

S/No	Pesticides	Kola nut
1	Dieldrin	√√
2	Chlorpyrifos	√√√√
3	Cypermethrin	√
4	Dichlorvos	√√
5	Profenofos	√
6	Dimethoate	ND

ND = Not Detected

Table 3: Corresponding molecule of kola nut sample with reference to mass spectroscopy data

Line	Retention Time (min.)	Base peak fragment m/z+	Corresponding molecule	Corresponding pesticides standard
1	31.275	161.15	1-(4-methoxyphenyl)-4-methyl-1-penten-3-one	Cypermethrin
2	32.225	194.15	Tetrahydrocytisine	Chlorpyrifos
3	33.812	194.20	2-bromo-1,3-difluorobenzene	Profenofos
4	54.228	207.15	N-methyl-4-chlorobenzenesulfonamide	Dieldrin
5	55.278	207.15	2-benzyloxy-beta-dimethylalmino-6-nitrostyrene	Chlorpyrifos
6	57.635	208.15	Ethyl- α -fluoro-4-methylcinnamate	Dichlorvos
7	57.725	208.15	Ethyl- α -fluoro-4-methylcinnamate	Chlorpyrifos
8	58.550	227.25	Methylesterpalmitic acids	Diclorvos
9	67.693	253.15	12,13-epoxy-3,4,7,15-tetrahydroxy-(3, α ,4-beta,7- α)trichothec-9-en-8-one	Dieldrin
10	57.577	253.10	1,3,5-trimethoxy-2-(1Z)-2-nitro-1-propenylbenzene	Chlorpyrifos

The most highly concentrated pesticide in the fresh kola nut was Chlorpyrifos (0.076±0.011 mg/kg) and this concentration was higher than the European maximum tolerable residue limit of 0.0001mg/kg. Also the concentration of the pesticide residue detected in the sample include cypermethrin 0.001143±0.000484 mg/kg profenofos

0.003166667±0.001814754 mg/kg, dichlorvos 0.025±0.0043589 mg/kg and dieldrin, 0.003±0.00349476mg/kg. Amongst the entire pesticides residue, only dieldrin, cypermethrin and profenofos were much lower compared than the European maximum residue limit for each of the residue.

Table 4: Pesticides residue in Kola nut sample

Mean value	Organophosphorus pesticides			Organochlorine pesticides		Pyretheroids pesticides
	Chlorpyrifos	Dimethoate	Profenofos	Dieldrin	Dichlorvos	Cypermethrinn
Mariri (mg/kg)	0.0786	ND	0.0029	0.0043	0.02	0.001
EUMRL(mg/kg)	0.05	0.01	0.05	0.01	0.01	0.07

EU MRL= European Union Maximum Residue Limit

DISCUSSIONS

In the first phase of the study, the survey revealed the level of knowledge and pesticide usage in the study area, types of pesticides used and reasons for selecting pesticides. The result shows that 93.7% have heard of pesticide while only 6% never heard of it at all. This shows that majority of the traders in the study area use pesticide for kola nut preservation to enhance its shelve

life. This is in agreement with the findings of Olabode *et al* 2011 which says pesticide have served as a tools to enhance the shelve life of agricultural produce. The predominance of chlorpyrifos (26%) and dichlorvos (25%) in an open market indicated that they are widely used in protection of fresh Kola nut against insect pests. The frequency of these pesticides indicated that they are still available in Mariri market.

Most of the detected compounds in the fresh kola nut sample were organophosphorus pesticide (chlorpyrifos) and Organochlorine pesticide (dichlorvos). Ten compounds were detected from comparison of data with reference to mass spectrometry data. (NIST. Lib) and significant occurrence in the sample which had a corresponding molecules affiliated to some pesticide standard detected in fresh kola nut. Chlorpyrifos was the most frequently detected in the sample (n=4), while dichlorvos (n=2). Their detection was not surprising as they are commonly used as post harvest pesticides on kola nut in the study area. The presence of these pesticides residue in kola nut could be attributed to the inappropriate application such as over dosage of the said pesticides and indiscriminate acts to achieve set goals (Chedi *et al* 2010).

Improper use of pesticide may result in high Pesticide residue above the EUMRLs that will be hazardous to the consumer and lead to the emergence of resistant pest populations which would be insensitive to normal pesticide dosage (Chedi *et al.*, 2010).

Since kola nut are eaten raw in all cultures, and most of the pesticides made to preserve kola nut are been applied directly, this lead to the contamination of the kola nut.

The pesticide residues in the kola nut samples revealed that chlorpyrifos and dichlorvos have the higher mean concentrations above the EUMRLs which might be due to the post-application of this pesticides after harvest in the study area. This also supports Li, (2009) from Zimbabwe who says that illiteracy, poor or bad attitude of farmers on danger of pesticides contributed to higher level of dichlorvos. Hence, this could be a similar trend happening at Mariri market in Kano State. Although, profenofos and dieldrin are below tolerable limit of EUMRLS.

The detection of pesticides in the kola nut samples agrees with the report of Khuhro and Nizamani (2000), which states that the farmers repeatedly spray different pesticides on food crops to protect their crop from various pests without any awareness about their hazardous effect on health. It is also routine practice of farmers to send their produce to market based on the demand which causes contamination of residues beyond EUMRLs.

CONCLUSION

In conclusion, the result of the study established that 93.73% of the traders heard of pesticide in Mariri market. However, the survey also revealed that the major pesticide used in preserving their kola nut were chlorpyrifos (26.12%) and dichlorvos (25.82%). while the choice of pesticide usage was based on availability of the pesticide in the study area.

It also showed that ten different compounds were detected in kola nut in the market. The observed pesticide residues for chlorpyrifos and dichlorvos in kola nut in the study area were above the EU MRLs. Hence, there is urgency in need by federal government to train respondents on pesticide storage, the dosage and time of application to minimize the pesticide residues within the EU MRLS, and for consumer safety and to add value to our kola nut in the international market.

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