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ASSESSMENT OF AFLATOXIN AND PESTICIDE RESIDUE IN COWPEA (*VIGNA UNGUICULATA* L. WALP.) FROM NORTH CENTRAL NIGERIA

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ABSTRACT

Cowpea is a major staple food crop which serves as a source of protein for many Nigerians. Its production and preservation involve the application of pesticides to ensure optimum yield and prevent storage losses. The study evaluated aflatoxin and pesticide residues present in Cowpea grains obtained from retailers in major markets in six States of North Central and the Federal Capital Territory Abuja, Nigeria. The quantitative detection of pesticide residue was conducted through the use of Gas chromatography fitted with Electron Capture Detector (GC-ECD). Aflatoxin (AfB_1, AfB_2, AfG_1 and AfG_2) analysis was conducted by the use of thin layer chromatography (TLC). Twenty-three pesticide residues including organochlorine insecticides, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), fungicide (Etridiazole, chloroneb), herbicide (Simazine, atrazine) were detected in cowpea samples. Dichlorodiphenyldichloroethylene (DDE) was present in 95% of the samples while endosulfan sulfate and endosulfan II were detected in 85% of the cowpea samples. Endrin aldehyde, DDT and DDD were the least detected. Pesticides residues in cowpea grains tested ranged from 72.67 mg kg^{-1} in samples from Kogi State to 159.67 mg kg^{-1} in samples from Plateau State which were above the allowable limits of 20 mg kg^{-1}. Aflatoxin was below detectable limits in all the cowpea samples analyzed. The high amount of pesticide residues in the cowpea samples poses health hazards to unsuspecting cowpea consumers.

Keywords: Cowpea; aflatoxin; pesticide residue; chromatography; Endosulfan.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop used as food, cash crop and feedstuff for livestock. It has nitrogen fixing ability and aids in the prevention of hunger in Sub-Saharan Africa where food shortage is prevalent (Sariah, 2010). Cowpea is a popular legume which serves as a source of protein for many Nigerians who cannot afford animal protein (Animasaun *et al*., 2015). One hundred grams of raw Cowpea grain contains 60g carbohydrate, 23.52g protein, minerals and vitamins (Affrifah *et al*., 2021; Rangel *et al*, 2003; Goncalves *et al*. 2016). It also contains folic acid, lipids, essential and non-essential amino acids (Witthöft and Hefni, 2016).

The northern parts of Nigeria with less rainfall are suited for cowpea production (Dugje *et al*., 2009). Cowpea is either planted alone or as a companion crop when intercropped with cereals. The decay of its leaf litter, roots and root nodules produces nitrogen which enriches the soil (Okereke *et al*., 2006). Cowpea production is hampered by the presence of insect pests and diseases on the field and store (Omoigui *et al*., 2018). The bid to increase cowpea production and yield in Nigeria has resulted in the indiscriminate use of pesticides by cowpea farmers and traders giving rise to incidences of pesticide residues in cowpea sold in markets for human consumption (Arannilewa *et al*., 2006; Alakali *et al*., 2016).

Ninety five percent of cowpea grains produced in Northern Nigeria are randomly treated with insecticides by wholesale traders to prevent insect infestation in the store and during sale (Fakayode *et al*., 2014; Akintobi *et al*. 2018; Olufade *et al*., 2014). The rising awareness of possible presence of pesticide residues in food products especially cowpea which has a peculiar pest problem requiring frequent pesticide application in the field has necessitated the need to screen cowpea on sale for presence of pesticide residue. The possibility of high amount of pesticide residues in cowpea displayed for sale in some popular markets has become a serious concern for cowpea consumers who obtain cowpea from such markets (Olufade *et al*. 2014). Also, the increase in the amount of powdery substances suspected to be pesticide in cowpea purchased for consumption is alarming and requires investigation.

The contamination of foodstuff with aflatoxin has been linked to occurrences of malnutrition in South West Nigeria resulting in the death of some children who consumed the contaminated foodstuff (Ogungbemile *et al*., 2020). Hence the need for investigation of the presence of mycotoxins and pesticide residues in cowpea is necessary and gives insight to the safety status of cowpea grains consumed by a majority of people who cannot afford animal protein.

The study was conducted to evaluate the amount of aflatoxin B, aflatoxin G and pesticide residues in cowpea sold in major markets located in six North Central States of Nigeria and the Federal Capital Territory (FCT).

MATERIALS AND METHODS

The study was conducted in the North-Central geopolitical region of Nigeria which consists of six States, namely Benue, Plateau, Kogi, Nasarawa, Kwara, Niger and the Federal Capital Territory (FCT) Abuja, Nigeria (Figure 1). The North Central area of Nigeria lies between latitudes $6^{0}30''$ N - $11^{0}20''$ N and longitudes 7^{0}E - 10^{0}E (Adelaja *et al*., 2021).

Source: Stephens (2019); Abdulsalem (2020).
Figure 1: Map of Nigeria annotating North-Central States and the locations sampled.

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Sample Collection and Preparation

Sixty three samples of Cowpea grains sold in the major markets in the State capitals of the six North Central Nigerian States and the FCT were randomly collected between November 2019 and January 2020. The markets were namely: Benue (Wurukum, Wadata, Northbank), Nasarawa (Damarol, Modern Market, Alamis), Kogi (Kpata, Old market, New market), Kwara (Oja Oba, Oja Oloje, Ikpata), Niger (Bosso, Tunga, Gida Mangoro), Plateau (Terminus, Katako, Buruku) and FCT (Utako, Gwarinpa, Garki). These markets were chosen because they are popular and well patronized markets within the capital of each State in the study area. Two hundred grams (200g) of each cowpea sample was packaged and sent to the different laboratories for quantification of aflatoxin and pesticides residue.

Aflatoxin analysis

Twenty grams of each cowpea sample collected were analyzed for the presence of aflatoxin B1, B2, G1 and G3. The thin layer chromatography (TLC) method was used to quantify aflatoxins at the Pathology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria. The aflatoxin content determination was done following the procedure of Atehnkeng *et al*. (2008). A scanning densitometer (CAMAG TLC Scanner 3, Switzerland) was used to quantify the amount of aflatoxin present in cowpea samples.

Instrumentation, Standards and Gas chromatography GC–ECD conditions for pesticide residue detection

Cowpea samples were appropriately packaged, labeled and transferred to the Central Research Laboratory of the Federal University of Technology, Akure, Nigeria for the determination of the pesticide residue present. The detection of pesticide residues present in cowpea grains was conducted using the Gas Chromatography-Electron Capture Detector (GC-ECD) (Agilent Technologies; Santa Clara, CA, USA).

The gas chromatograph was fitted with a capillary column which had a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The gas chromatograph had Helium as the carrier gas flowing constantly at the rate of 2.0 mL/min while Nitrogen was used as the make- up gas flowing at the rate of 30 mL/min. The detector had base temperatures of 305°C and 280°C, the column temperature was 120°C held for one minute and later increased to 280°C for 8 minutes while the injector port was kept at 250°C.

The standard stock solution of 100 mg/L was prepared in Acetone while the calibration solutions for each pesticide were prepared from the stock solution through serial dilution with acetone to produce 6.00 mg/L, 8.00 mg/L and 10.00 mg/L solution. The solutions were preserved at 4°C in a refrigerator.

Sample preparation and clean-up

Forty milliliters of acetonitrile was added to ground cowpea samples and homogenized for two minutes and passed through a filter paper into a graduated cylinder with a stopper. Five (5) grams of Sodium Chloride was added into a graduated cylinder, stopped and shaken vigorously for two minutes and kept for 20 minutes. Ten milliliters of the supernatant was then added into a 100 mL flask and dried with a rotary evaporator at 40°C. The resulting residue was further diluted using 2 mL of a mixture of three parts of acetonitrile and one-part toluene. Afterwards the cartridge was conditioned with 5 mL of the 3:1 v/v mixture of acetonitrile and toluene and used for the solid phase extraction. Two milliliters of the extract was then applied onto the cartridge and the eluate was collected in a flask. The resulting analytes were then eluted with 15 mL of a mixture of acetone and hexane in a ratio of 3:1 (v/v). The eluate was then dried on a rotary evaporator at 40°C while the residue detected was dissolved in 5 mL acetone and analyzed by GC–ECD.

Statistical Analysis

Data from three replicates of each sample laid out in a completely randomized design were subjected to analysis of variance (ANOVA). Data analysis was done using the statistical package for social sciences (SPSS) version 16. Significant means were separated using Duncan's New Multiple Range Test (DNMRT) at 5% level of probability. Data were recorded as means ± standard deviation (SD).

RESULTS

The result of the aflatoxin analysis of cowpea samples indicated that the amount of Aflatoxin B_1, B_2, G_1 and G_2 in the cowpea grains were below detection limits of less than one part per billion (pbb).

Data presented in Table 1 shows the cyclodiene insecticide residues in cowpea samples. The concentration of aldrin residues in the samples ranged from 2.00 - 6.00 mg kg^{-1} and was absent in samples from Nasarawa State. Endrin residues had the highest mean concentration of 6.67 ± 0.58 mg kg^{-1} in Plateau and FCT while endrin aldehyde was present only in cowpea samples from Niger State (1.33 ± 2.31 mg kg^{-1}). Dieldrin recorded the highest level in cowpea samples from Kwara State (17.33± 15.14 mg kg^{-1}) and was lowest in samples from Niger State (5.00 ± 8.66 mg kg^{-1}). Endosulfan I residue concentration ranged from 1.67 ± 2.89 mg kg^{-1} to 2.33 ± 4.04 mg kg^{-1} and was not detected in cowpea samples from Benue, FCT and Kwara State. Endosulfan II had highest residue concentration in cowpea samples from Niger State (6.33 ± 0.58 mg kg^{-1}) and was not detected in cowpea samples from Benue State.

Endosulfan sulphate residue recorded highest concentration in samples from FCT (68.00 ± 44.17 mg kg^{-1}) and least concentration in cowpea samples from Kogi (0.00 ± 0.00 mg kg^{-1}) and Niger State (4.00 ± 6.93 mg kg^{-1}). The residue of heptachlor in the cowpea samples ranged from 0.33 ± 0.58 mg kg^{-1} in samples from Plateau State to 12.33 ± 2.31 mg kg^{-1}in samples from Niger State while it was not detected in cowpea samples from Nasarawa State. Cowpea samples from Nasarawa State recorded the highest concentration of g-chlordane of 6.67 ± 0.58 mg kg^{-1} and lowest residue concentration of 2.00 ± 3.46 mg kg^{-1} recorded in samples from Kwara and Plateau State.

Table 1: Cyclodiene insecticide (mg kg^{-1}) residues in cowpea samples from North Central Nigeria

OCP/ State	Benue	Nasarawa	FCT	Kogi	Kwara	Niger	Plateau
Aldrin	2.00 ^{ab} ± 3.46	0.00 ^{a} ±0.00	6.00 ^{b} ±0.00	4.00 ^{ab} ± 3.46	6.00 ^{b} ±0.00	4.00 ^{ab} ±3.46	2.00 ^{ab} ± 3.46
Endrin	2.33 ^{a} ± 4.04	6.33 ^{a} ±0.58	6.67 ^{a} ±0.58	6.33 ^{a} ±0.58	6.33 ^{a} ±0.58	4.67 ^{a} ±4.16	6.67 ^{a} ±1.16
Endrin Aldehyde	0.00 ^{a} ±0.00	0.00 ^{a} ±0.00	0.00 ^{a} ±0.00	0.00 ^{a} ±0.00	0.00 ^{a} ±0.00	1.33 ^{a} ±2.31	0.00 ^{a} ±0.00
Dieldrin	16.33 ^{a} ± 14.36	12.67 ^{a}±10.97	15.00 ^{a} ±13.00	7.33 ^{a} ± 12.70	17.33 ^{a} ± 15.14	5.00 ^{a} ± 8.66	5.33 ^{a} ±9.24
Endosulfan I	0.00 ^{a} ±0.00	2.00 ^{a} ±3.46	0.00 ^{a} ± 0.00	1.67 ^{a} ±2.89	0.00 ^{a} ± 0.00	2.33 ^{a} ±4.04	1.67 ^{a} ± 2.89

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Endosulfan II	0.00 ^{a} ± 0.00	2.00 ^{ab} ± 3.46	2.00 ^{ab} ± 3.46	4.33 ^{ab} ± 3.79	2.00 ^{ab} ± 3.46	6.33 ^{b} ± 0.58	4.33 ^{ab} ± 3.79
Endosulfan sulphate	30.33 ^{a} ± 52.54	40.67 ^{a} ± 47.59	68.00 ^{a} ± 44.17	0.00 ^{a} ± 0.00	46.67 ^{a} ± 49.74	4.00 ^{a} ± 6.93	37.33 ^{a} ± 9.87
Heptachlor	5.33 ^{abc} ± 4.73	0.00 ^{a} ± 0.00	4.33 ^{ab} ± 3.22	5.67 ^{abc} ±8.15	9.00 ^{bc} ± 0.00	12.33 ^{c} ± 2.31	0.33 ^{a} ± 0.58
g-Chlordane	6.33^{ab}± 0.58	6.67^{b}± 0.58	6.00 ^{ab} ±0.00	6.00 ^{ab} ±0.00	2.00 ^{a} ± 3.46	4.33 ^{ab} ± 3.79	2.00 ^{a} ± 3.46

OCPs = organochlorine pesticides. *Mean values with the same letter within the same row are not significantly different using DNMRT at 5% probability*

Data presented in Table 2 shows the concentration of hexachlorocyclohexane insecticide residues in cowpea samples from North Central Nigeria. Alpha-benzene hexachloride (α-BHC) residue was highest in cowpea samples from Niger State (5.67 ± 0.58 mg kg^{-1}) and lowest in samples from Plateau State (1.67 ± 2.89 mg kg^{-1}). Residues of β-BHC were highest in cowpea samples from Benue State (5.00 ± 0.00 mg kg^{-1}) while samples from Nasarawa and Plateau State recorded the least residue of β-BHC (1.33 ± 2.31 mg kg^{-1}). Trans-nonachlor was highest in samples from FCT (6.67± 0.58 mg kg^{-1}) followed by samples from Kwara State (6.33± 0.58 mg kg^{-1}) while it was not detected in samples from Plateau State.

Gamma BHC (Lindane) was detected in FCT (2.67± 4.62 mg kg^{-1}), Kogi State (1.00 ±1.73 mg kg^{-1}) and Niger State (0.67 ±1.16 mg kg^{-1}). Niger and Plateau States recorded the highest d-BHC residue of 5.67 ± 0.58 mg kg^{-1} followed by Benue State (5.33 ± 4.62 mg kg^{-1}). Delta BHC (d-BHC) was not detected in samples from Kogi State.

Table 2: Concentrations of hexachloro cyclohexane insecticides (mg kg^{-1}) residues in cowpea samples from North Central Nigeria

State/OCP	α-BHC	β-BHC	Trans-Nonachlor	g-BHC (Lindane)	d-BHC
Benue	4.67 ^{a} ± 4.16	5.00 ^{a} ± 0.00	2.00 ^{ab} ± 3.46	0.00 ^{a} ±0.00	5.33 ^{a} ± 4.62
Nasarawa	4.33 ^{a} ±4.04	1.33 ^{a} ± 2.31	2.00 ^{ab} ± 3.46	0.00 ^{a} ±0.00	1.67 ^{a} ±2.89
FCT	2.00 ^{a} ± 3.46	3.33 ^{a} ± 2.89	6.67 ^{b} ± 0.58	2.67 ^{a} ± 4.62	2.00 ^{a} ±3.46
Kogi	3.67 ^{a} ±3.22	4.67 ^{a} ± 0.58	4.00 ^{ab} ± 3.46	1.00 ^{a} ±1.73	0.00 ^{a} ±0.00
Kwara	2.00 ^{a} ± 3.46	3.67 ^{a} ± 3.22	6.33 ^{b} ± 0.58	0.00 ^{a} ±0.00	2.67 ^{a} ±4.62
Niger	5.67 ^{a} ± 0.58	3.67 ^{a} ± 3.22	4.33 ^{ab} ± 3.79	0.67 ^{a} ±1.16	5.67 ^{a} ±0.58
Plateau	1.67 ^{a} ± 2.89	1.33 ^{a} ± 2.31	0.00 ^{a} ± 0.00	0.00 ^{a} ±0.00	5.67 ^{a} ±0.58
Total	3.43 ± 3.14	3.29 ±2.43	3.62 ± 3.23	0.62 ±1.86	3.29 ± 3.32

OCP= Organochorine pesticide. *Mean values with the same letter within the same column are not significantly different using DNMRT at 5% probability*

Table 3 shows the concentrations of dichloro diphenylethene insecticides (mg kg^{-1}) present in cowpea sold in major markets in North Central Nigeria. The result indicates that dichlorodiphenyldichloroethylene (DDE) a metabolite of dichlorodiphenyltrichloroethane (DDT) was detected in all cowpea samples with highest mean concentration in samples from Benue State (6.67 ± 0.58 mg kg^{-1}) and lowest DDE residue in samples from Plateau (4.00 ±3.46 mg kg^{-1}). Although DDT and DDD were not detected in all the cowpea samples, their isomer, p,p' DDD was detected only in Plateau State (2.33 ± 2.08 mg kg^{-1}) while p, p' DDT was detected in Niger State (2.67± 2.52 mg kg^{-1}) and Plateau State (76.67 ± 61.00 mg kg^{-1}). Chlorobenzilate was not detected in any cowpea sample across the six States.

Table 3: Concentrations of dichloro diphenylethene insecticides (mg kg^{-1}) present in cowpea sold in Major markets in North Central Nigeria

State/OCP	DDE	DDT	DDD	P, p-DDD	P, p-DDT	Chlorobenzilate
Benue	6.67^{a} ±0.58	ND	ND	0.00^{a} ±0.00	0.00^{a}± 0.00	ND
Nasarawa	6.33^{a} ±0.58	ND	ND	0.00^{a} ±0.00	0.00^{a}± 0.00	ND
FCT	6.00^{a} ±0.00	ND	ND	0.00^{a} ±0.00	0.00^{a}± 0.00	ND
Kogi	6.00^{a} ±0.00	ND	ND	0.00^{a} ±0.00	0.00^{a}± 0.00	ND
Kwara	6.00^{a} ±0.00	ND	ND	0.00^{a} ±0.00	0.00^{a}± 0.00	ND
Niger	6.67^{a} ±1.16	ND	ND	2.33^{b} ± 2.08	2.67^{a}± 2.52	ND
Plateau	4.00^{a} ±3.46	ND	ND	0.00^{a} ±0.00	76.67^{b} ± 61.00	ND
Total	5.95±1.47	0.00±0.00	0.00±0.00	0.33±1.07	11.33±33.47	0.00±0.00

OCP= Organochorine pesticide. ND= Not detected

Data presented in Table 4 shows the concentration of thiadiazole fungicides (mg kg^{-1}) detected in cowpea samples from North Central Nigeria. The result indicates that the concentration of etridiazole residues detected in cowpea samples from Nasarawa State (5.67 ± 2.08 mg $kg^{-1)}$ were significantly higher ($P \leq 0.05$) compared with the etridiazole residue detected in cowpea samples from Benue State (1.67 ± 1.53 mg kg^{-1}), Niger State (1.00 ± 1.00 mg kg^{-1}), Kwara State (0.67 ± 1.16 mg kg^{-1}) and the FCT (0.67 ± 1.56 mg kg^{-1}).

Chloroneb recorded highest concentration in Benue State (5.52 ± 0.31mg kg^{-1}) followed by residue in samples from Nasarawa (5.36 ± 0.24 mg kg^{-1}), Plateau (5.36 ± 0.14 mg kg-1), FCT (5.29 ± 0.33 mg kg^{-1}) and recorded least concentration in samples from Niger State (1.70 ± 2.94 mg kg^{-1}). Chlorothalonil residue was highest in cowpea samples from FCT (3.67 ± 3.22 mg kg^{-1}), Niger State (3.33 ±2.89 mg kg^{-1}), Kwara State (2.00 ± 3.46 mg kg^{-1}), Benue and Kogi State (1.67 ±2.89 mg kg^{-1}). Chlorothalonil residue was not found in Nasarawa and Plateau State. The concentration of etridiazole was highest in samples from Nasarawa State (5.67 ±2.08 mg kg^{-1}) followed by samples in Benue State (1.67 ± 1.53 mg kg^{-1}), Niger State (1.00 ± 1.00 mg kg^{-1}), Kwara State (0.67 ±1.16 mg kg^{-1}) and FCT (0.67 ±1.56 mg kg^{-1}). Etridiazole was absent in cowpea samples from Kogi and Plateau States.

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Table 4: Concentration of thiadiazole fungicides (mg kg^{-1}) in cowpea samples from North Central Nigeria

State/OCP	Etridiazole	Chloroneb	Chlorothalonil
Benue	$1.67^{a} \pm 1.53$	$5.52^{a} \pm 0.31$	$1.67^{a} \pm 2.89$
Nasarawa	$5.67^{b} \pm 2.08$	$5.36^{a} \pm 0.24$	$0.00^{a} \pm 0.00$
FCT	$0.67^{a} \pm 1.56$	$5.29^{a} \pm 0.33$	$3.67^{a} \pm 3.22$
Kogi	$0.00^{a} \pm 0.00$	$3.75^{a} \pm 3.26$	$1.67^{a} \pm 2.89$
Kwara	$0.67^{a} \pm 1.16$	$3.72^{a} \pm 3.25$	$2.00^{a} \pm 3.46$
Niger	$1.00^{a} \pm 1.00$	$1.70^{a} \pm 2.94$	$3.33^{a} \pm 2.89$
Plateau	$0.00^{a} \pm 0.00$	$5.36^{a} \pm 0.14$	$0.00^{a} \pm 0.00$
Total	1.38± 2.13	4.38 ± 2.20	1.76±2.57

OCP= Oragnochlorine Pesticide.

The data shown in Table 5 presents the concentrations of organochlorine herbicides detected in cowpea samples from North Central Nigeria. Alachlor residues in cowpea samples from Niger and Plateau States (2.00 ± 3.46 mg kg^{-1}) were significantly ($P \leq 0.05$) lower than the other four States and the FCT which ranged from 6.00 ± 0.00 mg kg^{-1} to 6.33± 0.58 mg kg^{-1}. Atrazine residue recorded highest concentration in samples from Kogi State with 6.00± 0.00 mg kg^{-1} followed by Niger and Benue State (4.00± 3.46 mg kg^{-1}), FCT, Kwara and Plateau State (2.00 ± 3.46 mg kg^{-1}) and was not detected in Nasarawa State. Simazine residue which was not detected in samples from Nasarawa State had highest residue in samples from Niger State (6.00± 0.00 mg kg^{-1}) followed by samples from Benue State (4.33± 3.79 mg kg^{-1}), Kwara State (4.00 ± 3.46 mg kg^{-1}), Plateau State (3.33 ± 2.89 mg kg^{-1}), FCT (2.00 ± 3.46 mg kg^{-1}) and Kogi State (1.67 ± 2.89 mg kg^{-1}) .

Table 5: Mean concentrations of organochlorine herbicides (mg kg^{-1}) in cowpea samples from North Central Nigeria

State/OCP	Alachlor	Atrazine	Simazine
Benue	$6.00^{b} \pm 0.00$	$4.00^{ab} \pm 3.46$	$4.33^{ab} \pm 3.79$
Nasarawa	$6.00^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
FCT	$6.00^{b} \pm 0.00$	$2.00^{ab} \pm 3.46$	$2.00^{ab} \pm 3.46$
Kogi	$6.33^{b} \pm 0.58$	$6.00^{b} \pm 0.00$	$1.67^{ab} \pm 2.89$
Kwara	$6.33^{b} \pm 0.58$	$2.00^{ab} \pm 3.46$	$4.00^{ab} \pm 3.46$
Niger	$2.00^{a} \pm 3.46$	$4.00^{ab} \pm 3.46$	$6.00^{b} \pm 0.00$

Plateau	$2.00^{a} \pm 3.46$	$2.00^{ab} \pm 3.46$	$3.33^{ab} \pm 2.89$
Total	4.95 ± 2.48	2.86 ± 3.07	3.05 ± 3.01

OCP= Oragnochlorine Pesticide.

Figure 2 shows the total organochorine residues present in cowpea samples from North Central States. Cowpea samples from Plateau State recorded the highest organochlorine pesticide residue of 159.67 mg kg^{-1} followed by samples from FCT (146.33 mg kg^{-1}), Kwara State (127.00 mg kg^{-1}), Benue State (109.00 mg kg^{-1}), Nasarawa State (100.67 mg kg^{-1}), Niger State (91.00 mg kg^{-1}) and Kogi State (72.67 mg kg^{-1}).

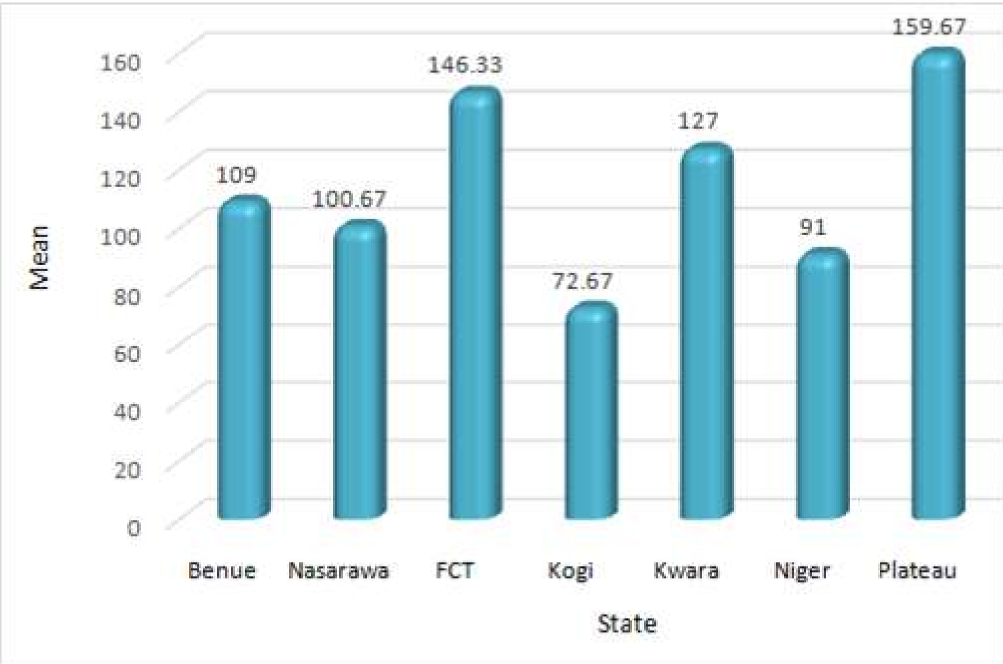

Figure 2: Total organochlorine pesticide present in Cowpea samples from North Central States in Nigeria

DISCUSSION

The present study has shown that cowpea grains sold in popular markets in the study area were free of aflatoxin B1, B2, G1 and G2 which are the four major aflatoxins contaminating food stuff and feed and are classified as a group 1 human carcinogens by the International Agency for Research on Cancer (IARC) (EFSA 2020). The absence of these aflatoxins may be due to the inhibitory action of the fungicides applied on cowpea in the field to the growth of the fungi responsible for aflatoxin formation. Kritzinger *et al*. (2003) also reported the absence of aflatoxin in cowpea samples from Benin Republic and South Africa. However, Houssou *et al*. (2009) reported only aflatoxin B1 in samples of stored cowpea seeds in the Republic of Benin.

The non- detection of aflatoxins in this study is similar to the report of Kritzinger *et al*. (2003) which did not find aflatoxins in cowpea from Benin Republic and South Africa. In contrast, Ogungbemile *et al*. (2020) however detected aflatoxins B1, B2, G1 and G2 in cowpea samples from Ibadan, South west Nigeria. Lindemann *et al*. (2022) attributed the non - detection of mycotoxins to the inability of fungi present to produce mycotoxin.

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The report of Njoroge *et al*. (2016) attributed the formation of aflatoxins to the genetic diversity and capabilities of fungi found on grains to produce toxin. This assertion is corroborated by the report of Karthikeyan *et al*. (2009) which observed that about 37 % of *Aspergillus flavus* isolated from maize did not produce Aflatoxin B1. The non-production of aflatoxin in this present study could be due to more proportion of atoxigenic strain of *A. flavus* than the toxigenic strains resulting in little or no aflatoxin production as corroborated by the report of Agbetiameh *et al*. (2018). Furthermore, Agbetiameh *et al*. (2018) observed that genotypes of the *A. flavus* L morphotype were more common and are usually atoxigenic due to defects in the genetic make - up of the fungus.

All the cowpea samples in this present study were contaminated with pesticide residues of various types. The detection of pesticides in this study is in line with the report of Adah *et al.* (2020) which reported a variety of banned pesticides including aldrin, dieldrin, endrin, and heptachlor in rice samples from Makurdi, Benue State, Nigeria. More insecticides were detected in this present study than fungicides and herbicides. This finding is corroborated by the report of Fakayode *et al*. (2014) and Akintobi *et al*. (2018) which identified insects as the major pests of cowpea hence the need for more insecticide. Similarly, Olufade *et al*. (2014) reported incessant application of insecticides such as lindane, DDT, endosulfan and aldrin by cowpea farmers in FCT, Abuja Nigeria. This frequent application could result in residual deposits on cowpea grains.

The detection of these banned pesticides in this study agrees with the report of Olufade *et al* (2014) which noted that banned organochlorine and other chemicals were still being used in agriculture in Nigeria and other African countries. The present study also indicated that pesticides such as endosulfan sulphate, dieldrin and p, p –DDT were above the allowable residual limit of pesticides recommended by the European Union. This has validated the claim of increasing use of several banned pesticides in Nigeria.

The increased detection of pesticide in cowpea grains has been largely attributed to the ease of availability of banned pesticide and their indiscriminate use by cowpea farmers who are unaware of the health implications. Fakayode *et al*. (2014) and Akintobi *et al.* (2018) reported the incessant application of insecticides among cowpea farmers and traders in FCT Abuja, Nigeria in order to control field and storage insect pests of cowpea grains. The continuous indiscriminate usage of these pesticides may have health implications on cowpea consumers.

The report of NRC (1982) indicated that the ingestion of aldrin and dieldrin at 25 mg/kg per day in the diet of mouse had significant effects on the reproduction of the test rodents and by extension humans. Chen (2014) had earlier reported lindane as a persistent organic insecticide while the report of Onuwa *et al*. (2017), confirmed the use of lindane by Nigerian farmers despite the pesticide being banned in Nigeria.

Hopkins (2008) reported the hospitalization of 112 cowpea consumers and the death of two people who consumed cowpea contaminated with pesticides. Furthermore, Hopkins (2008) observed that cowpea from markets in Benue and Taraba States recorded high levels of organophosphates and other toxic pesticides. Also, food poisoning of 120 students in a secondary School in Gombe State, Nigeria was reported after the consumption of cowpea with high residues of lindane commonly known as gammalin 20® (Hopkins, 2008).

Heptachlor which is used in insect and termite control poses potential health risk and an allowable limit of 0.01 parts per million for raw food crops has been recommended (ATSDR, 2007). Olufade *et al*. (2014) reported the high levels of heptachlor in cowpea grains from Ile Ife South West Nigeria. Atrazine which was also detected in this study is known to have low acute toxicity with potential of being carcinogenic in humans and is classified as 'not likely to be carcinogenic (Liu, 2014). This present study on cowpea identified aldrin which is a persistent organic pollutant seed and soil pesticide which reacts to form dieldrin. The report of Onuwa *et al*. (2017) also found aldrin in edible crops and soils in Makurdi, Nigeria. Aldrin is considered the most persistent of all pesticides banned for use in Agriculture in European countries more than forty years ago (NRC, 1982). According to NRC (1982) bioaccumulation of aldrin, dieldrin and heptachlor is linked to incidence of convulsions, tremors, seizures, incoordination, headache, dizziness, and gastrointestinal disturbances in humans. NRC (1982) reported that giving dieldrin in peanut oil orally at 6 mgkg^{-1} resulted in 41 percent mortality in rats and increased liver-to-body weight ratios and also reduced weight gain in mice.

Cowpea farmers engage in indiscriminate and illegal application of these banned pesticides leading to their persistence in the grains for consumption. Adah *et al*. (2020) noted that most of the pesticides have long half-life which makes them persistent in foodstuffs after a long period of application.

The half - life which is the time taken for one half of the different pesticides to disintegrate varies. Howard (2004) reported that dieldrin and endrin has a half- life of 5 years and 4 years respectively while p, p-DDT has a half-life of 2 years. Atrazine and endosulfan has half -life corresponding to 60 days and 50 days respectively while aldrin has a half- life of one year. Chlordane has a half- life of 10-20 years while its metabolite trans-nonachlor has been found in the adipose tissues of humans (Bondy, *et al*., 2000). Park *et al.* (2020) described etridiazole as a pesticide with a soil half- life of 20 days. According to EFSA (2017) etridiazole was detected in cucumber samples collected shortly after treatment with an MRL (maximum residue level) value of 0.4mg/kg in cucumber recorded within 6 days after application. The concentration of endrin in this present study (6.6701mgkg^{-1}) was higher than the recommended quantity of 0.01mgkg^{-1} (Adah *et al*., 2020).

The detection of endosulfan sulphate in 85% of cowpea samples and p, p'DDE in 95% of cowpea samples in this study is similar to the report of Kihampa *et al*. (2010) in which endosulfan sulphate was detected in 100% of soil samples while p,p'- DDE was reported in 46% of soil samples and p, p'- DDD was reported in 40% of the soil samples where tomato was grown in Tanzania.

In conclusion, the levels of the pesticide residues detected in the cowpea grains from the study areas are generally above the European Union maximum residue levels (EU-MRLs) of 0.01mgkg^{-1}, indicating that the foodstuffs may not be safe for human consumption and may pose health risks to the consumers of the contaminated cowpea.

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