

## **Risk assessment of residual pesticides of *Phaseolus Vulgaris L.* consumed in Port Harcourt, Rivers State, Nigeria**

### **ABSTRACT**

Pesticides are important and necessary in reducing the loss caused by insect infestation on grains but its toxicity and persistence in the environment is of health concern. Beans (*Phaseolus vulgaris L.*) preserved with insecticide are considered to be good for consumption if its content of insecticide is not higher than the maximum residue limits (MRLs). The aim of this research was to quantify the residue of pesticide in four beans samples and determine the health risk associated with consuming these foods by residents of Port Harcourt, Rivers State, Nigeria. Pesticides content of beans samples were analyzed using Gas Chromatography (GC). The results of the study showed the presence of 17 different pesticide residues in all samples of beans. 2-4-dichloro and DDVP were detected in all samples of beans and at levels above EU's MRL except in iron beans samples. Glyphosate was detected in all the samples at concentration above EU's MRL. The results of this study also showed that parboiling reduced the HRI of the pesticides and local beans was the only sample with HRI <1 in the parboiled state. This study showed that the beans samples monitored contain various concentrations of pesticide residues, many of which are already banned organochlorine pesticides, thus indicating potential health risk to consumers. This calls for the attention of regulatory agencies in foods and food products to effectively monitor the use and application of pesticides on foodstuff.

*Keywords: Pesticides, beans, maximum residue limits, health risk.*

## 1. INTRODUCTION

Agriculture is very essential in the economy of Nigeria, and it provides gainful employment for about 70% of the population [1]. Despite its importance, agriculture faces certain challenges in Nigeria and they are grouped into biotic and abiotic factors. The biotic factors include diseases, insects or pests which attack crops either on the field or in storage, thereby causing low crop production. In resolving this challenge, there has been an increase in the usage of pesticides since 1940s [2, 3].

Pesticides are poisonous substances which are produced and used because of their toxic effects on one or more pests [4, 5]. Pesticides are deliberately applied to the environment to control unwanted organisms like weeds, insects, and fungi [6, 2, 5]. It is therefore important to analyze pesticide residues in food as it helps assess consumers' exposure to these chemicals and the health risks involved in consuming the foodstuff [7, 8].

Pesticides protect crops while in the fields and during storage, ensuring a sufficient food supply. However, their active metabolites which may likely be absorbed and accumulated by the crops, can lead to harsh health conditions like skin disorders, hypertension, cardiovascular diseases, neurological damage, and even cancer [8] when the crops are consumed. Some researchers found certain stored grains contaminated by residues of chlorinated pesticides. Among the pesticides were: Aldrin, BHC, chlordane, DDT, dieldrin, HCB, and heptachlor [9, 10].

Organophosphate and carbamate pesticides are among the pesticides commonly used in stores and warehouses to protect grains from insect infestation.

These pesticides have prolonged insecticidal activity. Organophosphates are highly toxic, and there are records of acute poisoning incidents [11, 12, 8]. In contrast, carbamates are less toxic yet effective insecticides [7].

An example of foodstuff that is likely to contain high concentration of pesticides is beans [7, 13]. Beans are a vital protein source for people in the developing world, where most sources of protein are often expensive for common consumption. To prevent pest infestation during storage, beans are often treated directly with chemicals, raising concerns about potential high pesticide residue levels in this food.

Beans are safe to consume if free from foreign materials, odor, abnormal color, mycotoxins, live pests, and residual pesticide concentration are below the maximum residue limits (MRLs). This study aimed to determine pesticide residues in four beans (*Phaseolus vulgaris*) varieties consumed in Port Harcourt, Rivers State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

Four beans varieties (*Phaseolus vulgaris* L.) with local names: Patisco, Iron, Brown, Local (Figures 1 to 4) were bought from the sample area, Mile 3 market, Port Harcourt, using systemic random sampling. The samples were conveyed to the laboratory in four separate clean plastic containers. The beans samples were identified by Mr. Olaniyi Yinka a taxonomist at the International Herbarium, department of Botany, Obafemi Awolowo University (OAU) Ile-Ife with voucher numbers: Patisco beans (IFE, 16974), Iron beans (IFE, 16975), Brown beans (IFE, 16978) and Local beans (IFE, 16977). The foreign particles present in the beans were carefully removed by method of hand picking. Each of the samples were divided, into two and labeled: Raw samples and Parboiled samples. The Parboiled samples were processed by parboiling using the local method beans preparation before cooking. The Parboiled beans samples were placed in a pot containing distilled water and cooked for 8 minutes and dried at room temperature. Afterwards, the raw and parboiled samples were crushed using agate mortar.



Figure 1: Patisco beans



Figure 2: Brown beans



Figure 3: Iron beans



Figure 4: Local beans

## 2.2 Determination of Pesticides in Food

Pesticide concentrations of beans samples were determined using Gas Chromatography (GC) according to the method of AOAC [14].

## 2.3 Extraction of Pesticides from Beans Samples

Ten grams of the pulverized samples of *Phaseolus vulgaris* were weighed and placed into a 500 ml beaker. Then six grams (6 g) of  $\text{Na}_2\text{SO}_4$  was mixed with the sample and the mixture extracted using 300 ml n-hexane. Ten milliliter of acetonitrile was added to the homogenized sample and the mixture agitated for 2 min. Extra 10 ml of acetonitrile was added, and the separating funnel shut tightly and put on a horizontal shaker. It was then arranged to shake vigorously for 30 min at 300 rpm/min. For sufficient phase separation to be achieved, it was finally allowed to stand for 5 min. Furthermore, 2g anhydrous magnesium sulphate was applied in drying 10ml of the supernatant. Using filter paper, the mixture was filtered into a 50 ml round bottom flask and with the aid of rotary evaporator, it was concentrated to 1ml and made available for silica clean up step.

## 2.4 Extracts Clean-up (purification by applying Silica SPE cartridge)

One milliliter (1 ml) of filtered residue was dissolved in 50 ml of chloroform, and transferred to a 100 ml volumetric flask before diluting to the mark. At room temperature, most of the chloroform was diluted after which a mixture of

1 ml of the reagent which is 20 vol% benzene and 55 vol% methanol was added. The setup was firmly closed and heated at 40°C in water bath for 10 min. After heating the mixture, the organic phase was extracted with hexane and water, to achieve a final mixture of the reagent, hexane and water, in proportion of 1:1:1. The mixture was firmly agitated by hand for 2 min and by applying centrifugation, the emulsion was broken. Half of the top hexane phase was transferred to a small test tube for injection into GC column.

## 2.5 Gas Chromatographic conditions for Pesticide Determination

The extracts were analyzed using Buck M910 scientific gas chromatography equipped with Flame ionization detector (GC-FID). The temperature of the detector and injector were set at 280 °C and 250°C respectively. The temperature of the oven was set as follows: 120 °C held for 4 min, ramp at 10 °C/ min to 180 °C, held for 2 min, and finally ramp at 5 °C/ min to 300 °C. Helium served as carrier gas and at a flow rate of 1.0 ml/ min and detector make-up gas of 29 MI min<sup>-1</sup>. The volume of injection of the GC was 10.0 µl. Finally, the total run time for a sample was 43 min.

## 2.6 Estimation of Daily Intake (EDI)

The EDI of the various residues in beans were determined as described by the methods of Handa et al. [15].

Calculations of the EDIs of the samples were based on an average daily consumption rate (Cr) of 70g of *Phaseolus vulgaris* (beans) per person and a correction factor of 0.5. It is assumed that the Cr of 200 g beans meal two times weekly. Based on this the EDIs can be calculated thus:

$$EDI = R_m \times Cr \times 0.5$$

R<sub>m</sub> = residue pesticide concentration in samples

Cr = Beans consumption rate

## 2.7 Determination of Health Risk of Pesticides

Calculation of risk of exposure to pesticide residue for an individual (of a mean body weight of 70kg) was achieved using the formula of Akoto et al. [16].

$$HRI = \frac{EDI}{ADI}$$

HRI = means health risk index,

EDI = means estimated daily intake,

ADI = means acceptable daily intake.

Furthermore, the method of Akoto et al. [16], states that when HRI of the food containing the pesticides exceeds one (1), lifetime consumption could pose significant health risks.

## 2.8 Statistical analysis

The data obtained were expressed as mean±standard deviation of triplicate determinations, and the statistical significance ( $p < 0.05$ ) among the groups were derived using one way analysis of variance (ANOVA).

## 3. RESULTS

**Table 1:** Residual Pesticides of Raw Iron Beans and Parboiled Iron Beans

Component	Raw Iron Beans (mg/kg)	Parboiled Iron Beans (mg/kg)	EDI of Raw Iron Beans (mg/kg)	EDI of Parboiled Iron Beans (mg/kg)	HRI of Raw Iron Beans	HRI of Parboiled Iron Beans
2_4_dichloro	0.05±0.00 <sup>a</sup>	0.05±0.00 <sup>a</sup>	0.0001	0.0001	00.8	0.6
Aldrin	0.12±0.01	ND	0.0004	ND	4	ND
Carbofuran	0.43±0.03 <sup>b</sup>	0.23±0.10 <sup>a</sup>	0.0007	0.0007	0.7	0.7
DDVP	0.00±0.00	ND	0.0002	ND	0.01	ND
Dichlorovos	0.18±0.05 <sup>a</sup>	0.13±0.01 <sup>a</sup>	0.0004	0.0004	0.1	0.1
Endosulphan	0.18±0.01 <sup>b</sup>	0.09±0.01 <sup>a</sup>	0.0002	0.0005	0.03	0.08
Glyphosate	0.08±0.01 <sup>a</sup>	0.07±0.01 <sup>a</sup>	0.0002	0.0002	0.0002	0.0002
HCB	0.62±0.10 <sup>b</sup>	0.31±0.01 <sup>a</sup>	0.002	0.0009	0.9	0.5
Heptachlor	0.23±0.02	ND	0.0007	ND	7	ND
P, p'-DDD	0.09±0.01 <sup>a</sup>	0.09±0.01 <sup>a</sup>	0.0002	0.0002	0.02	0.02
Profenos	0.55±0.04 <sup>b</sup>	0.38±0.01 <sup>a</sup>	0.002	0.001	0.07	0.03
t-nonachlor	0.32±0.00 <sup>b</sup>	0.22±0.00 <sup>a</sup>	0.0009	0.0008	0.03	0.01

Values are Mean±SD of triplicate determinations. Columns having different superscripts are statically significant at  $p < 0.05$ .

**Table 2:** Residual Pesticides of Raw Patasco Beans and Parboiled Patasco Beans

Component	Raw Patasco (mg/kg)	Parboiled Patasco (mg/kg)	EDI of Raw Patasco (mg/kg)	EDI of Parboiled Patasco (mg/kg)	HRI of Raw Patasco Beans	HRI of Parboiled Patasco Beans
2_4_dichloro	0.42±0.01 <sup>b</sup>	0.17±0.02 <sup>a</sup>	0.001	0.0005	0.05	0.01
Aldrin	0.30 ±0.01 <sup>b</sup>	0.13±0.01 <sup>a</sup>	0	0.0009	0	9
Carbofuran	0.50 ±0.02 <sup>b</sup>	0.25±0.01 <sup>a</sup>	0.001	0.0007	1	0.7
DDVP	0.24 ±0.02 <sup>b</sup>	0.08±0.02 <sup>a</sup>	0.0007	0.0002	0.006	0.002



Dichlorovos	0.16 ±0.01 <sup>b</sup>	0.10±0.00 <sup>a</sup>	0.0004	0.0003	0.1	0.075
Endosulfan	0.11 ±0.01	ND	0.0003	ND	0.05	ND
Glyphosate	0.00±0.00	ND	0.00000	09	0.000009	ND
HCB	0.00±0.00	ND	0.00000	2	0.03	ND
Heptachlor	0.99 ±0.00 <sup>b</sup>	0.67±0.02 <sup>a</sup>	0.003	0.002	30	20
<b>P, p'-DDD</b>	0.29 ±0.00 <sup>b</sup>	0.17±0.01 <sup>a</sup>	0.0009	0.0005	0.09	0.05
t-nonachlor	0.00±0.00	ND	0.00000	2	0.003	0.01
DichloroBip hnyl	0.24±0.01 <sup>a</sup>	0.22±0.00 <sup>a</sup>	0.0007	0.0006	7	6
g-chlordane	0.15 ±0.02 <sup>b</sup>	0.11±0.00 <sup>a</sup>	0.0004	0.0003	0.8	0.6
Lindane	0.24 ±0.01 <sup>b</sup>	0.14±0.01 <sup>a</sup>	0.0007	0.0004	0.14	0.08

Values are Mean±SD of triplicate determinations. Columns having different superscripts are statically significant at  $p < 0.05$ .

**Table 3:** Residual Pesticides of Raw Brown Beans and Parboiled Brown Beans

Component	Raw Brown Beans	Parboiled Brown Beans	EDI of Raw Brown Beans (mg/kg)	EDI of Parboiled Brown Beans (mg/kg)	HRI of Raw Brown Beans	HRI of Parboiled Brown Beans
2_4-dichloro	0.2 ±0.00 <sup>b</sup>	0.12 ±0.01 <sup>a</sup>	0.0006	0.0003	0.004	0.001
Carbofuran	0.21 ±0.06 <sup>a</sup>	0.23 ± 0.05 <sup>a</sup>	0.0004	0.0008	0.4	0.8
DDVP	0.21 ±0.00 <sup>a</sup>	0.21 ±0.00 <sup>a</sup>	0.0006	0.0006	0.004	0.003
Dichlorvos	0.15 ±0.00 <sup>b</sup>	0.12 ±0.00 <sup>a</sup>	0.0004	0.0003	0.1	0.075
Endosulfan	0.11 ±0.02 <sup>a</sup>	0.11 ±0.02 <sup>a</sup>	ND	0.0003	ND	0.05
Glyphosate	0.13 ±0.01 <sup>b</sup>	0.10 ±0.00 <sup>a</sup>	0.0004	0.0003	0.0004	0.0003



Heptachlor	0.24 ±0.00 <sup>a</sup>	0.24 ±0.00 <sup>a</sup>	0.0007	0.0007	1	1
Profenos	0.20 ±0.00 <sup>b</sup>	0.10 ±0.00 <sup>a</sup>	0.0006	0.0003	0.02	0.01
t-nonachlor	0.13 ±0.00 <sup>a</sup>	0.13 ±0.00 <sup>a</sup>	0.0004	0.0004	0.6	0.4
DichhloroBiph nyl	0.39 ±0.01 <sup>a</sup>	0.36 ±0.00 <sup>a</sup>	0.001	0.001	10	10
g-chlordane	0.26 ±0.00 <sup>b</sup>	0.12 ±0.00 <sup>a</sup>	0.0007	0.0003	1.4	0.6
Biphenyl	0.15 ±0.01	ND	0.0004	0	0.01	0

Values are Mean±SD of triplicate determinations. Columns having different superscripts are statically significant at  $p < 0.05$ .

**Table 4:** Residual Pesticides of Raw Local Beans and Parboiled Local Beans

Component	Raw Local Beans	Parboiled Local Beans	EDI of Raw Local Beans (mg/kg)	EDI of Parboiled Local Beans (mg/kg)	HRI of Raw Local Beans	HRI of Parboiled Local Beans
2_4-dichloro	0.16±0.01 <sup>b</sup>	0.10±0.00 <sup>a</sup>	0.0005	0.0003	0.7	0.5
Carbofuran	0.43±0.03 <sup>b</sup>	0.21±0.01 <sup>a</sup>	0.001	0.0006	1	0.6
DDVP	0.15±0.00 <sup>b</sup>	0.15±0.00 <sup>a</sup>	0.0004	0.0004	0.6	0.45
Dichlorvos	0.18±0.02 <sup>a</sup>	0.12±0.00 <sup>a</sup>	0.0005	0.0003	0.125	0.075
Glyphosate	0.19±0.00 <sup>b</sup>	0.14±0.00 <sup>a</sup>	0.0005	0.0004	0.0005	0.0004
HCB	0.15±0.00 <sup>b</sup>	0.10±0.00 <sup>a</sup>	0.0004	0.0003		
<b>P, p'-DDD</b>	0.11±0.00 <sup>b</sup>	0.07±0.00 <sup>a</sup>	0.0003	0.0002	0.03	0.02
Profenofos	0.34±0.00 <sup>a</sup>	0.34±0.00 <sup>a</sup>	0.001	0.001	0.03	0.03
DichhloroBiphnyl	0.18±0.01 <sup>a</sup>	0.18±0.00 <sup>a</sup>	0.0005	0.0005	0.5	0.5
g-chlordane	0.10±0.00 <sup>a</sup>	0.10±0.00 <sup>a</sup>	0.0003	0.0003	0.6	0.6
Lindane	0.15±0.00 <sup>a</sup>	0.15±0.00 <sup>a</sup>	0.0004	0.0004	0.08	0.08
Isopropylamine	0.02±0.00 <sup>a</sup>	0.02±0.00 <sup>a</sup>	ND	0.00005	ND	0.0001

Values are Mean $\pm$ SD of triplicate determinations. Columns having different superscripts are statically significant at  $p < 0.05$ .

## DISCUSSION

The use of pesticides for the preservation of agricultural food crops is a common practice in Nigeria. Therefore studies on the concentration of pesticide residues absorbed by food crop are necessary as it provides an insight on the safety of the population consuming such food crop.

In the present study, 17 pesticide residues were detected in the samples of *Phaseolus vulgaris* (Table 1 - Table 4). The detected pesticide residues belong to the four main classes, which are Organochlorines, Organophosphates, Carbamates and Polychlorinated biphenyls.

The organochlorine pesticides detected include Aldrin, 2,4-dichloro, DDVP, Lindane, Endosulfan, p,p' DDT, t-nonachlor, g-chlordane and Heptachlor.

All the samples of beans had 2,4-dichloro and DDVP with concentrations ranging from  $0.42 \pm 0.01$  mg/kg to  $0.05 \pm 0.00$  mg/kg and  $0.24 \pm 0.02$  mg/kg to  $0.00 \pm 0.00$  mg/kg respectively. The value of 2,4-dichloro and DDVP were below the EU's MRL in only Iron beans samples. This implies that the pesticides 2,4-dichloro and DDVP were still used in preserving the samples. Studies indicate that consumption foodstuffs contaminated with 2,4-dichloro and DDVP pesticides could have health effects such as neurological damage, hypertension, cardiovascular diseases and skin disorders and cancer in human [8, 17].

Endosulfan and t-nonachlor were detected in all the samples except the local beans samples. These residues occurred at concentrations ranging from  $0.11 \pm 0.02$  mg/kg to  $0.18 \pm 0.01$  mg/kg and  $0.01 \pm 0.00$  mg/kg to  $0.32 \pm 0.00$  mg/kg respectively in the raw samples and 0 to  $0.09 \pm 0.01$  and 0 to  $0.22 \pm 0.02$  in the parboiled samples. These values were above the EU's MRL. The presence of endosulfan and t-nonachlor in these beans samples, which are banned organochlorine pesticides indicate their continued use in preserving foodstuff in Nigeria. Exposure to endosulfan and t-nonachlor has been linked to severe health conditions like liver lesions and reproductive disruptions, as well as presenting potential carcinogenic risks [17, 18].

Similarly, organochlorine pesticides were detected in beans from markets situated in Ile-Ife, Nigeria [18]. Also the research of Obida et al. [19] and Ogah et al. [7] detected organochlorines like dieldrin, aldrin and DDT at concentrations exceeding the EU's maximum residue level (MRL) in bean samples from Maiduguri and Lagos respectively. Furthermore, Iliya et al. [20] reported high concentrations of endosulfan in samples of beans in Jos, Nigeria.

Consumption of beans contaminated with DDT or any other organochlorines could cause liver lesions and may also disrupt reproductive functions as well as carcinogenic risks [21, 22].

Organophosphates detected in this study include Dichlorovos and glyphosate. They were detected in all the samples and occurred at concentrations ranging from  $0.15 \pm 0.00$  mg/kg to  $0.18 \pm 0.05$  mg/kg and  $0.00 \pm 0.00$  mg/kg to  $0.08 \pm 0.01$  mg/kg in the raw samples respectively and  $0.10 \pm 0.00$  mg/kg to  $0.13 \pm 0.01$  mg/kg and  $0$  to  $0.08 \pm 0.01$  mg/kg in parboiled samples respectively. Previous research by Iliya et al. [20] showed that organophosphates were in samples of beans sold in Jos, Nigeria. The results of their work, showed the residues to be below their respective EU's MRL, which does not agree with the results of this study.

Carbamates detected in this study, include Carbofuran. In this study, all the samples of beans had Carbofuran ranging from  $0.21 \pm 0.06$  mg/kg to  $0.50 \pm 0.02$  mg/kg in the raw samples and  $0.21 \pm 0.01$  mg/kg to  $0.25 \pm 0.01$  mg/kg in the parboiled samples. The residues were above EU's MRL value of 0.1 mg/kg. This result agrees with the work of Oshatunberu et al. [23] that found carbofuran as the only carbamate in some selected beans samples and carbofuran concentration in the beans samples of this study was also above the EU's MRL value. From this study, the low concentration of organophosphate and carbamate residues in the samples suggests good storage practices and safe pesticide application was observed to prevent insect infestation.

The polychlorinated biphenyls pesticides detected in this study include dichlorobiphenyl and biphenyl. Dichlorobiphenyl was detected in all samples of beans except in Iron beans and the concentrations ranged from  $0.18 \pm 0.01$  mg/kg to  $0.39 \pm 0.01$  mg/kg in the raw samples and  $0.18 \pm 0.00$  mg/kg to  $0.36 \pm 0.00$  mg/kg in the parboiled samples. These residues were above the EU's MRL in brown and patasco beans samples. The result of this study is in tandem with

the work of Usman et al. [24] that reported polychlorinated biphenyls to be above the EU's MRL in some beans samples in Gombe State, Nigeria. Previous studies linked PCB species to endocrine disruption and toxic health effects, cancerous and noncancerous in animals [25].

The estimated health risk of a sample is the risk associated with consuming the sample. From the work of Akoto et al. [16], when HRI exceeds one, consumption of the food sample could pose significant health risks.

Aldrin and Heptaclor in raw iron beans have a health risk index (HRI) of 4 and 7 respectively, but were not detected in the parboiled iron beans sample. This implies that parboiling and decanting the water, removed aldrin from the sample. Other pesticide residues had HRI below 1.

In raw Patisco beans, DichloroBiphenyl and Heptaclor have HRI of 7 and 30 respectively, but after parboiling the HRI reduced to 6 and 20 respectively. This means that parboiling and decanting the water, reduced these pesticides residue but not to a level less than 1. The residues of dichloroBiphenyl and heptaclor investigated in this study could pose potential toxicity risk to liver and kidney for adult consumers of beans. This is in line with the study of IRIS [22].

In raw Brown beans, DichloroBiphenyl and Heptaclor have a HRI of 10 and 1 respectively, but after parboiling, the HRI reduced to 8 and 0.8 respectively. This implies that parboiling and decanting the water, reduced these pesticide residues although the HRI value of DichloroBiphenyl was still greater than 1. Consuming this sample could pose significant health risk on the consumers which include liver lesions IRIS [22].

In raw Local beans, Carbofuran has a HRI value of 1, but after parboiling it reduced while other residues have HRI below 1. This means that parboiling and decanting the water, affected the carbofuran concentration. It is observed that the HRI value of the pesticide residues in this sample after parboiling was below 1. Therefore consuming this sample poses no significant health risk.

## **CONCLUSION**

The results of the concentration of pesticide residues in the four varieties of beans consumed in Port Harcourt, Rivers State, Nigeria showed that the beans

samples monitored contain various concentrations of the pesticide residues. Many already banned organochlorine pesticides were found in most of the samples which could be as a result of direct application of these pesticides to the samples or the soil on which they grew had been contaminated by these pesticides. The detection of pesticides in beans raises health concerns, as accumulation over time can lead to medical issues, highlighting the importance of safe and proper pesticide use and continued efforts by the government to address the problem.

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