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Research Article
Risk Assessment of Organochlorine Pesticide Residue in *Phaseolus vulgaris* Purchased in Igbara-oke, Ondo State, Nigeria

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Abstract

Background and Objective: Preservation of agricultural products remains a hallmark of all farmers as a result, both pesticides and herbicides are being applied during planting and after harvesting with the sole aim of maximizing profits. Research had shown the various degree of toxicity of organochlorine pesticides residues, the objective of the research was to identify the organochlorine pesticide residues, analyze their risk assessment vis-a-vis, Hazard Index (HI), Estimate Dietary Intake (EDI), Target Hazard Quotient (THQ) and compare the results with Acceptable Dietary Intake (ADI), Reference dose standard (Rfd) and Maximum Residue Limit (MRL) and characterized the identified organochlorine pesticides residue for their toxicological properties. **Materials and Methods:** *Phaseolus vulgaris* were purchased in a local market in Igbara -Oke, Ondo state Nigeria, the sample was powdered using a grinder (Sumeet CM/L 2128945) and solid phase extraction techniques were employed, the extract was subjected to fractionation into two fractions of aliphatic hydrocarbons and the pesticides. The pesticide extract was subjected to characterization using gas chromatography-mass spectrophotometer. **Results:** Total 4 organochlorine pesticide residues were identified and the contaminant rates (mg kg^{-1}) were less than 1. Furthermore, EDI values were lower than the ADI, MRL, also, the THQ values were less than 1, an indication that the *Phaseolus vulgaris* was safe for consumption. **Conclusion:** The research had shown no toxicity of the *Phaseolus vulgaris* purchased from the local market and it shows compliance by the local farmers on the application of pesticides to the food crop by obeying the recommended dose.

Key words: Organochlorine pesticide residue, contaminant rate, risk assessment, reference dose, maximum residue limit, acceptable dietary intake, target hazard quotient, estimated dietary intake

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Preservation of agricultural products remains the major focus for all the farmers both subsistence and commercial farmers as a result, both pesticides and herbicides are being applied during planting and after harvesting with the sole aim of maximizing profits. The effects of applying these chemicals remain a major challenge to the concerned authority because of the various diseases associated with the consumption of the food crops. Moreover, a major challenge identified by the agricultural experts is how to quantify the appropriate dosage for applications. However, pesticides can cause environmental pollutions[1], also it is well documented that chronic exposure to pesticides increases the risk of damage organs, causes mutagenic and carcinogenic transformation and endocrine[2-4]. Organochlorine (OC) pesticides belong to one of the classifications of pesticides and they are chlorinated hydrocarbon derivatives that have wider applications both industrially and agriculturally. Research documents have shown the various degree of toxicity of OC pesticides such as high, slow degradation and bioaccumulation[5], organochlorinated residue effects include disruption of deoxyribonucleic acid in unborn babies, damaging the nerve and brains[6,7] and these have compelled the various government to ban the use of the pesticides, however, they still find their ways to the commercial markets. The World Health Organization had classified toxicity of pesticides into class I (highly hazardous), class II (slightly toxic) and it has been reported to be poisonous, hazardous and toxic to humans[6-9].

Phaseolus vulgaris belongs to the family of leguminous plants and can be found in many African countries, Asia, the Middle East and South America. They are highly nutritious food and the nutritional qualities have been extensively discussed according to Geil and Anderson[10].

The study was aimed at identifying organochlorine pesticide residue in *Phaseolus vulgaris* and analyse the health risks associated with the identified pesticide residue.

MATERIALS AND METHODS

Study area: The research was conducted in the chemistry laboratory, Afe Babalola University, Ado Ekiti, Nigeria between the periods of 15th June-16th July, 2020.

Sample source: The *Phaseolus vulgaris* were purchased at a local market in Igbara-oke, Ifedore local Government area, Ondo state on the 15th of May, 2020.

Sample preparation: Dried *Phaseolus vulgaris* were powdered using a laboratory-scale grinder (Sumeet CM/L 2128945) and sifted through 300 µm sieve to obtain the *Phaseolus vulgaris* flour. The flour samples were sealed and packed in airtight containers for further analysis.

Sample preparation and analysis procedure: *Phaseolus vulgaris* were prepared following a procedure described by Wang *et al.*[11] with little modification. A total of 10 g of the sample was measured in using Mettler Toledo analytical weighing into 60 mL volume beaker and 40 mL dichloromethane (DCM) was added. The samples were sonicated for 30 min in a (360 W Grant ultrasonic bath) and filtered using Whatman 1 filter paper (Sigma Aldrich, USA). The procedure was repeated three times for each of the samples. The extract was later subjected to fractionation using a glass column packed with silica-alumina in the ratio of 2:1 into two fractions of aliphatic hydrocarbons and the pesticides, using 7:3 volumes of hexane/dichloromethane. The resulting extracts were concentrated using a rotary evaporator (model rotavapor R-100, Buchi, Switzerland) at 30°C to 1 mL. The concentrated extracts were transferred into 2 mL volume clean chromatographic vials (Vials, screw top with solid green melamine cap, preassembled brand, Supelco, USA) and reduced to 0.5 mL under a gentle stream of nitrogen and subjected to gas chromatographic determination.

Identification of residue using Gc-Ms

Gas chromatographic detection: The gas chromatography was coupled to mass spectrometry using the Agilent 7890 model fitted with HP-5MS column of fused silica (30×0.25×0.25 mid film thickness) and the carrier gas used was helium (99.99%). The injection was splitless and the split time was 1 min after injection using auto-sampler and the injection temperature was 250°C the pesticides. The temperature programme was from 100 (held for 1 min)-200°C at 100°C min^{-1} (held for 2 min) and to 280°C at 10°C min^{-1} (held for 2 min). The pesticide residues were identified by comparing the retention time of sample peaks with that of the standards.

Risk assessment

- **Health Risk Index (HRI):**

$$HRI = \frac{EDI}{ADI} \quad (1)$$

This was done based on the levels of the OCP residues found in the food samples. Estimated Daily Intakes (EDI) were

determined and compared with the established Acceptable Daily Intake (ADI)[12-14]. Estimated daily intake was found by multiplying the residual pesticide concentration (mg kg^{-1}) by the food consumption rate (0.027 kg/day) and dividing by body weight[14]. Calculations were performed for adults. Adults were considered to have an average weight of 60 kg[15,16].

- **Estimation of dietary intake:**

$$EDI = \frac{F \times C_r}{\text{Mean body weight}} \quad (2)$$

Where:

F = Food consumption data

Cr = Is the concentration of the residue in the food sample

- **Target Hazard Quotient (THQ) estimation:** THQ was calculated with the USEPA standard method of estimating the risk of non-carcinogenic effects and Body Weight (BW) for adult 60 kg was used. The THQ was determined based on the method by Chien *et al.*[17]. Where THQ is the target hazard quotient, Fr is exposure frequency (365 days per year), Dex is the exposure consumption rate of 0.027 kg/person/day). The food consumption rates were based on the World Health Organisation's Global Environmental Monitoring System. C is the pesticide concentration in food (mg kg^{-1}), Bw is the average body weight 70 kg for adults, Rfd is the reference dose which is considered to be safe levels of exposure over the lifetime[17,18] and Ta is the averaging exposure time for non-carcinogens (365 days per year x number of exposure years[17,18], assuming 70 years in this study[19]

The following equation describes it:

$$THQ = \frac{Fr. \times Dex \times Ir \times C}{Rfd \times Bw \times Ta} \times 10^{-3} \quad (3)$$

RESULTS AND DISCUSSION

From the result of Fig. 1, the chromatogram showed different peaks of organochlorine pesticide residues with varying degrees of abundance and various retention times. Twenty peaks of OCP were identified and Table 1 showed the identified compounds at a concentration of 10 ppm as standard, compound p,p'-DDE was identified at retention time 18.622 min and an amount 0.0200078 ppm. Another OCP was elucidated at various retention times ranging from 10.028-23.405 min. Furthermore, Fig. 2 showed the chromatogram of OCP at 5 ppm standard and twenty OCP residues were also identified with various retention times ranging from 10.091-23.331 min as shown in Table 2. The Fig. 3 showed the chromatogram of the standard at 2.55 ppm with various

Table 1: List of organochlorine compounds identified in chromatogram at 10 parts per million

Compound names	RT (min)	Area	Amount	Units	Q-value
.delta.-Pentachlorocyclohexene	10.028	353817	10	ppm	96
.alpha.-Lindane	11.738	129752	10	ppm	98
.delta.-Lindane	14.29	1142875	10	ppm	94
Endosulfan ether	14.519	276180	10	ppm	97
Heptachlor	15.229	2515872	10	ppm	99
Aldrin	16.047	1901251	10	ppm	99
Isodrin	16.654	86294	10	ppm	95
Heptachlor epoxide	17.128	2447491	10	ppm	100
trans-Chlordane	17.769	3529360	10	ppm	100
DDMU	18.055	357696	10	ppm	95
trans-Nonachlor	18.422	2710779	10	ppm	100
p,p'-DDE	18.622	6311	0.0200078	ppm	60
Dieldrin	18.879	1473560	10	ppm	99
p,p'-DDE	19.057	3154273	0	ppm	97
Endrin	19.48	598174	10	ppm	98
Endosulfan	19.892	745763	10	ppm	98
Mitotane	20.482	4536	10	ppm	11
Endosulfan sulfate	21.191	579813	10	ppm	98
Endrin ketone	22.564	777102	10	ppm	100
Methoxychlor	23.405	5700249	10	ppm	99

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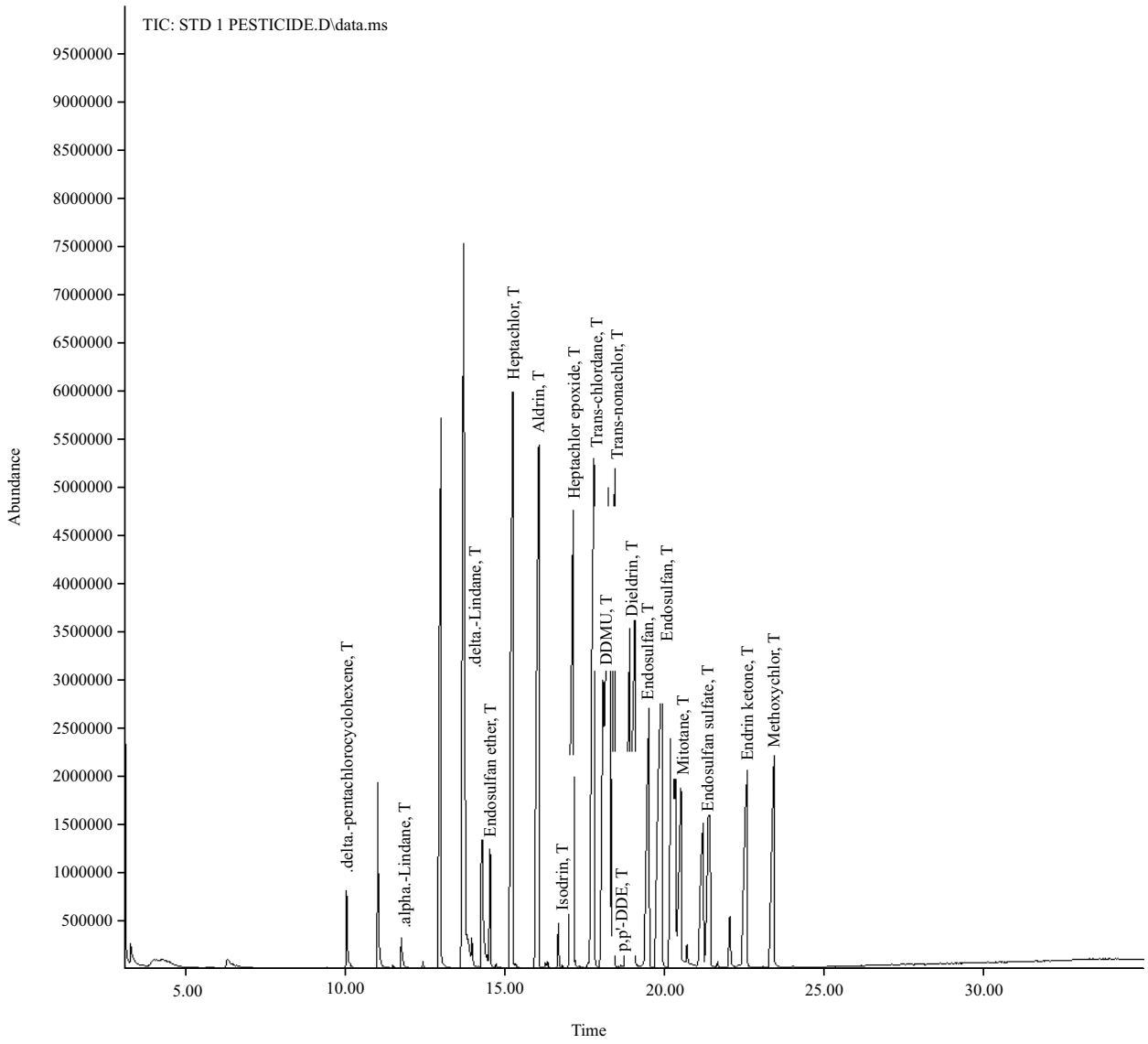

Fig. 1: Chromatogram of compounds at 10 parts per million concentration standards

abundance, seventeen peaks were identified and Table 3 showed the identified OCP compounds with various Q values and retention times. The Table 4 showed the identification and quantification of the OCP in the sample analyzed. Four OCP compounds were identified and these were lindane, having amount 0.005337 ppm, endosulfan, .004997 ppm, dieldrin 0.000977 ppm and mitotane 0.352734 ppm.

The Table 5 showed the estimated dietary intake, Hazard index of the identified OCP in the sample, reference dose (Rfd) and target hazard quotient (THQ) and it is worthy to note that the HI were all lower than 1 (HI<1.0) and this indicate that the lifetime consumption of the beans containing the measured level of OCP residue could not pose any health risk[12].

The data of Table 6 showed the toxicological characterization of the OCP residue in the beans sample being analysed, it is significant to know that the identified OCP compounds exhibited various toxicological properties such as endosulfan with high mutagenic, irritability and reproductive effect, all the compounds showed the negative sign to drug-likeness, an indication that they are not drug candidates for human consumption.

Olutona[25] had shown that the mean concentration of dieldrin, endosulfan and endrin residues in beans in Bodija and Oje in Ibadan, Oyo state Nigeria to be (Dieldrin 0.99±0.01, Endrin 1.00±0.00 and Endosulfan I 5.28±1.4 mg kg^{-1}), respectively. Moreover, Olutona[24] had shown the list of commonly used pesticides in Nigeria to include

Table 2: List of organochlorine pesticides identified in chromatogram at 5 parts per million

Compound names	RT (min)	Area	Amount	Units	Q-value
.delta.-Pentachlorocyclohexene	10.091	184096	5	ppm	80
.alpha.-Lindane	12.952	1601096	5	ppm	99
.delta.-Lindane	14.256	632480	5	ppm	97
Endosulfan ether	14.485	211366	5	ppm	97
Heptachlor	15.189	1638025	5	ppm	99
Aldrin	16.001	1250909	5	ppm	99
Isodrin	16.654	63491	5	ppm	98
Heptachlor epoxide	17.077	1636474	5	ppm	99
trans-Chlordane	17.729	2426278	5	ppm	100
.alpha.-Endosulfan	18.067	520362	5	ppm	90
cis-Chlordane	18.193	1795094	5	ppm	99
Dieldrin	18.799	1218772	5	ppm	99
p,p'-DDE	18.977	2307882	5	ppm	99
Endrin	19.395	471249	5	ppm	98
Endosulfan	19.795	543956	5	ppm	99
Mitotane	20.241	2865631	5	ppm	85
Endosulfan sulfate	21.088	395546	5	ppm	97
p,p'-DDT	21.323	2031779	5	ppm	99
Endrin ketone	22.473	513615	5	ppm	99
Methoxychlor	23.331	3635796	5	ppm	99

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Table 3: List of organochlorine pesticide compounds at 2.55 ppm

Compound names	RT (min)	Area	Amount	Units	Q-value
.delta.-Pentachlorocyclohexene	10.159	100359	2.5	ppm	65
.alpha.-Lindane	12.923	839323	2.5	ppm	98
.delta.-Lindane	14.279	285072	2.5	ppm	94
Endosulfan ether	14.474	117269	2.5	ppm	97
Heptachlor	15.155	854440	2.5	ppm	98
Aldrin	15.967	682374	2.5	ppm	98
Heptachlor epoxide	17.043	855120	2.5	ppm	98
Trans-Chlordane	17.689	1316219	2.5	ppm	99
.alpha.-Endosulfan	18.038	347299	2.5	ppm	99
Chlordane	18.13	1021528	2.5	ppm	99
Dieldrin	18.742	696366	2.5	ppm	99
p,p'-DDE	18.908	1277524	2.5	ppm	99
Endrin	19.349	257557	2.5	ppm	97
Endosulfan	19.732	297686	2.5	ppm	97
Mitotane	20.178	1254666	2.5	ppm	98
Endosulfan sulfate	21.037	216606	2.5	ppm	98
Endrin ketone	22.416	302161	2.5	ppm	97

Data File Name: C:\msdchem\1\methods\OldMethods\PESTICIDE 6217.M\STD 3 PESTICIDE.D, Acquired Date: 26 Jun 2019 11:34, Method Name: C:\msdchem\1\methods\OldMethods\new scan.M, Sample name: STD 3 PESTICIDE, Footnote: RT: Retention time

organochlorine pesticides such as DDT, heptachlor and aldrin. It is imperative to analyze the risk associated with dietary exposure to organochlorine pesticides. The contaminant rate of the organochlorine pesticide residues was all lower than the Maximum residue limit allowed for pulse foods, also the estimated dietary intakes were lower than the Acceptable Dietary Intake (ADI) as established by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) food standard program Joint Meeting on Pesticide Residue and the Australian government to estimate the human health risk from consumption of foodstuffs[25,26]. The implication of the lower EDI and HI show that the consumption of the beans possesses no health risk to consumers and it is a good practice on the part of farmers who are diligently following the directive of the federal ministry of Agriculture in Nigeria about the consequences of overdose of pesticides in the preservation of agricultural products. Wu[27] had shown that the consequences of the irresponsible application of pesticides may lead to pesticide residues which may lead to environmental and agricultural problems and Akomea[28] and Elgueta[29] had confirmed that exceeding the maximum residue limit pose a possible health risk to consumers. The implication of this study had shown that the *Phaseolus vulgaris* purchased in Igbara-Oke, Ondo state,

Table 4: Quantification of organochlorine pesticides in the *Phaseolus vulgaris*

Compound names	RT (min)	Area	Amount	Units	Q-value
alpha.-Lindane	0	0	0	PPM	0
delta.-Lindane	14.268	610	0.005337	PPM	25
Endosulfan ether	14.502	138	0.004997	PPM	38
Heptachlor	0	0	0	PPM	0
Aldrin	0	0	0	PPM	0
Isodrin	0	0	0	PPM	0
Heptachlor epoxide	0	0	0	PPM	0
trans-Chlordane	0	0	0	PPM	0
DDMU	0	0	0	PPM	0
trans-Chlordane	0	0	0	PPM	0
trans-Nonachlor	0	0	0	PPM	0
p,p'-DDE	0	0	0	PPM	0
Dieldrin	18.879	144	0.000977	PPM	1
p,p'-DDE	0	0	0	PPM	0
Endrin	0	0	0	PPM	0
Endosulfan	0	0	0	PPM	0
Mitotane	20.247	160	0.352734	PPM	28
Endosulfan sulfate	0	0	0	PPM	0
Endrin ketone	0	0	0	PPM	0
Methoxychlor	0	0	0	PPM	0

Footnote: The highlighted compounds are the identified organochlorine residue in the *Phaseolus vulgaris*, RT: retention time, Data File Name: C:\msdchem\1\methods\OldMethods\PESTICIDE 6217.M\sample, Acquired Date: 3 Jul 2020 10:02, Method Name: C:\MSDCHEM\1\METHODS\OLDMETHODS\OCP TEST2.M, Sample name: Sample c

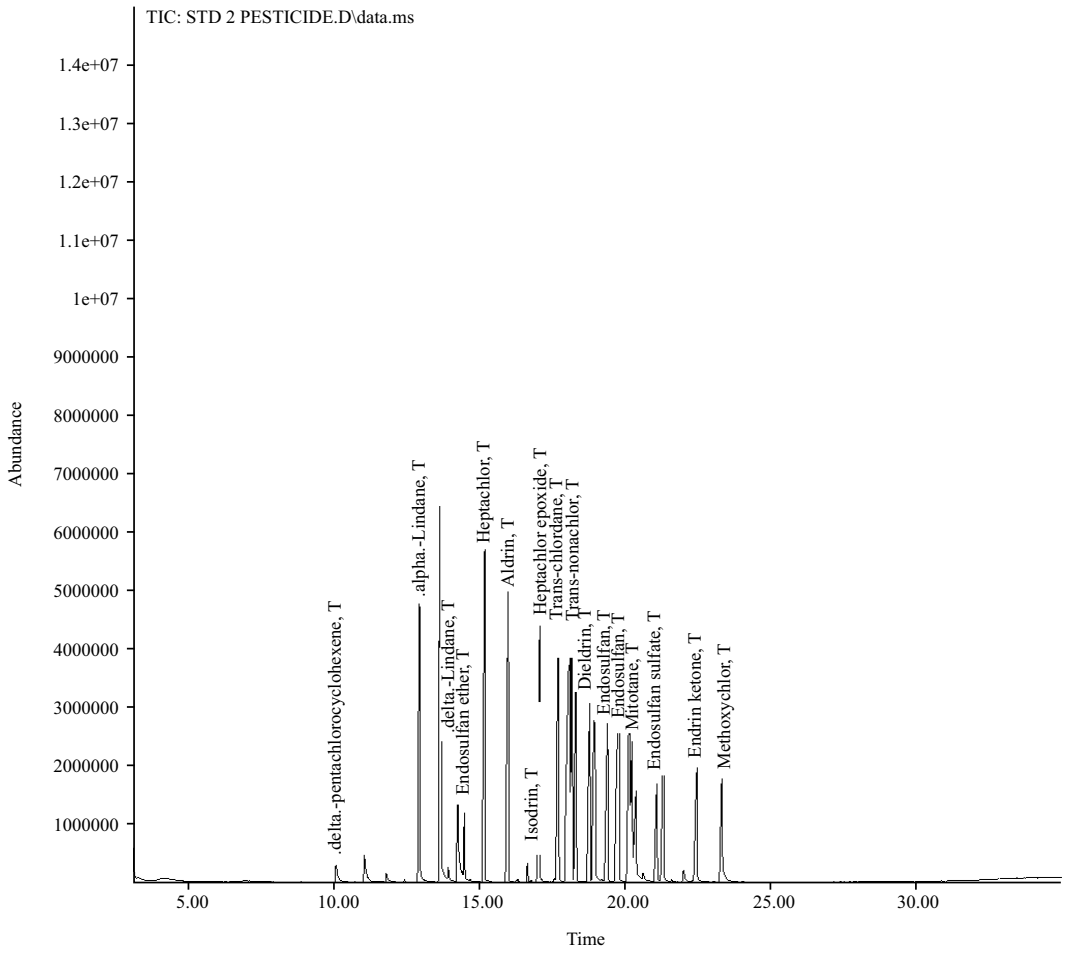

Fig. 2: Chromatogram of the standard at 5 parts per million concentrations standard

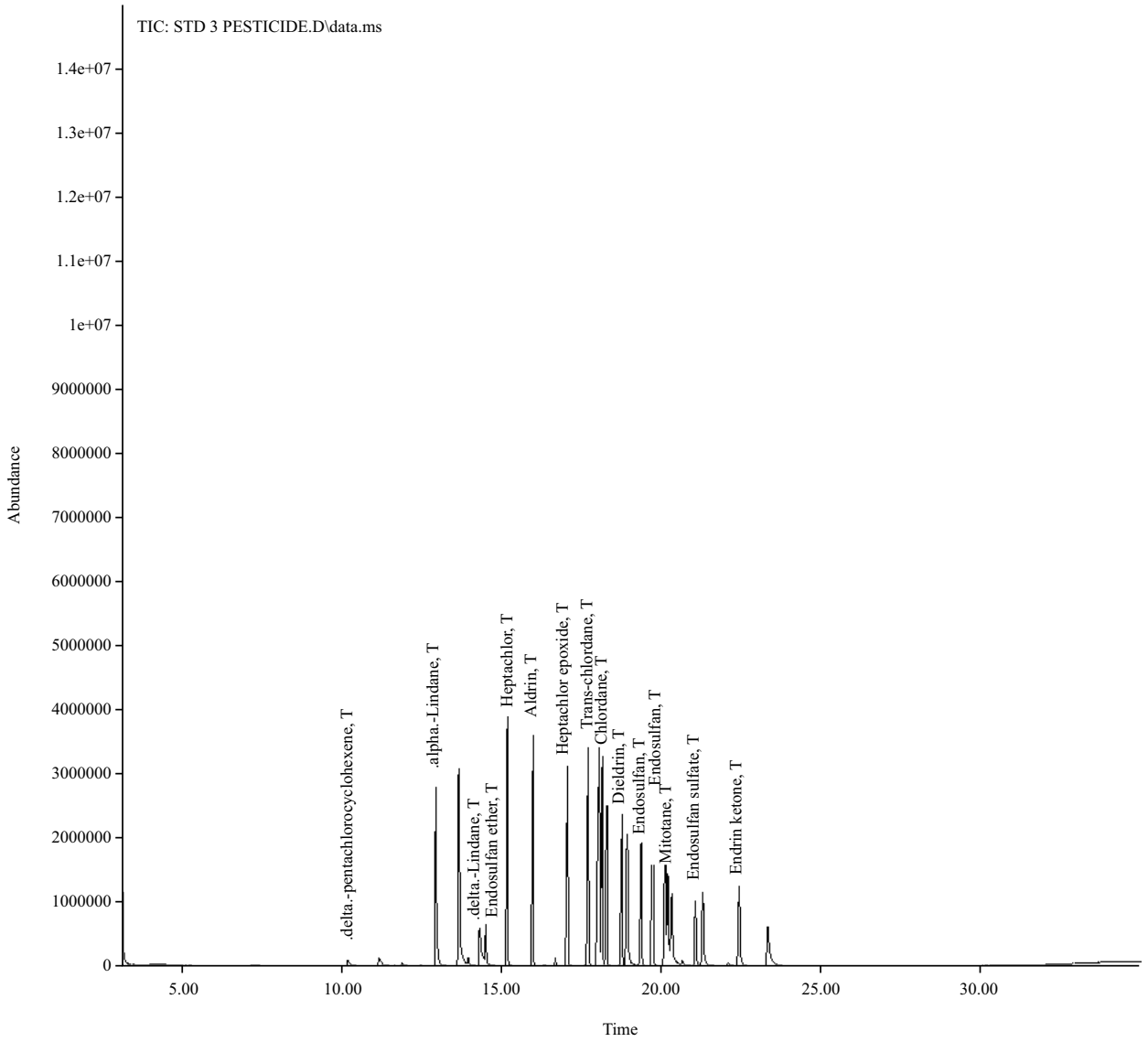

Fig. 3: Chromatogram of 2.55 parts per million of concentration standard

Table 5: Contaminant rates of the OCP in the *Phaseolus vulgaris*, their Maximum Residue Limit (MRL), Acceptable Dietary Intake (ADI) mg kg^{-1}, Hazard Index (HI), RFD, THQ and Hazard ratio

Compound name	Amount (ppm)	Contaminant rate (mg kg^{-1})	MRL (mg kg^{-1}) day[12]	ADI (mg kg^{-1}) day/b.wt.[20]	EDI (mg kg^{-1}) day	HI	Rfd (mg kg^{-1}) day[21]	THQ	HRI[22]
delta.-Lindane	0.005337	0.005337	1.00	0.003	2.4×10^{-6}	2.4×10^{-4}	3×10^{-4}	4.5×10^{-7}	No
Endosulfan ether	0.004997	0.004997	0.5	0.0075	2.25×10^{-6}	2.25×10^{-4}	6×10^{-3}	4.50×10^{-5}	No
Dieldrin	0.000977	0.000977	0.05	0.001	4.4×10^{-7}	4.4×10^{-4}	5×10^{-5}	8.79×10^{-6}	No
Mitotane	0.352734	0.352734	0.05	0.01	1.59×10^{-7}	1.59×10^{-2}	5×10^{-4}	3.17×10^{-4}	No

Footnote: References[20,21,22], MRL: Maximum Residue Limit; ADI: Acceptable Dietary Intake; EDI: Estimated Dietary Intake; HI: Hazard Index; Rfd: Reference dose standard; THQ: target hazard quotient; HRI: Health Risk Index, b.wt: Body weight

Table 6: Toxicological characterization of identified organochlorine pesticide residue showing mutagenic, reproductive effect and drug-likeness

Compounds	Drug likeness	Mutagenic	Tumorigenic	Irritability	Reproductive effect
Endosulfan	-1.0005	High	Low	High	High
delta.-Lindane	-0.92105	Low	Low	Low	Low
Dieldrin	-0.044038	High	Low	High	High
Mitotane	-1.3066	High	High	High	High

Footnote: Thomas *et al.*[23]

Nigeria had no associated health risk index has shown above, also the contaminant rate and estimated dietary intakes of identified organochlorine pesticide residue were lower when compared to MRL and ADI.

The application of organochlorine pesticide in the preservation of *Phaseolus vulgaris* as well complied with and it is recommended that farmers in Igbara-oke should continue with the recommended dose for the preservation of *Phaseolus vulgaris* in their farms. However, Agricultural experts should keep on monitoring the application of dosage required as this is a major task in order to protect the consumer's health.

CONCLUSION

The research had shown the level of toxicity of organochlorine pesticide residues available in *Phaseolus vulgaris* bought from Igbara-oke, Ondo state, Nigeria, the study had shown the compliance with the application of organochlorine pesticides as recommended by the Ministry of Agriculture, Ondo state, Nigeria as all the analysed parameters such as contaminant rates, estimated dietary intake, target quotient index and hazard index were all lower than the standard values such as Maximum Residue Limit, Acceptable Dietary Limit and Reference Dose Standard, an indicating tool that the Phaseolus vulgaris was safe for human consumption.

SIGNIFICANCE STATEMENT

This study discovered the compliance with the recommended amount of pesticides applications to farm crops by the lowering of the Maximum Residue Limit and Estimated Dietary Intake, this study will help the researchers to uncover the critical areas of risk assessment associated with the use of organochlorine pesticide applications and toxicological characterizations that many researchers were not able to explore. Thus, a new theory on monitoring and evaluation of organochlorine pesticide residues may be arrived at.

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