



Contamination levels, occurrence pattern and associated risk of organophosphate pesticide residues in spices and herbs sold in southern Nigeria

Duke Okoro^a, Oghenenyerhovwo E. Ididama^a, Godswill O. Tesi^{a,*}, Jude C. Ossai^b

^aDepartment of Chemistry, Federal University of Petroleum Resources, Effurun, Nigeria

^bAdvanced Research Centre, Delta State University, Abraka, Nigeria

Abstract

This study investigated the contamination levels, occurrence pattern and associated health risks of organophosphate pesticide residues in spices and herbs sold in southern Nigeria. Fifty-two samples of commonly consumed spices and herbs were purchased from multiple locations in southern Nigeria and subjected to extraction using a 1:1 mixture of dichloromethane and n-hexane. Thereafter, the extracts were analyzed for 14 OPPs on a gas chromatograph-mass spectrometer. The contamination levels of the Σ 14 OPPs in the spices and herbs ranged between 4.96 and 795 ng/g. The average OPP concentrations in the spices and herbs were in the order of diazinone > chlorpyrifos methyl > pyrazophos > isazophos > quinalphos > phosalone > pirimiphos methyl > chlorpyrifos > azinphos ethyl > pirimiphos ethyl > EPN > fenitrothion > pyraclofos > triphenyl phosphate. The mean concentrations of eight OPPs in the spices and herbs were above their maximum residue limits (MRLs) established by the Food and Agriculture Organization/World Health Organization. The risk estimated using the hazard index indicated the absence of non-carcinogenic risk from the intake of these spices and herbs, except for seven samples in the case of children's intake. Although the findings suggest minimal health risks from OPPs through the intake of these spices and herbs, we recommend that there is a need for continuous monitoring of spices and herbs sold generally in Nigerian markets for pesticide contamination and the appropriate regulators should establish MRLs for OPPs in foodstuffs in Nigeria and ensure strict compliance with the MRLs of contaminants in food and drugs.

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1. Introduction

Spices and herbs have made a remarkable history in nations as they have a wide variety of uses. They played significant roles in the civilization of countries like India, Egypt, China etc.

However, most spices and herbs originated from tropical Asian [1]. The use of spices and herbs was initially for sacrifices to gods and in burials. They impact aroma, colour and taste to food preparations and sometimes mask undesirable odour in food [2]. Spices are obtained from parts of plants such as roots, stems, leaves, rhizomes, bark, flowers, fruits and seeds, while herbs are leafy spices [3]. They are also used as medicines, preservatives, perfumes and serving a nutritional purpose [4].

*Corresponding author Tel. No: +234-703-531-2293.

Email address: godswillinfodesk@yahoo.com (Godswill O. Tesi)

Due to the increasing demand for spices and herbs by consumers, the global market for spices and herbs is continuously growing and has created numerous opportunities for spices and herbs producers and exporters. However, in order to meet the increasing demand, production of spices and herbs dependency on insecticides and pesticides is apparent. The improper use of chemical input, mostly organophosphate pesticides (OPPs) over the years, has led to a severe environmental threat [5].

Organophosphates are a group of chemical compounds that are formed by the esterification of phosphoric acid and alcohol and have applications in pesticides, insecticides, herbicides, and as nerve agents in chemical warfare [6, 7]. Globally, OPPs are the most widely used pesticides, accounting for 45% of the total world market due to their broad-spectrum insecticidal activity, high efficiency, chemical stability and low cost [8, 9]. Organochlorine pesticides were substituted with OPPs to improve crop yield and to protect crops from attacks of pests [10]. OPPs have contaminated several ecosystems worldwide due to their indiscriminate and excessive usage. Exposure to OPPs can be through food, inhalation, skin or direct ingestion and it can lead to different health risks such as nausea, vomiting, malaise, weakness, memory loss, speech impairment, lack of coordination, and peripheral polyneuropathy [7, 11, 13, 14].

Maximum residue limits (MRLs) for different pesticides have been set by different regulatory bodies; however, in Nigeria, where food safety regulations are frequently not properly monitored and enforced, chronic dietary exposure to OPPs through spices may present long-term health concerns to consumers [15]. The potential of pesticide contamination is further increased by Nigeria's informal spice trade, which is marked by unregulated distribution, packaging, and processing [16]. Research on OPP residues has been conducted on a number of food items in Nigeria such as vegetables and fruits [14, 17], canned fish [18], vegetable oils [19], herbal mixtures [20], honey [21], banana [22], grains - beans, maize, wheat and rice [23] as well as cereals and pulses [17]. However, there are no available studies of OPPs in spices and herbs, which are used extensively in Nigerian cuisine, to the best of our knowledge. This is an important gap in knowledge that needs to be filled. The purpose of this study, therefore, is to assess the contamination levels, occurrence pattern and associated risks of OPPs in spices and herbs sold and consumed in southern Nigeria with a view to achieving Sustainable Development Goal 3, which is ensuring healthy lives and well-being of all.

2. Materials and method

2.1. Sample collection

A total of 52 different spices and herbs were collected, comprising of local and imported branded spices and herbs, as well as local dried and fresh unbranded ones. The spices and herbs were purchased from different markets in southern, Nigeria. The samples were carefully chosen to reflect popular spices and herbs consumed by different income classes and were also selected based on their availability during the study period. The information regarding the spices is shown in Table S1. All collected samples were labelled, kept in aluminium foil and taken

to the laboratory where they were stored in the refrigerator for two days before analysis.

2.2. Reagents

All reagents used were HPLC grade. Alumina, silica gel and anhydrous sodium sulphate were acquired from BDH (Poole, UK). Hexane and dichloromethane (DCM) were acquired from Aldrich (USA). Accu Standard, USA, provided a mixed standard of fourteen (14) OPPs, including Pyrachlofos, azinphos ethyl, pyrazophos, phosalone, EPN, triphenyl phosphate, chlorpyrifos, quinalphos, pirimiphos ethyl, fenitrothion, pirimiphos methyl, chlorpyrifos methyl, isazophos and diazinone, while Merck (Darmstadt, Germany) provided anhydrous sodium sulphate.

2.3. Extraction of OPPs from spices and herbs

The OPPs were extracted from the spices following the USEPA-3550C method of extraction. A mass of 20 g of the spices was mixed with 20 g of anhydrous Na_2SO_4 in an extraction thimble. Twenty-five millilitres each of n-hexane and DCM were added to the mixture and sonicated at 30°C for 30 minutes. The organic extract was removed, and the extraction was done two times more with fresh n-hexane and DCM. The pooled extract was reduced to about 1 mL by passing it through a stream of N_2 gas. The concentrated extract was purified in a multi-layer column containing Na_2SO_4 , alumina and silica gel packed from top to bottom. OPPs were eluted from the column with 40 mL n-hexane and dichloromethane (1:1 v/v), and the eluate was concentrated to approximately 2 mL with a rotary evaporator. Thereafter, the extract was kept in a 2 mL vial ready for analysis.

2.4. Chromatographic analysis

The detection and quantification of OPPs in the extract were performed with a gas chromatograph equipped with mass spectrometry (Agilent, Palo Alto, CA, USA). An HP-5 column with 5% phenyl methyl siloxane (30 m \times 0.25 μm \times 0.25 mm) was employed for compound separation. Helium, at a constant flow rate of 1.8 mL/min, served as the carrier gas. Column temperature programme was initiated at 150°C and held for 1.2 mins before being ramped up at a rate of 15°C/min to 200°C, where it was maintained for 2 minutes. It was then further taken to 300°C at 15°C/min and held for 4 minutes. The temperature of 280°C, 150°C, 230°C, and 250, 230, 150 and 280°C were set for the injection port, ion source, quadruple and transfer lines, respectively. A splitless injection mode was used to introduce a 1 μL sample into the system. The mass spectrometer worked at 918 EmVolts, and data acquisition was performed in ion monitoring mode. By comparing OPPs retention time with that of genuine standards, OPPs were identified.

2.5. Quality control

The European Union [23] guidelines for quality control were followed in this study. Blank determination was carried out in addition to spiked recovery study. The spices and herbs were spiked with 5, 10 and 20 ng g^{-1} . OPP concentrations in

Table 1. Summary statistics of OPPs concentrations (ng/g) in the studied spices and herbs

OPPs	Mean	SD	Median	Min	Max	% >MRL
Diazinone	42.0	48.2	25.4	0.38	219	63
Isazophos	17.8	27.4	8.72	0.01	138	48
Chlorpyrifos-methyl	29.1	58.1	2.45	0.02	262	37
Pirimiphos-methyl	11.6	21.4	3.14	0.05	122	29
Fenitrothion	7.08	18.3	0.75	0.06	112	15
Pirimiphos ethyl	8.53	12.5	1.59	0.05	47.9	29
Quinalphos	13.1	26.3	3.25	0.09	129	29
Chlorpyrifos	10.4	27.1	1.01	0.02	146	19
Triphenyl phosphate	3.99	12.0	0.53	0.06	76.3	10
EPN	7.86	11.6	4.33	0.10	46.7	17
Phosalone	12.8	17.6	9.76	0.42	121	46
Pyrazophos	18.4	38.8	2.20	0.01	179	23
Azinphos-ethyl	9.19	13.5	4.08	0.04	69.3	23
Pyraclofos	4.57	8.26	2.67	0.11	58.0	12
Σ14 OPPs	196	150	167	4.96	795	98

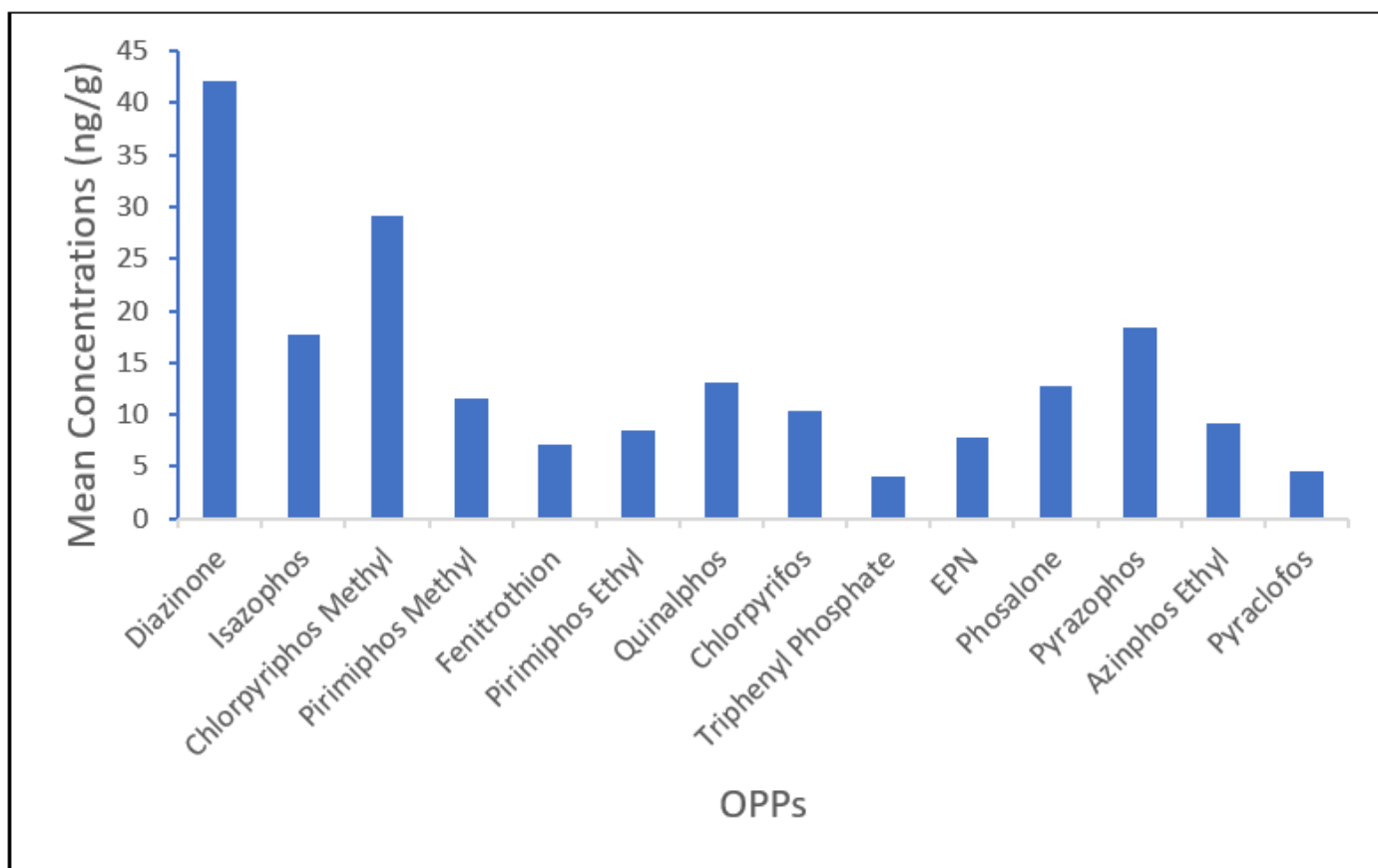


Figure 1. Mean concentrations of OPPs in the spices and herbs.

blank samples were below their limit of quantification. The per cent of OPPs recovered ranged between 88.4 % and 105 %. The limit of detection (LOD) was gotten from the OPP concentration that gave a signal-to-noise ratio = 3, while the limit of quantification (LOQ) was taken as $3 \times \text{LOD}$. The LOD of OPPs varied from 0.003-0.006 ng/g while the LOQ varied from 0.01-0.02 ng/g.

2.6. Statistical analysis

The analysis of variance (ANOVA) was utilized to ascertain the level of variation in OPPs concentrations in the spices/herbs using a 95 % confidence limit. The statistical test was done with version 23 of SPSS software.

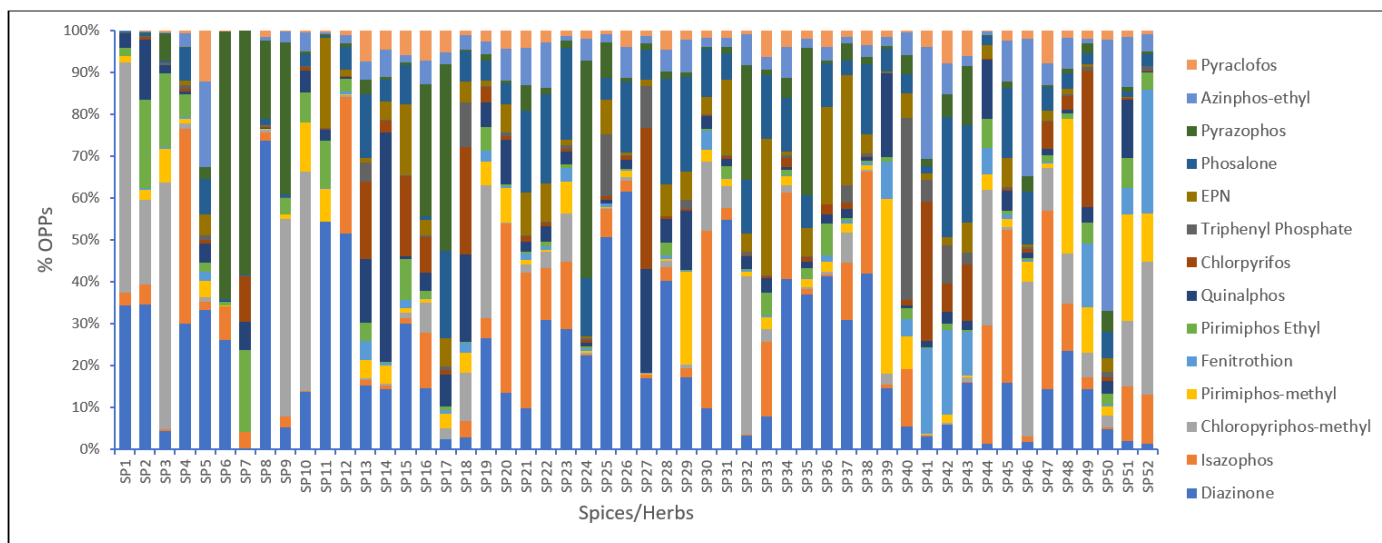


Figure 2. Percentage composition of OPPs in the spices and herbs.

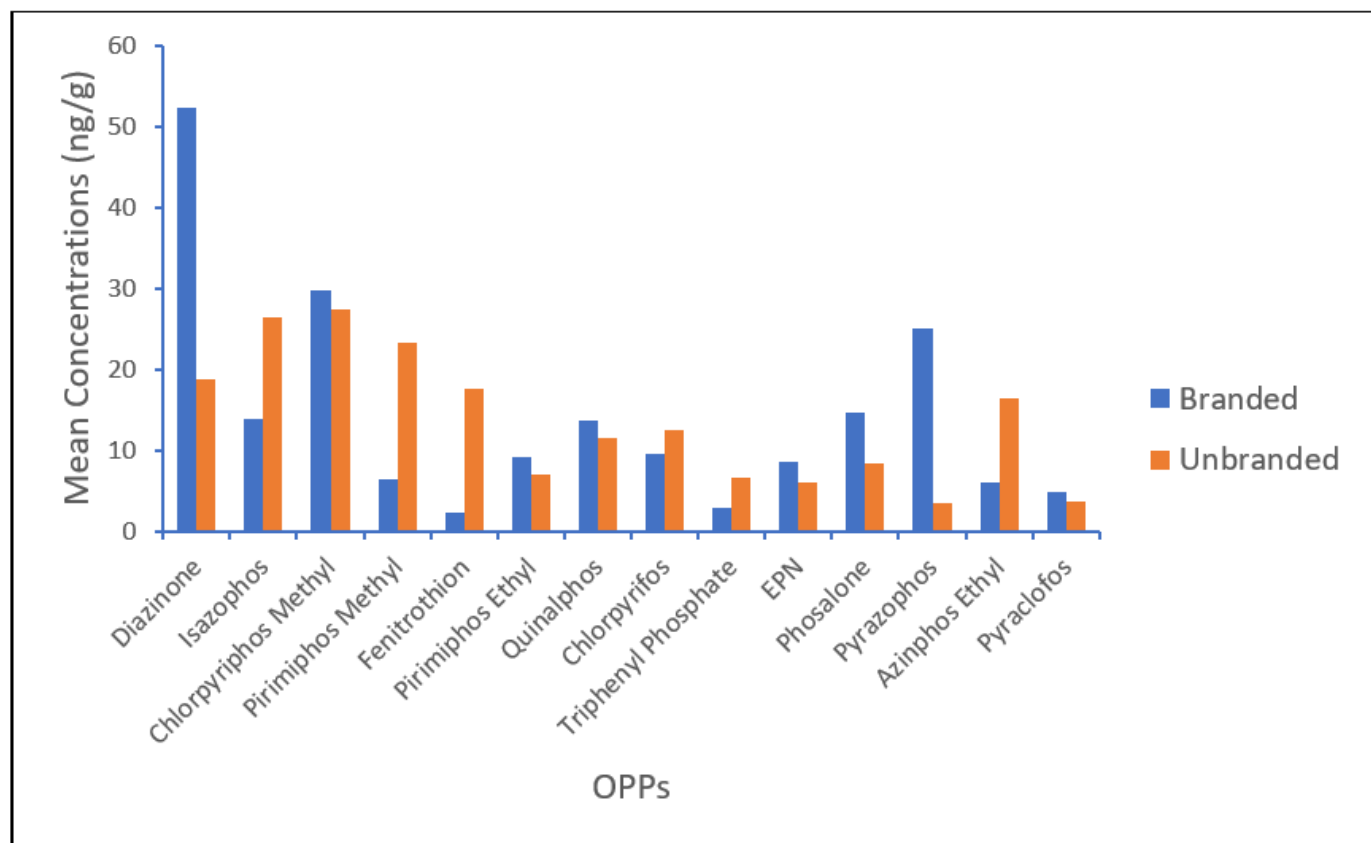


Figure 3. Occurrence pattern of unbranded and branded spices and herbs.

2.6.1. Estimation of Daily Dietary Intake (DDI)

The DDI of OPPs in the spices and herbs was computed using equation (1).

$$\text{Daily Dietary Intake (ng/g bw/day)} = \frac{\text{Concentration of OPPs in spices/herbs} \times \text{Ingestion Rate (IR)}}{\text{Body Weight (BW)}} \quad (1)$$

The ingestion rate used was 8.5 g/day based on the spices/herbs per capita consumption of Nigeria, which is 3.10 kg [12] and a BW of 60 kg for adults and 15 kg for children [13].

2.6.2. Estimation of non-carcinogenic risk

The non-carcinogenic risk associated with OPPs via the intake of the spices/herbs was assessed as hazard index (HI) using

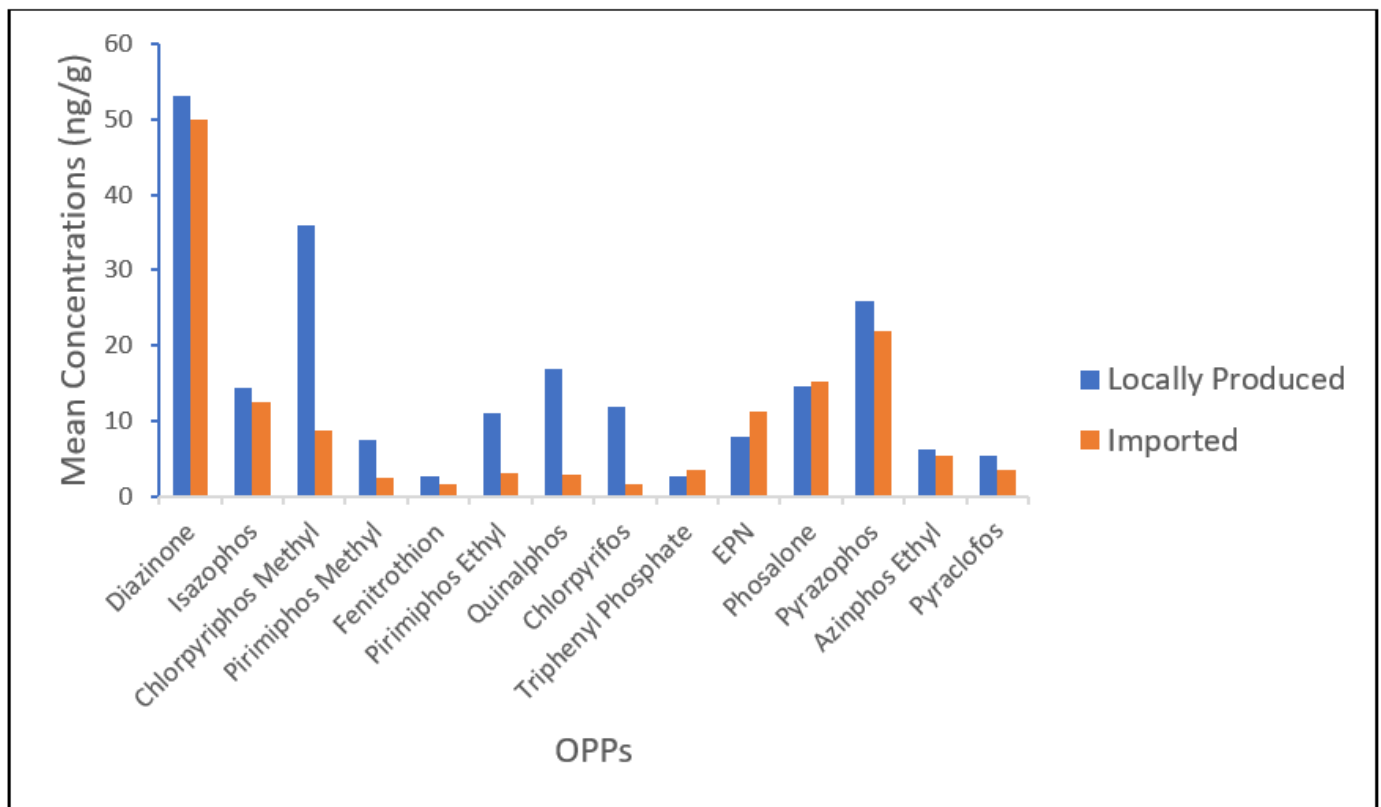


Figure 4. Occurrence pattern of local and imported spices and herbs.

equation 2. A hazard index value below 1 designates there is the absence of non-carcinogenic risk [24].

$$HI = \left[\frac{\text{Concentration of OPPs} \times IR \times EF \times ED}{BW \times AT_{nc}} \right] \times 10^{-6} / RfD, \quad (2)$$

where, EF = exposure frequency (day/yr) = 350; ED = exposure duration (years) = 6 and 30 years for children and adults, respectively; AT_{nc} (days) = averaging time for non-cancer = ED × 365 and RfD (mg/kg/d) = oral reference dose [25]. The RfD values used were obtained from [24].

3. Result and discussion

3.1. Contamination levels of OPPs in the spices and herbs

The summary statistics of the contamination levels of OPPs in the spices and herbs are shown in Table 1 while the contamination levels of individual samples are shown in Table S2. The 14 OPPs investigated were found in the 52 spices and herbs analyzed which amounted to a 100 % detection rate. This 100 % detection recorded in this study may be due to the use of OPPs in the cultivation of the spices and herbs and during storage processes. The high proportion of spices and herbs with OPPs in the present study is in consonance with previous studies that reported pesticide residues in 37 to 100 % of herbs in Poland,

Egypt, Italy, Tunisia, China and Nigeria [20, 26–34]. The concentration of the Σ 14 OPPs in the spices and herbs ranged between 4.96 and 795 ng/g. The highest concentration was observed in sample SP13, whereas the lowest concentration was observed in sample SP5.

ANOVA indicated a significant variation ($p < 0.05$) in the OPP concentrations in these spices and herbs. This variation might be attributed to the genetic features of the spices and herbs and OPPs characteristics [14, 20]. The average OPP concentrations in the spices and herbs were in the order of diazinone > chlorpyrifos methyl > pyrazophos > isazophos > quinalphos > phosalone > pirimiphos methyl > chlorpyrifos > azinphos ethyl > pirimiphos ethyl > EPN > fenitrothion > pyraclofos > triphenyl phosphate (Figures 1 and 2). The Food and Agriculture Organization/World Health Organization (FAO/WHO) has set an MRL of 10 ng/g for OPPs in foodstuffs [35]. The average concentrations of diazinone, quinalphos, chlorpyrifos, phosalone, pirimiphos methyl, pyrazophos, chlorpyrifos methyl and isazophos were above the FAO/WHO MRL. Whereas, the average concentrations of fenitrothion, pirimiphos ethyl, triphenyl phosphate, EPN, azinphos ethyl and pyraclofos were below the FAO/WHO MRL. The concentrations of diazinone, pirimiphos methyl, fenitrothion, pirimiphos ethyl and azinphos ethyl in these spices and herbs were below their respective MRLs of 500, 4000, 500, 50, 100 and 100 ng/g set by the European Pharmacopeia [36].

The levels of chlorpyrifos and chlorpyrifos methyl were also lower than their respective MRLs of 500 ng/g and 1000

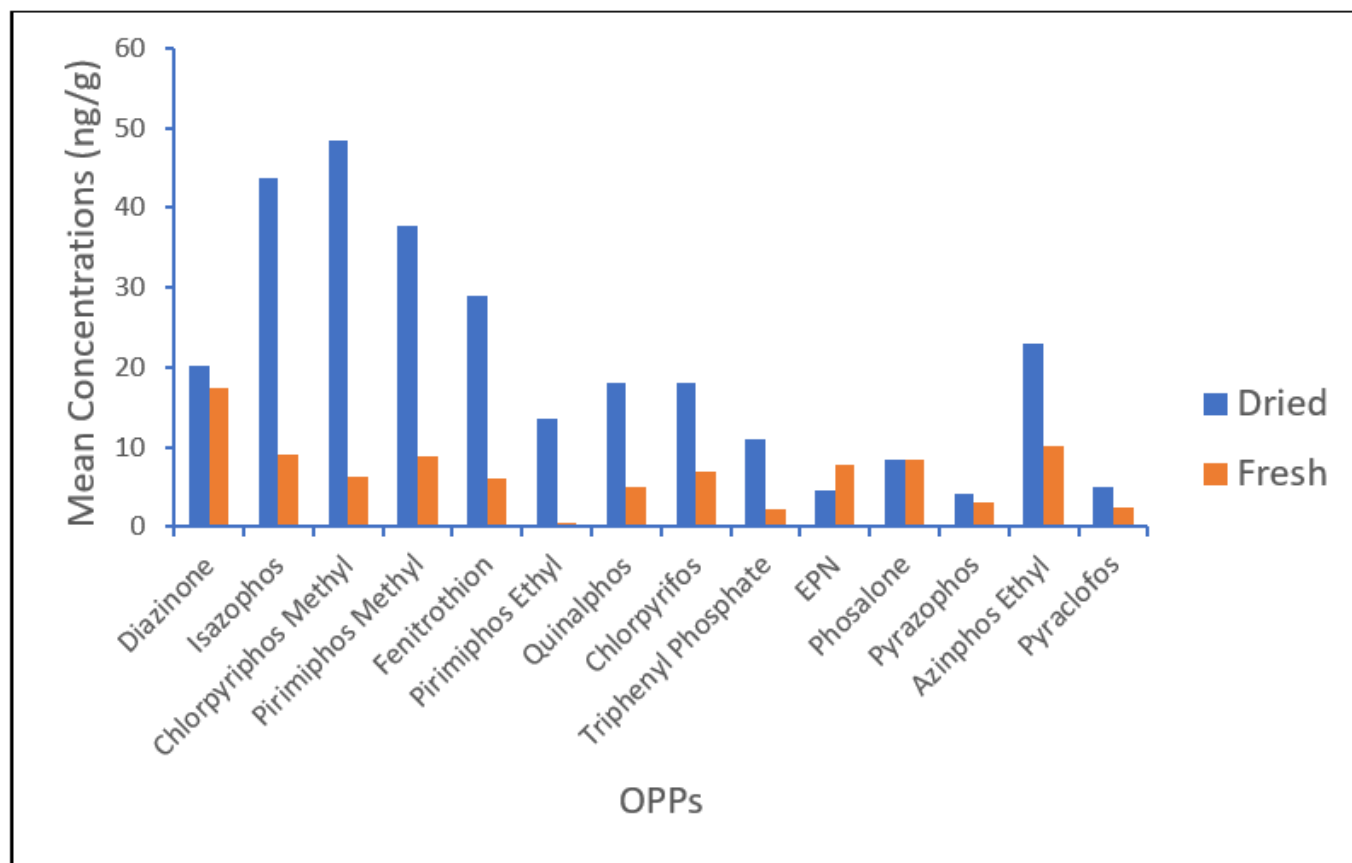


Figure 5. Estimated DDI of OPPs in the spices and herbs.

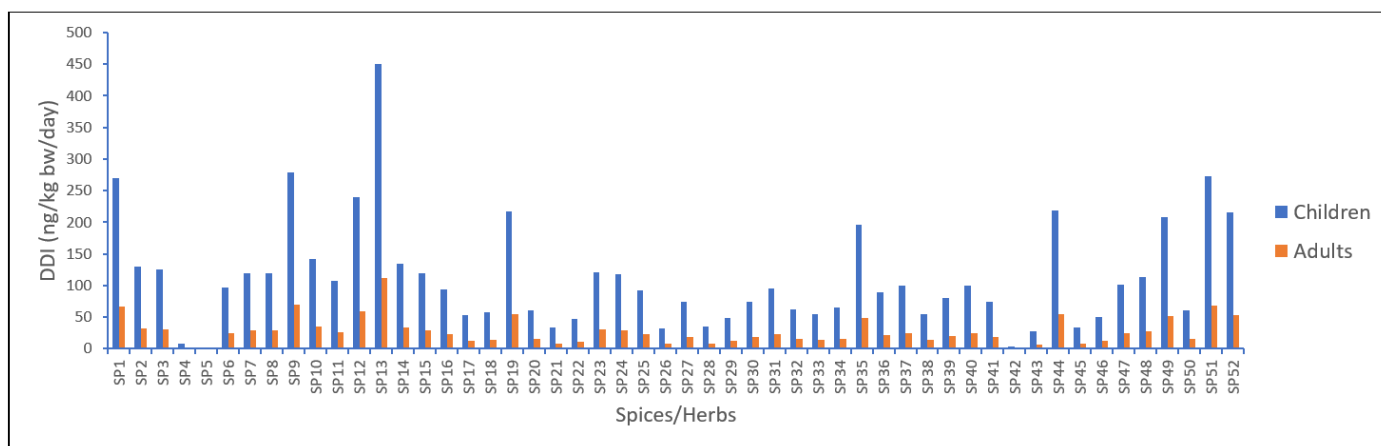


Figure 6. Daily dietary intake of OPPs for the spices and herbs.

ng/g set by the European Union. Comparing the results of this study with the few available studies of OPPs in literature, we found that the levels of OPPs recorded in our study was similar to those previously reported in spices and herbs from Thailand and Egypt. For instance, Farag *et al.* [33] reported levels of 10-147 ng/g for chlorpyrifos, 10 ng/g for diazinone and 16 ng/g for chlorpyrifos methyl in Egyptian herbs. Similarly, Abou-Arab and Abou-Dania [37] recorded pirimiphos methyl levels of 4.0 – 122 ng/g in Egyptian leafy spices. Furthermore, Am-

bus [38] reported chlorpyrifos levels of 20 – 50 ng/g in a number of spices analysed during 2005–2008 in Thailand.

3.2. Occurrence pattern of OPPs in the spices and herbs

3.2.1. OPPs in unbranded versus branded spices and herbs

The results of OPPs in the branded and unbranded spices and herbs are shown in Figure 3 and Table S3. On average, the unbranded spices and herbs have a higher mean concentration (200 ng/g) of $\Sigma 14$ OPPs than the branded (189 ng/g).

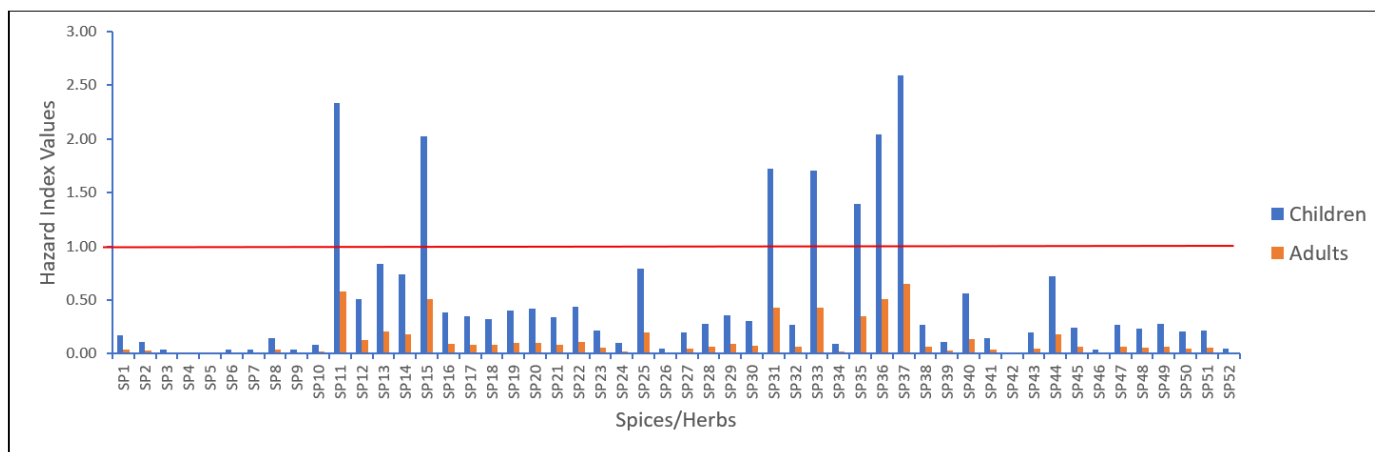


Figure 7. Hazard index of OPPs from the intake of the spices and herbs.

The lower levels found in the branded spices may be due to modernized methods of preparation and/or improved processing techniques and better-quality control measures put in place by the manufacturers. The unbranded spices and herbs have higher mean concentrations of diazinone, chlorpyrifos methyl, Pirimiphos ethyl, quinalphos, EPN, phosalone, pyrazophos and pyraclofos. Whereas, isazophos, pirimiphos methyl, fenitrothion, chlorpyrifos, triphenyl phosphate and azinphos ethyl have higher mean concentrations in the branded spices and herbs which may be due to the processing techniques.

3.2.2. OPPs in local versus imported spices and herbs

Figure 4 and Table S4 showed the results of OPPs in spices and herbs produced locally and those imported. The mean concentration of $\sum 14$ OPPs in the locally produced spices and herbs was 216 ng/g, while that of imported spices and herbs was 143 ng/g. Diazinone was the predominant OPP compound in both the locally produced and imported spices and herbs. The locally produced spices and herbs have a significantly higher mean concentration of $\sum 14$ OPPs than the imported spices and herbs. The mean concentrations of all the OPP compounds except triphenyl phosphate, EPN and phosalone were higher in the locally produced spices and herbs than the imported ones. The lower concentrations of OPPs found in the imported spices and herbs could be attributed to the environment where the spices are grown.

3.2.3. OPPs in dried versus fresh spices and herbs

Figure 5 and Table S5 showed the results of PAHs in dried and fresh spices. The mean concentration of $\sum 14$ OPPs in the dried spices and herbs was 284 ng/g, while that of fresh spices and herbs was 94 ng/g. Chlorpyrifos methyl and diazinone have the highest concentrations for dried and fresh spices, respectively. The dried spices have significantly higher mean levels of $\sum 14$ OPPs than the fresh spices. The higher levels of OPPs found in the dried spices could be attributed to the traditional processing method, mostly open-air drying, and may bring about atmospheric deposition of OPPs. The mean concentrations of all the OPP compounds except EPN were signif-

icantly higher in the dried spices and herbs than in the fresh ones.

3.2.4. Estimated DDI of OPPs in the spices and herbs

The calculated DDI of OPPs from spices and herbs consumption by children and adults is displayed in Figure 6 and Table S6. The DDI values based on the $\sum 14$ OPPs by children varied from 2.81 to 451 ng/kg bw/day. Moreover, the DDI for adults varied from 0.70 to 113 ng/kg bw/day. It was observed that the highest DDI was found in SP13, while the lowest was found in SP5. The DDI of children was thrice that of adults, which confirmed the findings of Tesi *et al.* [14, 20] and Suarez-Lopez *et al.* [39] that children are more prone to pesticides exposure.

3.2.5. Non-carcinogenic risks of OPPs in the spices and herbs

The estimated HI values associated with human exposure to OPPs from the intakes of these spices and herbs are displayed in Figure 7 and Table S7.

The hazard index values of the OPPs in all the spices and herbs ranged from 7.99×10^{-3} to 2.59 for children's intake and 2.00×10^{-3} to 6.48×10^{-1} for adults' intake. The highest and lowest HI values were observed in SP37 and SP42, respectively. The HI values for children were four times those of adults, suggesting that children will be more susceptible to any risks from OPPs in the spices and herbs. With the exception of samples SP11, SP15, SP31, SP33, SP35, SP36, and SP37 for children intake, the HI values of OPPs in these spices and herbs were < 1 , which is an indication of the absence of adverse non-carcinogenic risks from the intake of these spices and herbs. The OPP compound that contributed most to the HI was EPN while chlorpyrifos methyl contributed the least. Though the OPPs in the spices and herbs in this study pose no health challenges presently, chronic low-dose exposure to OPPs can have cumulative health effects, such as neurotoxicity, endocrine disruption, and carcinogenic risk.

4. Conclusion

This work provided background data on the contamination levels, occurrence pattern and associated risk of OPPs in spices and herbs sold and consumed in southern Nigeria. The study revealed that all analysed samples of spices and herbs were all contaminated by the 14 OPPs investigated. The computed HI values showed that there was no non-carcinogenic risk from the consumption of these spices and herbs except, for seven samples in the case of children's intake. Although the findings suggest minimal health risks from OPPs through the intake of the spices and herbs, it is therefore recommended that there is a need for continuous monitoring of spices and herbs sold generally in Nigeria markets for pesticide contamination. The National Agency for Food and Drugs Administration and Control should establish MRLs for OPPs in foodstuffs in Nigeria and ensure strict compliance with the MRLs of contaminants in food and drugs.

Data availability

The data used and/or analysed in this study are given in the work and Supplementary materials.

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