Health Risk Assessment of Organophosphate Pesticidal Residue in Selected Daily Consumed Vegetables in Akure Metropolis

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Abstract: **Farmers highly depend on chemical pesticides in recent farming practices to ensure expected yield is met, and organophosphates pesticides has been the common pesticides used on fruits and vegetables, this pesticide's instruction are not dully followed by farmers. Thereby the possibility of residue of these pesticides will continually be a concern. Acceptable daily intake values are being set by WHO and Food and Drug Agency using Cordex, and also the maximum residual limits of each pesticides in each fruit and vegetables to ensure safe level is maintained. But the farmers might not be informed about the possibility of the dangers of long-term exposure to the pesticide. This study shows the concentration level of organophosphate residue in fruits eaten almost every day in relation to the health risk values. The concentration ranges from Fenamiphos with a mean concentration of 0.08mg/kg showing the lowest while bromophos-methyl showed a mean concentration of1.136mg/kg as the highest mean concentration followed by azinfos-methyl with a mean concentration of 1.056mg/kg, then Chlorpyrifos with mean concentration of 1.046mg/kg. Bromophos-ethyl was only detected in tomatoes and onions, Etrimfos was not detected in carrot and carrot has the least pesticides summation of the detected pesticide while garden egg sample shows the highest concentration summation followed by tomato, a daily consumed vegetable/fruit in the southern part of Nigeria. It also revealed that the health risk index for children is of concern than other population therefore cooking these vegetable and fruits is recommended to greatly lower the residues.**

Keywords: **Vegetables, Organophosphate, Pesticides, Health Risk, Principal Component Analysis.**

I. INTRODUCTION

Food is essential and it is part of life itself and thus as become a major contributor to life sustenance, with different type of food, the consumption of fruits and vegetables is increasing. These class of food is considered rich in vitamins, minerals and dietary fiber and has been favoured among nutritionist to be taken regularly. When vegetables are included in diet, it has led to reduction of the possibility of chronic ailments such as cancer, stroke and cardiovascular disease. (Leon2011). But the production of these classes of food involve the use of chemicals such as for protection against pest and diseases, fertilizers to ensure good yield per harvest. Pesticides may be regarded as economical and effective substances or mixture of substances used to prevent, repel and destroy any pest (EPA 2021). These pesticides can leave behind their presence even when used in with good agricultural practices as residue on the crops they used on (Iya, and Kwaghe, 2007). Pesticides are numerous and are classified based on the pest they control, chemical structure and their mode of action.

While organophosphate pesticides (OPC)are insecticides or nerve agent acting on the enzyme acetyl cholinesterase. This is often used to describe all organic phosphorus (V) containing compound especially when dealing with neurotoxin compound. These insecticides are esters, amides or sample derivatives of phosphoric or thiosulphuric acid. Insecticides against internal and external parasites in animals are being treated with some of the less toxic compound of organophosphates. (Akpagu,*et al.,*2015).Their toxicity lies in the way they function (inhibiting of cholinesterase activity i.e., acethylcholinesterase and butylrylcholinestease especially acetylcholinesterase enzyme which controlling the functions of the nervous system(Neupane, and Brandt, 2014).The OPs can be permanently bound to the group hydroxylating the enzyme, which then prevents acetylcholinesterase from decomposing and gain more amount of acetylcholine (concentrated) at the synapses then leading to a state of hyper arousal, paralysis of the muscles and the main respiratory centre. Long-term exposure to pesticides is increasingly suspected of being linked to a broad spectrum of medical problems such as cancer, neurotoxic effects, reproductive health concerns and endocrine disruption, particularly for specific populations(Mostafalou, and Abdollahi2013,George and Shukla, 2011). Apart from farmers, those working on the farm and those living within the vicinity of the farm (they are at risk of greatest exposure) that these pesticides are used, consumers are also at risk of these pesticidal residues in fruits and vegetables because their major mode of exposure is through consumption (Wolejko*et.al.,* 2014) of the agriculture products that this class of pesticides are used on.This research's aim is to determine the organophosphate residue in the everyday eaten vegetables and fruits, its level of health risk to human.

II. MATERIALS AND METHODS

2.1.1 Samples collections

The fruits and vegetables were purchased from Oja Oba market, Akure, Ondo State. The method of extraction used for the extraction of the fruits and vegetables was the USEPA method 3510 for extracting pesticide residues in non-fatty crops, using ethyl acetate as solvent.

2.2 Methodology

2.2.1 Extraction

The samples were washed thoroughly with distilled water and placed in a mortar, the fruit and vegetables were converted into paste separately by using mortar and pestle, then spatula was used to move them to separate conical flask after which 20g of each sample was weighed on the weighing balance then 40ml of ethyl acetate was added and shaken thoroughly.Consequently, 5g of sodium hydrogen carbonate was added to neutralize any acid present in the mixture followed by 20g of anhydrous sodium sulphate (to remove water from the sample matrix.) and the entire mixture was shaken vigorously for 1 hour. The process was to ensure that enough of the pesticide residue dissolved in ethyl acetate. The procedure was repeated for other samples and the mixture was filtered into a labelled container before centrifuging at a speed of 1800rpm for 5 minutes. The organic layer was decanted into a conical flask, dried with rotary evaporator and a water bath and a 1:1 mixture of 5m of ethyl acetate and cyclohaxane was added(Akan *et al.*, 2013).

2.2.2 Cleaning up of vegetables extract.

A 10mm chromatographic column was filled with 3g activated silica gel and topped up with 2 to 3g of anhydrous sodium sulphate, and 5 ml of n-hexane was added to the column. The residue in 2 ml n-hexane was transferred onto the column and the extract was rinsed thrice with 2 ml hexane. The procedure was repeated for all the samples. The sample was collected in a 2 ml vial, sealed and placed in the refrigerator in the laboratory with temperature below normal room temperature, to prevent evaporation of the ethyl acetate (Akan *et al.*, 2013).

2.3. Qualitative Identification and Quantitative Estimation of the Organophosphate Pesticides

Qualitative identification and quantitative estimation of the pesticide residues were performed by reconstituting the dried sample eluents with 1 mL n-hexane. With the aid of a micro syringe, the injection of 1 μL of the purified eluents was performed in a splitless injection mode on to the injection port of an Agilent 5977B Gas Chromatograph (GC) system equipped with Electron Capture Detector (ECD). Carrier gas was Helium at a flow rate of 1.2 mL/min and make up gas was Nitrogen. The run time was 25 minutes. The identification of OPPs was done by comparing the retention times of the peaks with those obtained from standard mixture of OPPs, while the quantification was based on external calibration curves prepared from the serially diluted standard solution of each of the OPPs. The separation was performed on a fused silica capillary column (DB-17, 30 m long x 0.250 mm internal diameter and film thickness of 0.25 μm). The temperatures of the injector and detector were 250^{o}C and 290^{o}C respectively. Oven temperatures programme started from 150^{o}C(for 8 mins) and increased to 280^{o}C at 6^{o}C per minute for 5mins. The instrumental analysis was done at the Nigeria Institute of Oceanographyand Marine Research (NIOMR) Laboratory, Victoria Island, Lagos, Nigeria.

III. RESULT AND DISCUSSION

3.1 Concentrations (mg/kg) of Organophosphate Pesticides in Vegetables

Table 1 shows the occurrence and distribution of organophosphate pesticides in the studied vegetables. Minimal variations existed in the concentrations of the identified organophosphate pesticides in some of the studied samples. Dichlorvos had a mean concentration of 0.168±0.004 mg/kg in the samples. Mevinfos had the highest concentration of 0.28 mg/kg in garden egg and the least concentration of 0.17 mg/kg in carrot. Dimethoate was highest in garden egg (0.53 mg/kg) while it maintained a fairly constant concentration in the other samples. Diazinon had a mean concentration of 0.268±0.008 mg/kg. Etrimfos was not detected in carrot, but had a mean concentration of 0.184±0.103mg/kg. Phosphamidon had a mean concentration of 0.202±0.022 mg/kg. Parathion-methyl was highest in okro (0.46mg/kg) followed by onions (0.45mg/kg) and had a mean concentration of 0.348±0.104 mg/kg in all the samples. Malathion exhibited a fairly constant concentration in the studied samples with a mean concentration of 0.212±0.013 mg/kg. Chlorpyrifos was relatively high in Okro (2.13 mg/kg) with carrot showing the lowest concentration (0.24mk/kg). Generally, the sum of the organophosphate pesticides in the studied samples followed the order: Garden egg > Tomatoes > Onions > Okro > Carrot. The mean concentrations of the organophosphate pesticides followed the order: Bromophos-methyl > Azinfos-methyl > Chlorpyrifos > Fenitrothion > Parathion-methyl > Dimethoate > Diazinon > Pirimiphos-methyl > Mevinfos > Bromophos-ethyl > Chlorfenvifos > Malathion > Phosphamidon > Etrimfos > Dichlorvos > Carbofenothion >Ethion > Fenamiphos. The concentrations of chlorpyrifos detected in all the samples exceeded their maximum residue limit (MRL) of 0.05 mg/kg (Elgueta*et al.*, 2017). Its high occurrence could be attributed to its persistence and accumulation in food matrices (Angioni *et al.*, 2011). The high relative occurrence of chlorpyrifos in the vegetables is consistent with the reports of previous findings(Srivastava *et al.*, 2014; Ong-Artborirak*et al.*, 2017; Fatunsin *et al.*, 2020). The mean concentrations of organophosphate pesticides reported in this study are generally higher than those reported in a similar study (Njoku *et al.*, 2017). The relative upsurge in the concentrations of the organophosphate pesticides may be due to unauthorized or increased usage of pesticides during the cultivation and storage of the vegetables (Elgueta *et al.*, 2017). The non-compliance of the pesticide levels with maximum residue levels is an indication of insufficient knowledge of good agricultural practices and pesticide use (Lehmann *et al.*, 2017). In addition to the potential health risks that might be accrued from increased pesticide levels, prejudicial economic limitations could emanate from the upsurge in the pesticide levels of the vegetables. There is a growing demand for vegetables in developed countries. However, residual pesticide levels in the vegetables grown in developing countries like Nigeria may be a good reason why its export may be rejected at the international market(Bempah *et al.*, 2011

Table 1: Concentrations (mg/kg) of Organophosphate Pesticides in Vegetables

Pesticides	Tomatoes	Okro	Onions	Garden egg	Carrot	Mean±SD
Dichlorvos	0.17	0.16	0.17	0.17	0.17	0.168±0.004
Mevinfos	0.19	0.26	0.23	0.28	0.17	0.226±0.046
Dimethoate	0.32	0.27	0.3	0.53	0.27	0.338±0.109
Diazinon	0.26	0.27	0.28	0.26	0.27	0.268±0.008
Etrimfos	0.23	0.23	0.23	0.23	nd	0.184±0.103
Phosphamidon	0.22	0.19	0.22	0.21	0.17	0.202±0.022
Parathion-methyl	0.32	0.46	0.45	0.29	0.22	0.348±0.104
Fenitrothion	0.43	0.47	0.38	0.43	0.25	0.392±0.086
Pirimiphos-methyl	0.27	0.27	0.27	0.26	0.26	0.266±0.005
Malathion	0.23	0.22	0.21	0.2	0.2	0.212±0.013
Chlorpyrifos	1.14	2.13	0.85	0.87	0.24	1.046±0.690
Bromophos-ethyl	0.55	nd	0.55	nd	nd	0.22±0.301
Chlorfenvifos	0.19	0.21	0.2	0.27	0.19	0.212±0.033
Bromophos-methyl	1.95	0.32	1.69	1.62	0.1	1.136±0.858
Fenamiphos	0.08	0.09	0.09	0.08	0.06	0.08±0.012
Ethion	0.11	0.33	0.1	0.07	0.07	0.136±0.11
Carbofenothion	0.16	0.14	0.12	0.16	0.13	0.142±0.017
Azinfos-methyl	0.85	0.86	0.8	1.97	0.8	1.056±0.512
$\sum OPPs$	7.67	6.88	7.14	7.9	3.57	

OPP = organophosphate pesticide, SD = standard deviation

3.2 Principal Component Analysis of Organophosphate pesticides in studied samples

Table 2 shows the principal component analysis of the organophosphate pesticides studied. Principal Component Analysis (PCA) is a data reduction technique that creates components that can be meaningfully interpreted. The number of components that should be retained was determined using eigen values while the scree plot (showing the eigen values) and a plot of the extracted components are shown in Figures 1 and 2 respectively. Varimax method of rotation was used for the factor analysis and six principal components were identified. The six principal components accounted for 88.616 % of the data variance. Factor loadings of 0.5 and above are considered strong and significant (Ravindra *et al.*, 2008). Principal component 1 (which accounted for 29.995 % of the total variance) had high factor loadings of 0.958, 0.899, 0.820, 0.767, 0.741, 0.724, and 0.572 for fenitrothion, mevinfos, pirimiphos-methyl, fenamiphos, parathion-methyl, bromophos-methyl, and chlorpyrifos respectively. This is in consonance with the observed close clustering relationship of the group in the dendrogram, as also evident in their component plot. The close loading of the pesticides might be indicative of concurrent usage during cultivation, storage and/or transportation. Principal component 2 (which accounted for 19.616 % of the total variance) had high factor loadings of 0.895, 0.80, 0.757, and 0.703 for malathion, bromophos-ethyl, etrimfos, and diazinon respectively. Principal component 3 (which accounted for 15.935 % of the total variance) had factor loadings of 0.915, 0.813, 0.705, and 0.683 for ethion, azinfos-methyl, carbofenothion, and chlorpyrifos respectively. The remaining principal components did not make significant contribution to the data variance.

Table 2: Principal Component Analysis of Organophosphate pesticides in studied samples

Pesticides	Component					
	1	2	3	4	5	6
Fenitrothion	*0.958*	-0.054	0.031	0.042	0.134	0.161
Mevinfos	*0.899*	-0.188	0.085	0.140	-0.099	-0.121
Pirimiphos-methyl	*0.820*	0.312	-0.087	-0.214	0.177	-0.223
Fenamiphos	*0.767*	-0.433	0.285	-0.112	-0.193	0.209
Parathion-methyl	*0.741*	0.155	0.317	-0.287	0.329	0.191
Bromophos-methyl	*0.724*	0.155	-0.108	0.424	-0.247	-0.189
Dichlorvos	-0.676	-0.072	0.372	0.239	-0.131	-0.042
Malathion	0.044	*0.895*	0.035	-0.062	0.018	-0.128
Bromophos-ethyl	-0.122	*0.800*	0.056	-0.024	-0.185	0.484
Etrimfos	0.375	*0.757*	-0.054	0.283	0.313	-0.133
Diazinon	-0.196	*0.703*	0.291	-0.310	-0.005	-0.059
Ethion	0.000	0.122	*0.915*	-0.234	0.097	0.125
Azinfos-methyl	-0.245	0.309	*0.813*	0.293	-0.014	0.083
Carbofenothion	0.219	-0.014	*0.705*	-0.046	*0.517*	-0.086
Chlorpyrifos	*0.572*	-0.288	*0.683*	-0.233	0.055	0.104
Dimethoate	-0.106	-0.112	-0.071	*0.953*	0.118	-0.041
Chlorfenvifos	0.010	0.040	0.166	0.116	*0.888*	0.260
Phosphamidon	0.051	-0.075	0.107	-0.062	0.259	*0.944*
Eigen values	5.399	3.531	2.868	1.656	1.473	1.024
% data variance	29.995	19.616	15.935	9.202	8.181	5.687
Cumulative data variance	29.995	49.611	65.545	74.748	82.929	88.616

❖ Bold indicates significant factor loadings

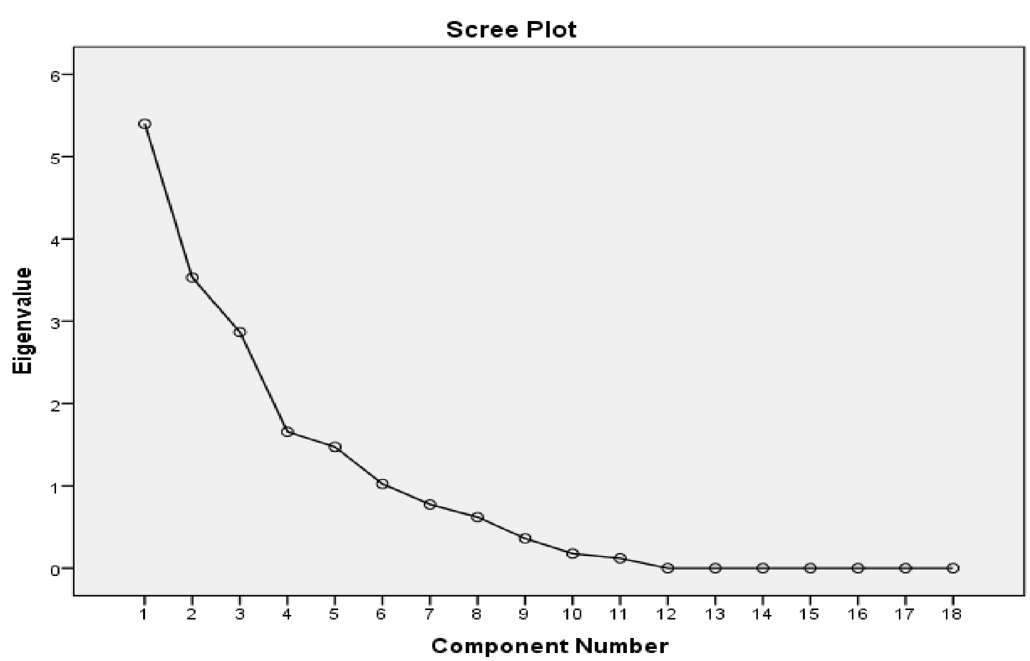

Figure 1: Scree plot showing eigen values of principal components

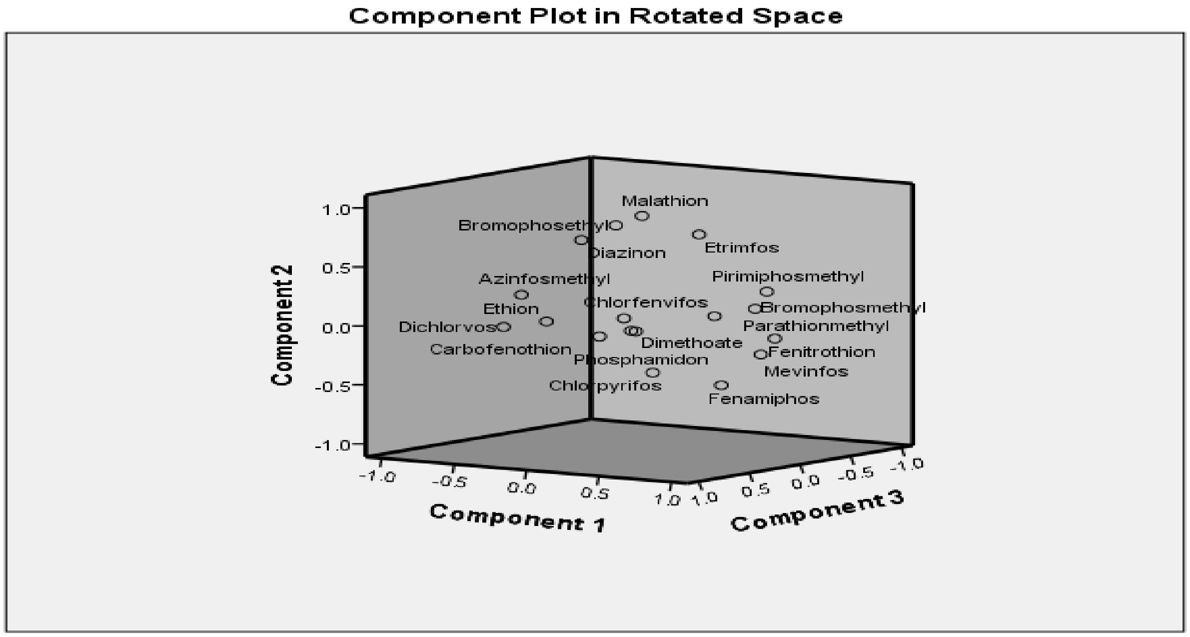

Figure 2: Component plot of factors

3.3 Health risk assessment of organophosphate pesticides in the vegetable samples

The estimated daily intake and health hazard index were represented in Table 3. The human health risk assessment associated with exposure to organophosphate pesticides via the ingestion of fruits and vegetables by adults and children was estimated. Notable observations in the health hazard index include the absence of malathion and fenitrothion related health risks due to the consumption of all the fruit and vegetables samples by adults (HHI<1). The children were observed to have higher HHI values than adults, indicating that they are relatively more susceptible to the pesticide contaminants than adults. This is consistent with the reports of earlier findings (Bempah *et al.*, 2011; Kumari and John, 2019). The highest risk of noncarcinogenic and carcinogenic health risks is related with exposure to diazinon, due to its relatively higher HHI values. Cooking and chafing of these vegetables is suggested to remain safeespecially for the children(Zhao & Liu 2020).

Table 3: Health risk assessment of organophosphate pesticides in the vegetable samples

Estimated Daily Intake (EDI)						
Pesticides	Population	Tomatoes	Okro	Onions	Garden egg	Carrot
Chlorpyrifos	Adults	0.005	0.01	0.004	0.004	0.001
	Children	0.046	0.01	0.03	0.012	0.043
Malathion	Adults	0.001	0.001	0.001	0.0009	0.0009
	Children	0.003	0.002	0.003	0.003	0.003
Ethion	Adults	0.0005	0.001	0.0004	0.0003	0.0003
	Children	0.02	0.001	0.004	0.001	0.002
Parathion	Adults	0.001	0.002	0.002	0.001	0.001
	Children	0.007	0.004	0.006	0.006	0.007
Diazinon	Adults	0.001	0.001	0.001	0.001	0.001
	Children	0.004	0.004	0.003	0.004	0.003
Fenitrothion	Adults	0.002	0.002	0.001	0.002	0.001
	Children	0.006	0.004	0.006	0.005	0.011
Health Hazard Index (HHI)						
Chlorpyrifos	Adults	0.561	1.049	0.418	0.428	0.118
	Children	1.653	3.088	1.232	1.261	0.348
Malathion	Adults	0.003	0.003	0.003	0.003	0.003
	Children	0.011	0.01	0.01	0.009	0.009
Ethion	Adults	0.271	0.813	0.245	0.172	0.172
	Children	0.797	2.392	0.725	0.507	0.507
Parathion	Adults	1.577	2.267	2.217	1.429	1.084
	Children	4.64	6.67	6.525	4.205	3.19
Diazinon	Adults	6.407	6.653	6.9	6.407	6.653
	Children	18.85	19.575	20.3	18.85	19.575
Fenitrothion	Adults	0.423	0.463	0.374	0.423	0.246
	Children	1.247	1.363	1.102	1.247	0.725

IV. CONCLUSION

The study which was carried out on organophosphate pesticide in the vegetable samples shows that the organophosphate pesticides in these samples is higher than normal. The increase in organophosphate pesticide in the selected vegetable samples may be due to the poor knowledge of the farmers on how to quantitatively apply the pesticide and this lack of knowledge by the farmers makes the exportation of such foods and vegetables to be difficult. According to the health hazard analysis that was done, the children are prone to pesticide contaminants than the adults so care must be made in order to minimize the intake of such fruits and vegetables by the children.

In order to reduce the effects and the potent of organophosphate pesticides in the vegetable the first thing to be done before eating any vegetable is thorough washing before eating, thereby reducing its effect. A seminar should be organized to teach the farmers how to quantitatively apply pesticides.

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