DETERMINATION OF SELECTED PESTICIDE RESIDUES IN LEAFY VEGETABLES (*AMARANTHUS SPINOSUS*) CONSUMED IN DONGA, TARABA STATE

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ABSTRACT: *Pesticides are a numerous and diverse group of chemical compounds which are used to eliminate pests in crop yields and households. But owing to this and other injudicious practice related to pesticide usage, pesticides become the inner part of vegetables in the shape of residues, which could be consumed by consumers thus creating health hazards. Hence, we investigated the presence of selected pesticide residues in leafy vegetable (Amaranthus spinosus) samples consumed in Donga, Taraba state, Nigeria. Five samples of leafy vegetable 50g each (AmaranthusSpinosus) which is an extensively grown vegetable in Donga local government area were collected from a vegetable farm situated about Ten meters away from Donga River. Information on the types of pesticides used on the collected samples was obtained from the farmer via oral interview. After the samples were collected, dusts on the samples were removed by washing with distilled water prior to placing them into polythene nylons. The samples were double packed in order to prevent any other contamination from coming in contact with them. The packaged samples were then labeled properly. The samples were transported to the laboratory using a cold box for analysis. The Acetate-buffered QuEChERS sample preparation method for pesticides (AOAC official method 2007.01) was used for preparing the samples, while for liquid chromatography analysis; an Agilent 1100 HPLC system was used. Nineteen pesticide residues were found present in the leafy vegetable samples analyzed. The descending order of their concentrations in the analyzed samples read; Gamma-HCH > alpha-HCH > methoxychlor > beta-HCH > DDT > DDD > perthane > cyclohexane > toxaphene > mirex > cyclodienes > chlordane > aldrin > heptachlor > dieldrin > endosulfan > dicofol > hexachlorobenzene > pentachlorobenzene, with Pentachlorobenzene having the lowest mean concentration (0.360 ± 0.063μg/kg) and gamma- HCH having the highest mean concentration (1.866 ± 0.483μg/kg). The concentrations of all these pesticide residues detected in the analyzed samples were found to be above the maximum residue limits set by FAO and WHO. This study revealed the presence of various pesticide residues in the analyzed samples of Amaranthus spinosus with organochlorines being the most present. This confirms the wide use of organochlorine pesticides in the study area. It was also observed that all the pesticide residues found present in the analyzed samples exceeded the Maximum Residue Limits (MRLs) and Acceptable Dietary Intake (ADI) which connotes the extensive use and prevalence of pesticides in the study area.*

KEYWORDS*:* pesticides, pesticide residues, leafy vegetable, residue concentration, maximum residue limits.

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ULR: https://doi.org/10.37745/ijbbbs.15

INTRODUCTION

Leafy vegetables are an important part of a healthy diet as they are a significant source of vitamins and minerals. However, they can also be a source of noxious toxic substances-pesticides **[1]**. Pesticides are a numerous and diverse group of chemical compounds, which are used to eliminate pests in crop yields and households **[2]**. Owing to this and other injudicious practice related to pesticide usage, pesticides become the inner part of vegetables in the shape of residues, which could be used by consumers thus creating health hazards **[3].** These Pesticide residues are the deposits of pesticide active ingredient, its metabolites or breakdown products present in some component of the environment and of its application, spillage or dumping **[4].**

The Application of pesticides to crops may leave residues in or on food, and Many of these chemical residues, especially derivatives of chlorinated pesticides, exhibit bioaccumulation which could build up to harmful levels in the body as well as in the environment **[5].** These bioaccumulative or Persistent chemicals can be magnified through the food chain and have been detected in products ranging from meat, poultry, and fish, to vegetable oils, nuts, and various fruits and vegetables **[6]**. Exposure of the general population to these residues most commonly occurs through consumption of treated food sources, or being in close contact to areas treated with pesticides such as farms or lawns around houses **[7].** This exposure is strongly associated with acute and chronic health effects in humans. However, total elimination of the use of pesticides is not possible even though it would be preferable **[8].**

To ensure the proper use of pesticides, the levels of pesticides residues in food stuffs are generally legislated so as to minimize the exposure of the consumer to harmful or unnecessary intakes of pesticides **[9]**. The levels selected pesticide residues in leafy vegetable (*Amaranthus Spinosus*) samples is yet to be elucidated. Therefore, this research was undertaken to investigate selected pesticide residues in leafy vegetable (*amaranthus spinosus*) samples consumed in Donga, Taraba state

MATERIALS AND METHODS

Sample collection

Five samples of leafy vegetable (*Amaranthus Spinosus*) which is an extensively grown vegetable in Donga local government area were collected from a vegetable farm situated about Ten meters away from Donga river.

Sample preparation

The Acetate-buffered QuEChERS sample preparation method for pesticides (AOAC official method 2007.01) was applied to all the samples after homogenization with a house-hold mill (equipped with stainless steel knives). 15g portion of each of the homogenized samples were weighed into 50ml polytetrafluoroethylene(PTFE) tubes and 100ml of 50mg/ml triphenyl phosphate (TPP) surrogate standard solution in acetonitrile was added to each of the tubes followed by 15ml of acetonitrile containing 1% acetic acid (v/v not accounting for purity). Then, 6g magnesium sulphate and 2.5g sodium acetate trihydrate (equivalent to 1.5g of form) were added and the samples were shaken and

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centrifuged at 4000rpm for 5min and 5ml of the supernatant from each of the tubes were transferred to a 15ml PTFE tube to which 750mg magnesium sulphate and 250mg PSA were added to each of the PTFE tube. The extracts were shaken using a vortex mixer for 20seconds and centrifuged at 4000rpm again for 5minutes, approximately 3ml of the supernatants were filtered through a 0.45mm PTFE filter (13mm diameter) and 800ml portions were transferred to autosampler vials. The extracts were evaporated to dryness under a stream of argon and reconstituted in 800ml acetonitrile/water (20/80, v/v) for the LC-MS/MS analysis. For the matrix-matched and standard addition calibrations, 4 to 80ml of reconstituted samples were transferred into autosampler glass inserts and 20ml portions of 0, 250, 500 and 1250ng/ml and standard solutions containing pesticides in 25/75 acetonitrile/water (v/v) were added to reach the final additional concentrates of 0, 50, 100 and 250ng/ml equivalents respectively.

Liquid chromatography-Mass spectrometry analysis (LC-MS analysis)

For liquid chromatography analysis, an Agilent 1100 HPLC system was used. It contained a binary pump, degasser, column thermostat and an autosampler. A reverse-phase C8 analytical column of 150mm x 4.6mm internal diameter and 5µ particle size and a guard column of 125mm x 4.6mm and 5mm particle size were coupled to the LC system. Deionized water containing 0.1% formic acid (mobile phase component A) and acetonitrile (component B) were employed for the gradient programme, which started with 20% B for 3minutes and was linearly increased to 100% B in 27 minutes (held for 3minutes). The column was then re-equilibrated for 12 minutes back to the 20% B.Thus, the total run time took 45minutes. The flow rate was constant at 0.6ml/min, and injection volume was 10µl. for the MS/MS analysis, an applied biosystems 3200 QTRAP system was used. Applied biosystems analyst 114.2 software was used for instrument control and data processing, for the determination of pesticides, the commercial method of biosystems (2005) and its library was used.

Statistical analysis

The data were analyzed using statistical package for social science (SPSS) software version 21 and the group mean were compared for significance at $p \leq 0.05$. Data were presented as mean ± standard deviation.

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RESULTS

Table 1 shows the levels of selected pesticide residues detected in *Amaranthus spinosus* harvested from Donga Local Government Area, Taraba State, Nigeria. The mean ± standard mean errors of pesticide residue concentrations were measured based on pentaplicate determination. In all, nineteen pesticide residues namely, cyclodienes, cyclohexanes, DDT, DDD, dicofol, perthane, endosulfan, pentachlorobenzene, mirex, toxaphene, hexachlorobenzene, methoxychlor, aldrin, dieldrin, alpha-HCH, beta-HCH, gamma-HCH, chlordane and heptachlor were detected in the analyzed samples.

From the result, the lowest to highest pesticide residue concentration range from 0.360µg/kg to 1.866µg/kg with pentachlorobenzene having the lowest mean concentration (0.360 ± 0.063µg/kg) and gamma- HCH having the highest mean concentration (1.866 ± 0.483µg/kg) (Table 1). The mean concentrations of Dicofol (0.386µg/kg), Dieldrin (0.694µg/kg), endosulfan (0.538µg/kg) and Hexachlorobenzene (0.362µg/kg) are relatively low compared to the mean concentrations of other pesticide residues detected. It was also observed that the mean concentrations of perthane, chlordane, mirex and toxaphene were (1.226 ± 0.333µg/kg), (1.086 ± 0.307µg/kg), (1.102 ± 0.288µg/kg) and (1.130 ± 0.306µg/kg) respectively with perthane having the highest mean concentration and chlordane having the lowest mean concentration (**Table 1**).

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Print ISSN: 2397-7728,
Online ISSN: 2397-7736

Table 1: Result of pesticide residue analysis.

Pesticide residues	**Cyclodienes**	**Cyclohexanes**	**DDT**	**DDD**	**Dicofol**	**Perthane**	**Methoxychlor**	**Aldrin**	**Dieldrin**
Mean ± Std mean error (µg/kg)	1.088 ± 0.312	1.122 ± 0.444	1.412 ± 0.361	1.280 ± 0.317	0.386 ± 0.135	1.226 ± 0.333	1.450 ± 0.394	0.960 ± 0.283	0.694 ± 0.232

Heptachlor	**Chlordane**	**Endosulfan**	**Hexachlorobenzene**	**Pentachlorobenzene**	**Mirex**	**Toxaphene**	**Alpha-HCH**	**Beta-HCH**	**Gamma-HCH**
0.782 ± 0.274	1.086 ± 0.307	0.538 ± 0.160	0.362 ± 0.098	0.360 ± 0.063	1.102 ± 0.288	1.130 ± 0.306	1.596 ± 0.427	1.432 ± 0. 388	1.866 ± 0.483

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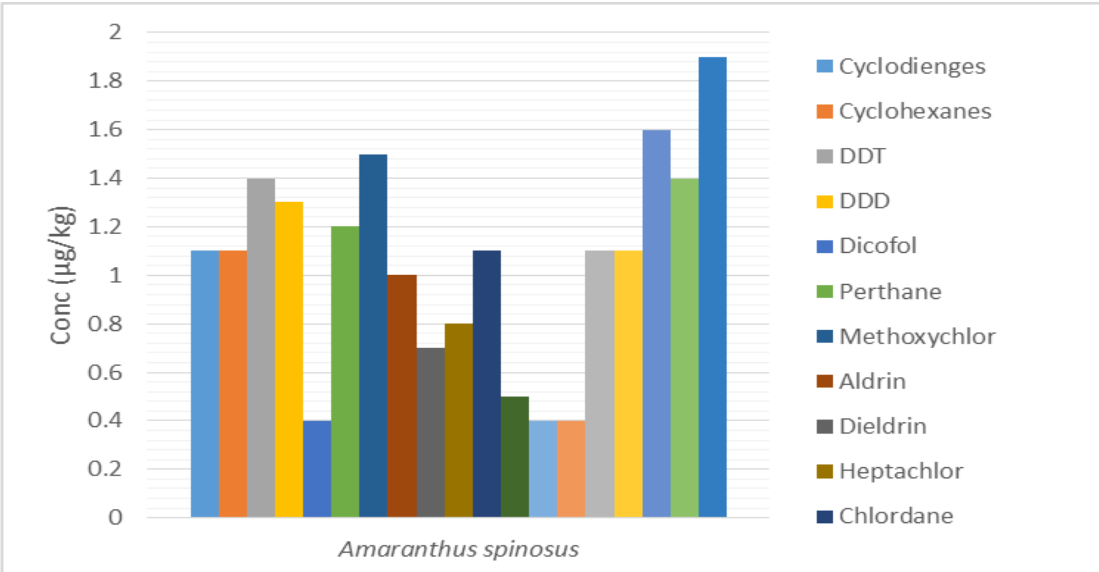

Figure 1: Mean concentrations of selected pesticide residues in *Amaranthus spinosus*

The order of pesticide residue concentrations in the analyzed samples was gamma-HCH > alpha-HCH > methoxychlor > beta-HCH > DDT > DDD > perthane > cyclohexane > toxaphene > mirex > cyclodienes > chlordane > aldrin > heptachlor > dieldrin > endosulfan > dicofol > hexachlorobenzene > pentachlorobenzene (**Figure 1**).

Generally, the mean concentrations of all the pesticide residues detected in the analyzed samples were above the maximum residue limits set by FAO and WHO. More precisely, the mean concentrations of DDT and its metabolite, DDD in the analyzed samples were higher than the WHO and FAO MRLs of 1.0µg/kg, indicating contamination of the samples by pesticides. Also, the mean concentrations of Dieldrin and Aldrin were found to be higher than the WHO/FAO MRLs of 0.2µg/kg. The mean concentrations of alpha-HCH, beta-HCH, and gamma HCH, were also observed to exceed the MRLs of 0.01µg/kg set by WHO/FAO.

DISCUSSION

The result of this study shows that the pesticide residues belong to class Organochlorines. Residues of organochlorine pesticides have the peculiar characteristics of relatively high chemical stability and persist in the environment for long periods **[10].**These chemicals have been banned by countries who signed the 2001 Stockholm Convention – an international treaty that aims to eliminate or restrict the production and use of persistent organic pollutants **[11].**The result of this study can be attributed to the

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high frequency of 0rganochlorine pesticide application on farms, the short time intervals allowed between the numerous applications **[12]** and the pesticide retention capacity of the leafy vegetable surfaces **[13]** since the mean concentrations of all the pesticide residues detected in the analyzed samples were above the maximum residue limits set by FAO and WHO.

A study by Lozowicka et al. **[14]** revealed the presence of organochlorine pesticides such as Aldrin, dieldrin, p,p'DDE and p,p'DDD, among others from samples of beans, cucumber, lupine, tomatoes, carrots, celery and parsley collected from Kazakstan and Poland. A report by Otitoju and Lewis **[15]** also shows the presence of similar organochlorine pesticides in bean samples from Wukari, Taraba state. Previous works have detected the presence of organochlorine pesticides such as Aldrin, dieldrin and DDT at concentrations higher than the MRL, set by European Union, in bean samples from Maiduguri and Lagos respectively **[16-17]** which is in tandem with the result of this study. This suggests the high use of organochlorine pesticides in the cultivation of leafy vegetables in Donga, Taraba state.

CONCLUSION

This study revealed the presence of various pesticide residues in the analyzed samples of *Amaranthus spinosus* with organochlorines being the most present. This confirms the wide use of organochlorine pesticides in the study area. It was also observed that all the pesticide residues found present in the analyzed samples exceeded the Maximum Residue Limits (MRLs) and Acceptable Dietary Intake (ADI) which connotes the extensive use and prevalence of pesticides in the study area. It could be reasonably concluded that the extensive use of these pesticides for the sake of improved yield and pest control by farmers with little or no knowledge on the effects of wrong use of pesticides could result in the transfer of these pesticides through exposure and consumption of foods containing their residues, and this can be deleterious to humans. Thus, improper use of pesticides by farmers with inadequate knowledge on these pesticides for the purpose of yield increase and pest control should be checked through adequate control of the trade and use of pesticides and the enforcement of appropriate sanctions.

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