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Screening of selected vegetables from Wudil, Farmlands In Kano State, Nigeria for organophosphorus and organochlorine pesticide residues

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ABSTRACT

Improper pesticide use in agriculture in rural areas portends huge health hazard to humans and other biological life forms. This present study was aimed at screening four vegetables randomly sampled from selected farmlands in Wudil, Kano State of Nigeria for pesticide residues and their derivatives with the intention of determining possible contamination from pesticides residues. The extraction was carried out by standard procedures and the identification and quantification of pesticide residues were carried out by gas chromatography–mass spectrometry analysis. The result revealed chlorpyrifos (an organophosphorus) as the only known active pesticide residue detected in all four vegetables. The mean levels of chlorpyrifos detected (1.42, 1.69, 1.86, and 2.10 mg/kg of sample) in spinach, carrot, cabbage, and garden egg, respectively, were significantly higher ($p < 0.05$) than the WHO/FAO maximum residual limit of 0.5–1.0 mg/kg of sample. Also, naphthalene and alkylben-zenes, which are volatile organic compounds described as co-formulants in pesticides were detected. This result is indicative of risk and public health concerns. Sensitization of farmers on best practice as well as strict monitoring and regulation of pesticide use is still hugely desired.

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Introduction

Pesticide residues are very small amounts of pesticides that can remain in or on a crop after harvesting or storage and make their way into the food chain. They can remain even when pesticides are applied in the right amount and at the right time. Pesticide residue refers not only to the active ingredient but also to any derivatives of pesticide, such as conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance [1].

In a country where large-scale agriculture contributes hugely to the economy and gross domestic product, control of pests is very central to the attainment of food security [2]. Pesticides are extensively used in agricultural production to check or control pests, diseases, weeds, and other plant pathogens in an effort to reduce or eliminate yield losses and preserve high product quality [3,4]. Although pesticides are manufactured under very strict regulatory processes to function with more specificity and minimal impact on human health and the environment, serious concerns have been raised about health risks resulting from residues in food and this is becoming a fast-growing global problem [3,4]. Most pesticides by their very nature show a high degree of toxicity because they are intended to kill certain organisms and thus create some risk of harm. They are also resistant to chemical and biological degradation and thus accumulate in both aquatic and terrestrial food webs [5,6]. Over 98% of sprayed insecticides and 95% herbicides reach a destination other than their target species, including non-target species, air, water, and soil [7]. It is within this framework that pesticide use has evoked grave concerns not only of potential effects on human health but also about

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impacts on wildlife and sensitive ecosystems. More than 700 pesticides are currently registered for use in the world and more continue to persist in the environment, even though they are no longer being applied. According to the Stockholm Convention on Persistent Organic Pollutants, 10 of the 12 most dangerous and persistent organic chemicals are pesticides [7]. Currently, organochlorine and organophosphate pesticides are widely used for agricultural and non-agricultural purposes in Nigeria even though the former have been banned in most countries due to their high persistence in the environment, their residues are still found in many matrices. More attention is usually devoted majorly to the toxicity and persistence of the pesticide active ingredient(s) and some of their degradation products. Relatively little research has been conducted to examine pesticide inactive ingredients, sometimes termed co-formulants or inert ingredients which constitute some environmental and consequently health hazards [8].

Fruits and vegetables are important components of the human diet after cereals. They are widely used for culinary purposes. They are of great importance in the diet because of the presence of vitamins and mineral salts and offer advantages over dietary supplements. A generous intake of fruits and vegetables still prevent various types of diseases and keep a person energetic and active all through life [9,10]. Residues in fruits and vegetables are of grave consequence considering the fact that they are eaten either raw or without further processing [11]. Some studies in Nigeria and other parts of the world have reported significant levels of pesticide residues detected in various vegetables despite regulation and sensitization on best practice in pesticide use in Agriculture; spinach and cabbage [4], maize [12] rice [13], carrot and cucumber [14], apples, grapes, pears, guava and eggplants [15], and a host of other agricultural crops. It is upon this premise that this present study was aimed at screening four randomly sampled vegetables from selected farmlands in Wudil, Kano State of Nigeria for pesticide residues.

Materials and Methods

The selected vegetables were carrot, spinach, garden egg, and cabbage.

Study area

Wudil is a small city in northern Nigeria, with the population close to 180,000 people. It is the capital city of a small district, Wudil district, and a small transportation knot near the Highway A237. Since the city is situated near Hadejia River, which has Wudil River as one of its tributaries, it is also an agricultural spot, with numerous farmlands and cattle companies operating in the city.

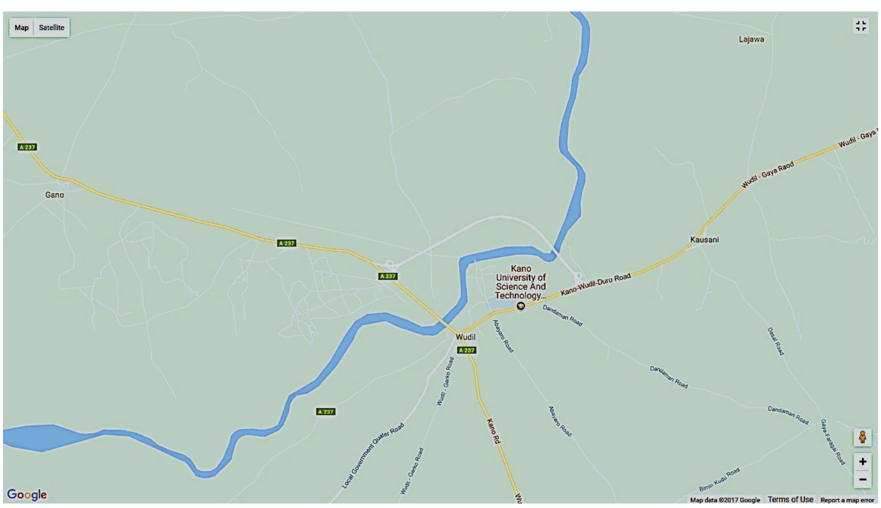
Reagents and solvents

The reagents used were of analytical grade. They include: chlorpyrifos pestanal, ethyl acetate, sodium hydrogen carbonate ($NaHCO_3$), anhydrous sodium sulfate (Na_2SO_4), cyclohexane, hexane, silica gel, and distilled water.

Sample collection and preparation

Collection

Collection of samples was carried out as described by Akan et al. [4] within the month of August 2017, being the middle of the farming season in the study



Map 1. Satellite map view of Wudil River area. Source: *Google map and GPS system*, (2017)[16].

area. At each selected sampling site, 20 g each of the four selected vegetables were collected from three randomly selected locations in each area to provide replicate samples of each crop. The vegetable samples were collected in a clean polyethylene bag, labeled, and transported to the Biochemistry and Forensic Science Department Laboratory in Nigeria Police Academy and preserved in a refrigerator at 4°C, until extraction.

Extraction of vegetable samples

The method of extraction used for the vegetables was the United State environmental protection agency method 3510 for extracting pesticide residues in non-fatty crops, using ethyl acetate as the solvent as described by Adusumalli [17]. $NaHCO_3$ was used to neutralize any acid that may be present and the vegetable samples were washed thoroughly with distilled water. Twenty grams (20 g) of each of the samples were weighed out, cut into smaller pieces, and Na_2SO_4 was used to remove water from the sample matrix. After reweighing, the samples were washed thoroughly with distilled water and placed in a mortar and ground to a paste using a pestle. The paste was transferred into a conical flask with the help of a spatula and 40 ml of ethyl acetate was added and shaken thoroughly. A 5 g of $NaHCO_3$ was added to the mixture, followed by 20 g of Na_2SO_4 and the entire mixture was shaken vigorously for 1 hour. This process was to ensure that enough of the pesticide residue dissolved in the ethyl acetate. The procedure was repeated for the samples from each area and the mixture was filtered into a labeled container before being centrifuged at a speed of 1,800 rpm for 5 minutes. The organic layer was decanted into a container and a 1:1 mixture of 5 ml ethyl acetate and cyclohexane was added.

Cleaning up of extracts

A 10 mm chromatographic column was filled with 3 g silica gel that was activated at 100°C in a microwave oven and topped up with 2–3 g of Na_2SO_4, 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 n-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.

Determination of pesticide residues

The analyses were performed using a Shimadzu gas chromatography–mass spectrometry (GC-MS) QP 2010 Ultra equipped with an mass selective detector, fused silica capillary column Rtx-5MS (30 m × 0.25 mm × 0.25 µm), and an autosampler [18]. The initial temperature program was 90°C, held for 2 minutes. The temperature was then ramped up to 260°C at a rate of 5°C/min and held for 5 minutes. The injector temperature was 250°C. The carrier gas was helium at the flow rate of 2.17 ml/minute with an average velocity of 54.6 cm/second. The GC-MS was operated in splitless mode with a purge flow of 3 ml/minute; the injection volume was 1 µl and the pressure was set at 150 kPa. The GC interface temperature was 300°C. The mass spectrometer was operated in full scan mode (45–500 m/z) on electron impact (EI) ionization at 0.2 V with ion source temperature of 230°C. The identification and quantification of the compounds were accomplished by comparing the retention times and mass spectra of analytes in samples to data from the National Institute of Standards and Technology (NIST) 14 mass hunter spectral library (US National Institute of Standards and Technology) and also that of reference standard (chlorpyrifos pestnal) run at the same conditions with the samples. A specific pesticide was identified if it had the same retention time as the standard (within a deviation of ±0.05 min) and their spectra data matches that from the NIST spectral library. The semi-quantification was done by comparing the peak area of the analyte (identified chlorpyrifos) in the sample with the peak area of the standard (chlorpyrifos pestanal) of a known concentration.

Results and Discussion

The result of the identification and semi-quantification of pesticide residues in four randomly selected vegetables from farms in Wudil is presented in Figure 1 and Table 1. The results revealed that an organophosphorus (chlorpyrifos), alkyl benzene (with varying substitution patterns), and naphthalene were identified in all the samples. Different methyl and ethyl substituted benzenes were identified in the different samples: 1,2,3,4-tetramethyl benzene in all samples; 1-ethyl-3,5-dimethlylbenzene in garden egg; 1,2,3-trimethylbenzene, 2-ethyl-1,4-dimethylbenzene in cabbage; 1-ethyl-,2,3-dimethylbenzene, 2-ethyl-1,4-dimethylbenzene in spinach; 4-ethyl-1,2-dimethylbenzene in carrot and garden egg; 1-ethyl-2,3-dimethylbenzene in cabbage and spinach; 1,3,5-trimethylbenzene in carrot, garden egg, and spinach.

Chlorpyrifos is a well-known broad-spectrum organophosphate pesticide commonly used in rural agricultural farmlands in Nigeria in different brands. Thus, this explains its detection in the samples. No organochlorine or pyrethroid pesticide residue was detected. This could be attributed to the fact that organochlorine pesticide is no longer favored in use in agriculture because of the perceived persistence in the environment. According to Abdulhamid et al. [7], this class of pesticides is banned and thus has resulted in the decline in its use. On the other hand, pyrethroid has greater photostability, enhanced insecticidal activity, and relatively low toxicity as compared with organochlorine and organophosphorus pesticides but are not readily affordable and available to some local farmers hence the decline in use. Organophosphorus is more favored in use and as such are detected or observed more frequently in samples. Alkylbenzene and naphthalene derivatives are not known as active ingredients in pesticides but are volatile organic compounds (VOCs) that included as co-formulants or inert ingredient in pesticide formulations. These are not usually contained in the material safety data sheets (MSDS) or official label documentations of the pesticide product [8]. This present study identified these VOCs in the samples studied with relatively high area percentages. The emission of these VOCs negatively impacts on the environment with a effect impact on ozone and ambient air quality [8]. The result showed that chlorpyrifos was the only known pesticide residue found in all four vegetables. The relative concentration of chlorpyrifos detected in the samples ranged from 1.42 to 2.01 mg/kg of the sample (Fig. 1). These levels observed were 2.84–4.02 fold higher than the FAO/WHO MLRs for chlorpyrifos [19]. This result is in line with findings of Mahugija et al. [18]. They reported that all the concentrations of chlorpyrifos detected in samples obtained in Dar es Salaam significantly exceeded the FAO/WHO MRLs. A similar result has been reported by Chilumuru et al. [20] and Jallow et al. [21]. This trend might have grave consequences on human health as these vegetables are consumed raw with little or no further processing. The selected vegetables in this study are usually consumed with little or no further processing, thus there will be little or no alteration in the level or chemical composition of contaminating residues on them. Thus, with the levels recorded, large consumption of these contaminated vegetables may lead to bioaccumulation of the pesticide residues to a level that might provoke toxicological effects. According to Akan et al. [4], the implications of high pesticide residue include muscle cell degeneration, which involves the respiratory muscles, and chronic exposure may damage the peripheral nervous system, affect patient's behavioral abilities and/or personality, cause chronic fatigue

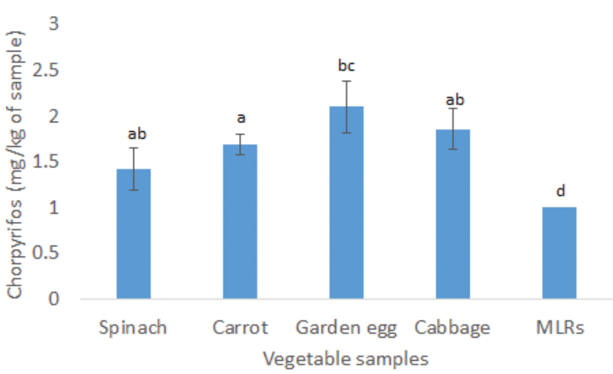


Figure 1. Mean concentrations of pesticide residues in samples.

Footnotes: Superscripts. Values bearing any different superscript are statistically ($p < 0.05$) different MRLs.

Table 1. Summary of data obtained from GC-MS analysis of garden egg, carrot, cabbage, and spinach samples.

Peak No.	R_t (minute)	Compounds	Area (%)			
			Garden egg	Carrot	Cabbage	Spinach
1	5.40	1,3,5-trimethylbenzene	12.49	32.21	9.84	16.27
2	5.63	1-ethyl-3,5-dimethylbenzene	17.15	26.07	18.97	20.18
3	5.86	4-ethyl-1,2-dimethylbenzene	19.94	16.59	23.33	29.09
4	6.15	1,2,3,4-tetramethylbenzene	16.27	1.11	17.92	13.86
5	6.70	Naphthalene	1.57	21.60	1.27	1.14
6	14.86	Chlorpyrifos	28.29	32.21	25.05	17.15

syndrome, and have cardiology effects. The contamination of these vegetables may be attributed to multiple reasons ranging from poor use of the pesticides (non-compliance to appropriate application time, majorly), natural persistence of the pesticides in the environment, run off from neighboring farm lands where there is heavy use of pesticides, and so on. Although the farmers during closed group interaction/discussion claimed they use the pesticides appropriately, however, we cannot rule out the fact that good knowledge of pesticide use best practice was still somewhat lacking. The idea of best practices seemed to differ among the different farmers. The study was limited by our inability to quantify the detected inert co-formulants for lack of standards.

In conclusion, this study investigated the levels of pesticide residues in four commonly eaten vegetables in Wudil in Kano. The results indicated that all four vegetable samples were contaminated with a particular organophosphorus pesticide—chlorpyrifos and some volatile organic compounds that are possibly co-formulants. The concentrations observed were significantly higher than the FAO/WHO MRLs. From a public health perspective, this observed level of pesticide residues may pose a potential health risk to consumers. Therefore, efforts should be devoted to sensitizing and enlightening farmers on the proper use of pesticides and best pesticide safety practices. Relevant agencies should tighten up regulations and monitoring of pesticide use in food crops. Regulations should also ensure that contaminated vegetables and food do not get into the general markets, this is particularly important in rural agricultural areas where poor use of pesticides is most common.

Abbreviations

FAO Food and Agricultural Organization;
WHO World Health Organization

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