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Assessment Of Levels Of Pesticide Residues And Their Degradation Products In Selected Grains From The Strategic Grain Reserve Facilities In Nigeria

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Abstract: **Samples of maize, millet and sorghum were collected from strategic grain reserve facilities located in eight states in Nigeria, while cowpea samples were collected from local open markets in Kano, Lagos and Akwa Ibom States of Nigeria. The samples were screened for the presence of fifteen pesticides (permethrin, bifenthrin, lamda-cyhalothrin, dicchlorvos, cypermethrin, DDT, parathion, malathion, aldrin, paraquat, diquart, chlordane, toxaphene, imidacoprid, actamiprid) using ultra high pressure liquid chromatography- coupled with mass spectrometry (UPLC-MS). The EPA Method 3540C solvent system in a Soxhlet extractor was applied for sample extraction and the method validation was satisfactory. Three pesticides were detected, viz permethrin, cypemethrin and dichlorvos and quantitative analyses of the pesticides, and their degradation products was carried out using gas chromatography coupled with electron capture detector (GC-ECD) and UPLC-MS. Spike recovery/reproducibility for GC-ECD was 90%,with LOD of 0.138ppm and LOQ of 0.46ppm. seven samples exceeded the WHO and FAO permissible levels for permethrin using GC-ECD, 14 samples exceeded the permissible levels of permethrin using UPLC-MS. 16 grain samples indicated the the presence of dichlorvos but below LOQ while 6 samples indicated the presence of cypermethrin but below LOQ. All cowpea samples had dichlorvos levels that exceeded the permissible levels by WHO and FAO (CODEX Alimentarius). Degradation products of permethrin and cypermethrin were detected in some samples. These results indicate the need for action to be taken to allow the revision of practices in the administration of pesticides during both cropping and storage of grain in Nigeria to reduce risks of pesticide poisoning.**

Keywords: **Pesticide residue, degradation product, Strategic grain reserve facility, Maize, millet, Sorghum, cowpea, GC-ECD, UPLC-MS.**

1. INTRODUCTION

Since the beginning of history, mankind has had to compete with more than one million other species for the earth's goods essential to survival [1], [2]. Insects have been one of man's more serious problems since prehistoric times until now [3]. A pest is any organism, usually an animal, judged as a threat to humans. The agricultural significance of pests and diseases of crop plants is that the damage they cause reduces the quality or quantity of yield [4]. The economic impact of insects is measured not only by the market value of products they destroy and the cost of damage they inflict but also by the money and resources expended on prevention and control of pest out breaks [5]. Pesticides are biocides especially designed to kill, repel, attract and mitigate the organisms, which are nuisance to humans and their agricultural and hygiene activities. The pesticides may be the organic molecules or inorganic synthetic molecules or the bio-pesticides [6].

Several reports show that global pesticide usage has increased significantly during the last three decades consequent with changes in farming practices and the increasing intensive agriculture [7], [8].

Nigeria grows several food grains from which the country has gained an impressive capacity to develop a food-grain policy. Strategic grain reserve facilities have been launched to provide first line of food relief internally and to friendly countries in times of disaster and to make food available at other times at affordable prices [9]. The preservation of grains in the silos requires the use of pesticides. In 2008 Nigeria reported that 112 people had been poisoned by pesticide contaminated food. Two children died as a result. Another report from Nigeria recorded 120 cases of poisoning of students who had eaten beans contaminated with lindane [10]. Also in Nigeria, scenarios have occurred in which contamination of grain by pesticides applied to preserve them caused sickness and death [11], [12]. These events suggest the possibility of health insecurity of food stuffs. To ascertain food security from contamination and associated health hazards, chemical analysis of food stuff drawn from the Nigerian strategic grain reserves is imperative to ascertain the safety of such food stuffs deployed for either trade or charity purposes.

A pesticide is any substance or mixture of substances used to destroy, suppress or interrupt the life cycle of any pest. In crop production, the farmer has run into problems of low yield and return arising from the facts that:

- Crops fail because of excessive pest infestation.
- Weeds compete with crops for nutrients and reduce yields.
- Pests reduce the quality of products on the field and in storage.
- Pests follow produce into storage and cause damage in storage.
    - To contain the damage done by pests and weeds to crop production, a need arises to combat pests both in the field and in storage.
    - In use, applied herbicides and other pesticides decompose to stable residues.
    - These residues persist in the environment to different extents and produce associated adverse effects on the environment and stored produce.

The aim of this research is to determine the levels of pesticide residues and their degradation products in stored grains and their impacts on the quality of the stored grains.

Since 1940, the production, marketing and ongoing use of various pesticides has increased to the present day. Exposure to pesticides, either at pre-harvest or post- harvest, can have adverse effects on health and the environment. [13]. In what was captioned "Nigerian Mystery Deaths" in April, 2015, by [14], whereby 18 persons died in South Western Nigeria and was suspected to arise from pesticide poisoning various incidents of food poisoning have been reported which has continued to increase as years go by. These deaths have been associated with pesticides used in storing the grains.

This has made it very necessary to ascertain accurately the levels of pesticide residues and their degradation products in stored grains from strategic grain reserve facilities in Nigeria.

II. MATERIALS AND METHODS

Chemicals and Reagents: All chemicals used in this research were of very high purity, analytical grade. Solvents applied for liquid and gas chromatography were of pesticide analytical grade. Deionized water was used generally to prepare sample and reagent solutions throughout the study.

Samples/Sampling: The samples analyzed are grains stored in Strategic Grain Reserve facilities in Nigeria. They include red and white maize (RM/WM), Brown and white sorghum (BS/WS), Millet (M) while all cowpea (CP) samples where obtained from the open markets in Kano, Akwa Ibom and Lagos. The Strategic Grain Reserve facilities that were sampled for this work are located in a spread of eight states in Nigeria including Abuja, Akwa Ibom, Gombe, Jigawa, Kano, Niger, Plateau and Lagos. To obtain a sample at each storage facility, a grain sampler was applied to fetch the grain from all aspects of the facility including the top, bottom, middle areas and side aspects into a polythene bucket. The collected grain was emptied on a cleaned, dried wooden platform. The grain was then coned and quartered and one quarter of each cone selected. From the selected quarter cones, about 300g sample was packaged in a polypropylene bag.

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Sample Storage/Sample Extraction: At the laboratory, the samples were blended in a coffee grinder and stored in dry sample bottles from which 5.0g was weighed out and used for the analysis.

5.0g of powdered sample was inserted in a 25mm x 80mm Whatman thimble and extracted with 100ml of a 1:1 hexane-acetone solvent mixture (EPA Method 3540C) in a Soxhlet extractor run at 93°C for 6 hours, using a magnetic stirrer to stir the mixture at 3000rpm. 1.0ml fractions of the extract were dispensed into GC vials. Each vial was capped and labeled for GC/ECD and LC/MS analyses. Sample blanks were similarly extracted for six hours.

Preparation of calibration graphs: The methods used to analyze pesticides in this research have to be calibrated. In each case, the analytical standards prepared for this purpose were measured and the signals recorded against the solution concentrations. These data are applied to construct a peak area versus concentration graph. Signals obtained by a method for a sample were interpolated on the graph to obtain the concentration of the pesticide(s) in the sample using the LINEST OUTPUT. [15].

Analysis by UPLC/MS: The sample extracts dispensed in vials labeled for UPLC/MS at the end of sample extraction were analyzed by the UPLC instrumentation. The results obtained, after calibration, are presented in Table 1.

Analysis by GC/ECD: The sample extracts were dispensed in vials and labeled for GC at the end of sample extraction and analyzed by the GC/ECD instrumentation. Calibration data are shown in Table 2 for the test pesticides. Apparently dichlorvos and cypermethrin occur only at extremely low levels in samples below the detection limits of the method/ instrument for these pesticides. During analysis, it was observed that this method/ instrument was sensitive to only permethrin in samples; it did not indicate other pesticides in samples. The method/instrument, however, responded to various levels of all tested pesticides in synthetic solutions.

Recovery and Reproducibility Studies for GC-ECD: 1ml of a concentrated solution of 400µg/ml permethrin standard pesticide was added to 5g of sample in an extraction thimble and extracted with 100ml of 1:1 hexane-acetone mixture for six hours in triplicate. This final mixture was dispensed for analysis by GC/ECD and LC/MS for the test pesticides. The level of permethrin measured in this spiked sample was compared with the amount that was purposely added as a spike and the % recovery calculated.

Determination of the Detection Limit/Limit of Quantification of GC/ECD for Permethrin in Grains

The regulatory limit for permethrin in grains is 0.05ppm; this is equivalent to 0.05µg in solid grain. If permethrin occurs at this regulatory value in a sample and 5.0g of the sample is extracted in 100.0ml of solution, this concentration becomes 0.05 x 5 i.e. 0.0025µg/ml. A synthetic solution with a concentration close to the detection limit was made and the measurement repeated in triplicate.

Limit of Detection for GC-ECD: In this work, the lowest concentration of permethrin detected on the GC-ECD was 0.04µg/ml; however, this concentration is not seen by the instrument most times or it had to be manually integrated on the instrument because the peak area is very small. So a concentration which is one order of magnitude higher i.e. 0.4 ug/ml was used for determining the detection limit. This solution was analyzed in triplicate by GC/ECD obtaining the peak areas 0.078 0.082 0.070.

The detection limit was calculated from the standard deviation (S) and the slope (M) of the standard plot using the equation

Detection Limit = 3S /M

= 3(0.00611) / 0.1321 i.e. 0.138ppm

Limit of Quantitation for GC-ECD

Limit of Quantification = 10S/M

= 10 × 0.00611/0.1321 = 0.463ppm

LOQ = 0.46ppm

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III. RESULTS AND DISCUSSION

Table 1: Pesticide Quantitation by UPLC-MS using LINEST Output

Silo	Sample	Pesticide Detected (ppm)		
		Permethrin	**Dichlorvos**	**Cypermethrin**
Abuja	R M	1.48	BLQ	BLQ
AkwaIbom	R M	ND	BLQ	ND
	W M	2.82	BLQ	ND
	C P	1.25	**4.70**	ND
Gombe	B S	1.49	BLQ	ND
	W S	ND	ND	BLQ
	W M	1.60	ND	BLQ
	M	9.65	BLQ	BLQ
Jigawa	W S	10.3	BLQ	BLQ
	B S	4.70	BLQ	BLQ
	M	1.43	BLQ	
Kano	B S	0.66	BLQ	ND
	W M	ND	BLQ	ND
	M	ND	BLQ	ND
	C P	ND	**0.19**	ND
Niger	B S	13.73	BLQ	ND
	W M	3.51	ND	ND
	R M	ND	BLQ	ND
Plateau	W M'12	8.83	BLQ	ND
	W M'16	0.82	BLQ	ND
Lagos	R M	ND	BLQ	ND
	C P	ND	**0.48**	ND

BLQ = Below Limits of quantitation, ND = Not Detected

Table 2: Results of sample analysis by GC-ECD using LINEST Output

Sample	Amount of permethrin (ppm)	WHO/FAO Permissible level
R M Abuja	0.35±0.092	0.05
B S Jigawa	2.9± 0.09	0.5
W S Jigawa	3.3±0.09	0.5
W M Niger	4.34± 0.089	0.05
R M Niger	1.26± 0.091	0.05
B S Niger	11.11± 0.085	0.5
W M Plateau'12	4.67± 0.088	0.05

Table 3: List of degradation products of permethrin and cypermethrin observed in sample extracts

Silo/sample	Degradation product/average peak area					
	3-phenoxybenzyl alcohol	3-hydroxybenzyl alcohol	Benzaldehyde	3-hydroxybenzaldehyde / benzoic acid	3-phenoxybe-nzaldehyde	Benzyl alcohol
Abuja						
RM	48984	ND	ND	ND	ND	ND
AkwaIbom						
CP	34653.59	22749.053	20674.8	ND	ND	ND
RM	ND	104231	ND	495114	ND	ND
WM	218324	330257	ND	239607		
Gombe						
BS	42764.67	29537.03	ND	19595.167	ND	ND
WS	ND	64779.68	1422117.293	7954.21	ND	ND
WM	56228	ND	ND	47910	ND	ND

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Jigawa						
BS	62157	4000648.33	1120720.333	56053.33	ND	ND
WS	65340	114164	590096.33	ND	31764	ND
M						
Kano						
BS	52279.2	722298.33	1647843.33	79432.333	ND	ND
CP	24291.333	ND	ND	ND	ND	ND
M	30902.333	ND	1180407.67	ND	ND	ND
WM	34620.667	143814	ND	86804.667	ND	ND
Niger						
BS	52994.667	286620.667	759975.33	41940	ND	ND
WM	37055	237236.667	1082163.33	79450.333	ND	52637.33
RM	47319.333	75955	1068700	80107.67	ND	22276.33
Plateau						
WM (2012)	100396	81325.667	143320.33	89178	ND	12070
WM (2016)	19359.5	389132	458237.5	244040	ND	59079.5
Lagos						
RM	61850.33	214767.33	885579	131877	ND	ND
CP	57863.33	9602.333	ND	ND	ND	ND

NB: No dichlorvos degredation product was detected; ND= Not Detected

From the analytical data obtained, after screening sample extracts for both pesticides and their degradation products , the degradation products actually observed in the samples by the test procedures are listed with their average peak areas as shown in Table 3. Average peak areas rather than concentrations were used because of the range of standards required and the additional calibration procedures involved. The peak areas however indicate the relative level of each degradation product observed, which are given in Hertz (Hz). No degradation products of dichlorvos was detected. The contamination of grains by these pesticides and pesticide degradation products have serious environmental, culinary and health impact (Gwary et al., 2012).

The optimum extraction time was deduced as the time at the onset of the extract peak area maximum. The value of extraction time was six hours which was routinely applied for sample extraction.

From all calibration data presented, satisfactory calibration of procedures was achieved: each calibration graph was a growth curve, initially including a long linear range. This long linear range was an advantage because it enabled sample extract concentrations to be interpolated without recourse to too many dilution steps. Sample results were obtained by interpolation of sample extract peak areas on the appropriate calibration graphs.

Out of fifteen pesticides screened in sample extracts, only three were indicated in the measurement by both methods.

GC-ECD indicated only permethrin in samples; UPLC-MS, however, indicated the permethrin and also dichlorvos and cypermethrin.

The results of sample analysis for permethrin using GC-ECD when evaluated against tolerance levels suggested in CODEX Alimentarius [16] are all prohibitively high.

The tolerances for permethrin residues in maize (corn) and sorghum grains are 0.05 and 0.5 ppm respectively. The observed levels in maize sample extracts are largely above the recommended tolerances; 7-fold, 87-fold, 25-fold and 92-fold respectively.

Similarly, the observed permethrin levels in the sorghum samples are all well above the allowable levels in the CODEX 5.8-fold, 6.6-fold and 22.2- fold.

A recovery of 90% of added permethrin spike was considered acceptable.

This recovery indicated that the grain matrix was inert towards permethrin so that added spike could be recovered again. This also suggests that measurements will be reproducible.

These studies need to be repeated for every new pesticide introduced for grain storage.

This technique was able to identify only permethrin, dichlorvos and cypermethrin. The permethrin concentrations in sample extracts when evaluated against allowable values are as offensive as similar results obtained by GC-ECD. Out of the 22 samples investigated by this procedure, 3 were free from dichlorvos (WS Gombe, WM Gombe, WM Niger).

Another 3 samples, all cow pea samples (CP Akwa Ibom, CP Kano, CP Lagos) had clearly measurable and quantifiable levels of dichlorvos. The other 16 samples, however, contained very low levels of dichlorvos, levels below the limit of quantification of the UPLC-MS procedure.

Results are also included for the determination of cypermethrin in samples. All the samples in which cypermethrin was indicated contain the pesticide at very low levels, levels below the limit of quantification (LOQ) of UPLC-MS. These included only six samples out of the twenty-two screened by this technique (RM Abuja, WS, WM, M Gombe, WS and BS Jigawa).

The findings from this work agrees with those of Abdlhamid et al. (2015) [17] in the determination of organochlorine and pyrethroid pesticides in some vegetables from farms located in Minna, as well as those of Ogah, Coker and Adepoju-Bello(2011) [18] who investigated organophosphate and carbamate pesticide residues in beans from markets in Lagos State Nigeria. In these works, as in the present work, abundant levels of test pesticides in the samples have been observed in the samples. While Abdlhamid et al. (2015) reported 0.51-9.95ug/ml cypermethrin in two samples of spinach, Ogah et. al. (2011), [19] reported 19.4 -455.9 ug/kg of residues of one or more organophosphate or carbamate pesticide in all samples analysed.

IV. CONCLUSION

- The sample results obtained in this work enable pesticide levels in stored grains in Nigeria and elsewhere to be easily determined, so that the quality of such stored grains is also easily determined. The outcome of this research is beneficial to agriculture by providing a basis for a more effective control of grain quality both in the field and in storage.
- Specifically, excesses of pesticides during use can be controlled and reduced; this reduces the chances of pick-up by the crop or grain during cropping or storage. Global health benefits immensely from such successful control.

The high levels of permethrin and dichlorvos detected by both procedures suggest that emergency action be taken to allow the revision of practices in the administration of pesticides during both cropping and storage of grain. During the suggested emergency action, best available practices for handling and application of pesticides should be defined and adopted as regulations. The practices in these regulations should be supervised and policed for strict compliance by local authority. In this respect, the concept of green chemistry could be invoked towards the elimination of residues and surpluses. Although levels of cypermethrin detected were below quantification, caution must be exercised in the administration of the pesticide both in cropping and storage. Environmental quality and health stand to benefit from the scheme to reduce residues and surpluses; the cleaner environment that will be attained predisposes to a healthy ecosystem in which the physical health of the environment and the health of the species inhabiting the ecosystem is enhanced. The food chain is protected when the levels of pesticide residues that threaten it are drastically reduced.

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