

## Pesticides Residue Contamination in commercial Maize (*Zymase*) and Cowpea (*Vigna unguiculata* L. Walp) crops from Mubi North Local Government Area of Adamawa State, Nigeria

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### Abstract

Pesticides especially organochlorines and organophosphates are known primarily as neurotoxins. However, reactive oxygen species (ROS) caused by organochlorines and organophosphates may be involved in the toxicity of various pesticides. Therefore, this study aimed to investigate the contamination levels of selected POPs (organochlorines, organophosphorus, and synthetic pyrethroids) in commercially sold maize and cowpea crops within the metropolis of Mubi North Local Government Area of Adamawa State, Nigeria. For this purpose three experiments were carried out to determine organochlorines, Organophosphorus and synthetic pyrethroids using Gas chromatography –mass spectrometry (Agilent GC-MS 7890A coupled with MSD 5975C) for the analysis from 10 randomly obtained samples of each sample. Limits of detection and quantification (LOD and LOQ) were both determined and the result shows both to be below the maximum residue limit of each pesticide. The results for organochlorines in cowpea and maize indicates the presence of Aldrin, Dieldrin, Endrin and Heptachlor in concentrations of  $0.073 \pm 0.002 \text{ mg/kg}$  and  $0.023 \pm 0.003 \text{ mg/kg}$  for Aldrin,  $0.098 \pm 0.005 \text{ mg/kg}$  and  $0.05 \pm 0.004 \text{ mg/kg}$  for Dieldrin and  $0.05 \pm 0.001 \text{ mg/kg}$  for Endrin in cowpea only with traces of Heptachlor in the sample. For Organophosphorus, chlorpyrifos was higher in both cowpea and maize ( $0.072 \pm 0.04 \text{ mg/kg}$  and  $0.063 \pm 0.03 \text{ mg/kg}$ ) and malathion was  $0.056 \pm 0.03 \text{ mg/kg}$  and  $0.032 \pm 0.07 \text{ mg/kg}$  respectively. Cypermethrin shows the highest concentration in all the samples with  $0.913 \pm 0.001 \text{ mg/kg}$  in cowpea. Indicators are high that maize and cowpea crops may be highly contaminated with pesticides with concentrations mostly close or above the maximum residue limits.

**Keywords:** Contamination; Pesticides; Organochlorines; Synthetic pyrethroids; Cowpea

### Introduction

In recent times, significant progress have been achieved in seeking better methods for pest control, weed control and yield optimization within the agricultural sector. However, these newer methods have over the years become a threat to human safety and to that of the environment at large. Persistent organic pollutants (POPs) are toxic, bio accumulate in adipose tissues, persistent and have long range transport potential. These chemicals are capable of causing serious health conditions even in minute concentrations due to their potential ‘cocktail effect’. Maize and cowpea crops are vulnerable to insect and pest attacks where proper storage facilities are lacking. In Nigeria, rural farmers resort to post harvest application of various pesticides directly on food crops which are sold and consumed before these chemicals are degraded into less toxic forms. Persistent Organic Pollutants are a group of naturally occurring or chemically synthesized organic compounds that have high resistance to biological, chemical or photolytic degradation (El-Shahawi *et al.*, 2010). A study

reported by Wang *et al.*, (2005), informs that these chemicals are being conveyed into the food chain, which poses a serious threat of contamination in the food chain. Mechanisms for such transfers into the

food chain include root uptake, particle deposition, diffusion etc amongst others (Anna *et al.*, 2010). Furthermore, high levels of POPs contaminants have been reported not only in food crops, but also in water (Akan *et al.*, 2014), sediments (Hai *et al.*, 2003), breast milk and fish (Jacobson, 1996) etc.

The use of pesticides, including insecticides, fungicides, herbicides, rodenticides and synthetic fertilizers for pest control, weed control or yield optimization has significantly reduced losses and improved the yields of crops, as well as protect animals from diseases and parasites. However, despite their large advantages, these agrochemicals pose a serious danger to the ecosystem (Fernando, 2006).

According to Sweetman *et al.*, (2000) (as cited in Luisa *et al.*, 2009), ingestion of contaminated food or water is the most significant route of human

exposure to these compounds, accounting for >90%, when juxtaposed with other ways such as inhalation and dermal contact.

Pesticides are almost always found in mixtures with other ones. For this reason, the combined toxicological effect of two or more components of pesticides mixtures can assume one of three forms; independent, dose-addition or interaction (Antonio *et al.*, 2012). Example of such may include potentiation of organophosphorus toxicity by organochlorine insecticides, potentiation of Malathion toxicity by isomalathion, antagonism between triazine with perchloraz etc.

Maize and cowpea crops are major food crops, most especially in northern Nigeria. Due to poor storage facilities, local farmers and traders rely on post-harvest application of pesticides in order to protect their crops from pest attacks and avoid economic loss (Olufade *et al.*, 2014). For instance, in Northern part of Nigeria, about 95% of stored cowpea is treated with insecticides at instances where they are not even required (Lowenberg-Deboer and Ibro, 2008, as cited in Olufade *et al.*, 2014).

This study was designed, as a preliminary survey, to investigate the occurrence levels of organochlorines, Organophosphorus and synthetic pyrethroids pesticides residues in maize and cowpea crops in whole sale markets of Michika and Mubi North Local government areas of Adamawa state, Nigeria. These two local governments are trade oriented, and their markets significant in grain supply within the state. This makes this study relevant and important

In a futile attempt to eliminate the prevalence of some of these organic pollutants in Nigeria, for instance, the National Agency for Food, Drug, Administration and Control (NAFDAC) has banned the importation and use of 30 different organochlorines in 2008 including aldrin, dieldrin, DDT, however, studies shows that they are still in use within the country due to their effectiveness and affordability (Akan *et al.*, 2014)

The major aim of this research is to ascertain the safety of these grains in terms of POPs contamination, so that appropriate measures can be adopted.

## Materials and Methods

### Sample collection

The maize and beans grains used for this research were obtained at the major markets within the metropolis of Michika and Mubi North local government areas of Adamawa state, Nigeria. Since the research intends to elicit information about maize and beans crops generally sold within the study area, maize and beans crops were randomly obtained from ten (10) different points within the

market. A composite of each (maize and beans) was made by weighing the same amount of the samples obtained from the various points and homogenized to obtain a representative sample.

### Sample preparation

The samples collected were first cleaned by picking out stones and other extraneous materials. 200.0g portion of sample from each unit was taken and grinded to 20 mesh particle size to produce a good homogenate. The produced samples was then stored in glass bottles with appropriate labels in a refrigerator at 4<sup>0</sup>C.

### Extraction of organochlorines pesticide residues.

Organochlorine residues was extracted according to methods of analysis of food pesticides residue described by Food safety and standard authority of india, (FSSAI, 2015). 30g particles of dry samples were mixed with 50mL aqueous acetonitrile (350ml H<sub>2</sub>O and 1L acetonitrile). The mixture was introduced into a blender and blended for 5 minutes. The homogenized mixture was filtered through suction filter. 250ml of the filtrate was transferred into a 1L separator. 100ml petroleum ether was added to the filtrate in the separator. The mixture was shaken vigorously for 1-2 minutes, to which 10ml of sodium chloride saturated with 650ml H<sub>2</sub>O was added. The separator was held in a horizontal position and its content mixed vigorously at 30-45<sup>0</sup>C. The mixture was left to separate, which formed two layers. The aqueous layer was separated and discarded, while the solvent layer was gently washed with 100ml portions of distilled water and the washings was discarded. Solvent layer was transferred to 100ml glass stoppered cylinder and the volume V was recorded. 15g anhydrous Sodium sulfate was added and shaken vigorously. The extract was allowed to remain with the sodium sulphate for about 45 minutes. This was because allowing the extract to stay with the sodium sulphate for more than an hour may result in loss of Organochlorine pesticides by adsorption. The extract was concentrated to about 10ml in a rotary evaporator.

### Clean- up procedure

The concentrated petroleum ether extract was taken into a 250 mL separator funnel. Concentrated sulphuric acid was added drop wise using a Pasteur pipette until the upper layer of petroleum ether becomes clear. The lower phase of the spent sulphuric acid was discarded while the upper layer with three 10mL portions of distilled water. The petroleum ether layer was dried over sodium sulphate to the desired volume. Take the concentrated petroleum ether extract into 250 mL separator funnel and drop wise 10mL concentrated sulphuric acid a Pasteur pipette till the upper layer of petroleum ether becomes clear, discard the lower

phase of spent sulphuric acid and wash the upper layer with three 10 mL portion of distilled water. The petroleum ether layer was dried over sodium sulphate and made to the desired volume (FSSAI, 2015).

#### **Multi residue method for determination of Organophosphorus pesticide residue**

##### **Sample extraction**

50g of dry sample was put into a high speed blender to which 50mL water was added. The blended sample was split in halves. To the first half, 100 mL of dichloromethane and 100 mL acetone and 10 g of sodium chloride was added and placed in a separator funnel. The solution was vigorously shaken for 1 minute until the sodium chloride dissolved. The two layers were allowed to separate. The aqueous layer was transferred into a second separator funnel. The organic layer was dried through sodium sulphate. 200 mL portion of dichloromethane was added to the second separator funnel and shaken vigorously. Sodium sulfate was rinsed with Ca 50 mL dichloromethane. The organic layer and washings were collected and concentrated to dryness using a rotary evaporator (40- 45 °C) water bath reduced pressure (FSSAI, 2015).

##### **Chromatographic column cleanup**

Glass column (2 cm id glass column) was filled with 2g celite followed by 4g of carbon celite (1+4) and top with glass wool plug. The column was washed with 20 mL benzene. The sample was quantitatively transferred into the column with small portions of benzene (Ca 2mL) and pesticide was eluted with 60mL of acetonitrile-benzene (1:1). The elute was concentrated to dryness in a rotary evaporator (45- 50 °C) water bath, reduced pressure). Suitable benzene was added before analysis (FSSAI, 2015).

##### **Multi-residue gas chromatography methods for determination of synthetic pyrethroids.**

Analytes was partitioned into hexane, evaporated to dryness, and dissolved in hexane. The extract was partitioned with acetonitrile and cleaned up on a deactivated Florisil column with 6 % ethyl ether in hexane. Analyte concentrations was determined by gas chromatography with mass spectrometer detector (GC-MS) and comparison with calibration standards.

##### **Preparation of standard solutions of pesticides.**

Stock solutions of pure standards of organophosphate, organochlorines and synthetic pyrethroids, containing the analytes of interest were prepared and then serially diluted to produce different concentrations of the pesticides. Stock standard solutions were stored in amber coloured bottles at 4°C in a refrigerator while working

standard solutions were prepared fresh before use (FSSAI, 2015).

##### **Gas chromatographic Analysis**

Gas chromatograph was employed to carry out the analysis. The upper limit of the quadruple mass analyser was of  $mz^{-1}$  1050. An electron ionization source was used to acquire the mass spectra. The ion source was held at 250°C, the filament voltage was set at 70eV, the voltage intensity was 100 mA. The analytes were acquired in Selected Ion Monitoring (SIM) mode. Separations of organophosphate compounds were performed through an Optima 5MS (5% diphenyl 95% dimethylpolysiloxane) capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness, MachereyNagel, DE). Separation was carried out injecting samples (1mL) in splitless mode at 250/C. Carrier gas (helium) flow was kept regular at 1 mL/min. The oven temperature was programmed as follows: isothermal at 120°C for 1 min, from 120°C to 170°C at 25°C/ min, hold for 2 min, from 170°C to 200°C at 4°C/ min, hold for 1 min, from 200 C to 320 C at 25C/ min and isothermal at 320 C for 5 min. The transfer line was set at 270°C (Daniela *et al.*, 2012).

##### **Determination of limit of detection (LOD)**

The limit of detection for each pesticide was calculated according to methods described by Ogah and Coker (2012). To determine the limit of detection of the equipment for each pesticide, an air blank sample was run under the experimental conditions to obtain the detector baseline noise. A detectable ion should produce a signal that is at least three times the baseline noise [that is, signal-to-noise (S/N) ratio = 3]. The LOD of each pesticide was determined by running serially diluted solutions of the working pesticide standard at the set chromatographic conditions and finding the concentration at which S/N = 3. This procedure was repeated for all the pesticide residues.

##### **Identification and Quantification**

Pesticide identification and quantification was carried out using the methods described by Ogah and Coker (2012). Pesticide residues were identified by matching their retention times and cast codes with those of the standards. Identified pesticides were quantified by the external standard method of comparing sample peak areas with those of the pesticide standards under the same conditions. The samples were analysed three times and the mean values obtained. The pesticide content of each sample was calculated as:

$$\text{Pesticide Content} = \text{As} \times \text{Vf} / \text{Wt} \times \text{CF}$$

Where As = peak area of sample

Vf = final volume of clean extract

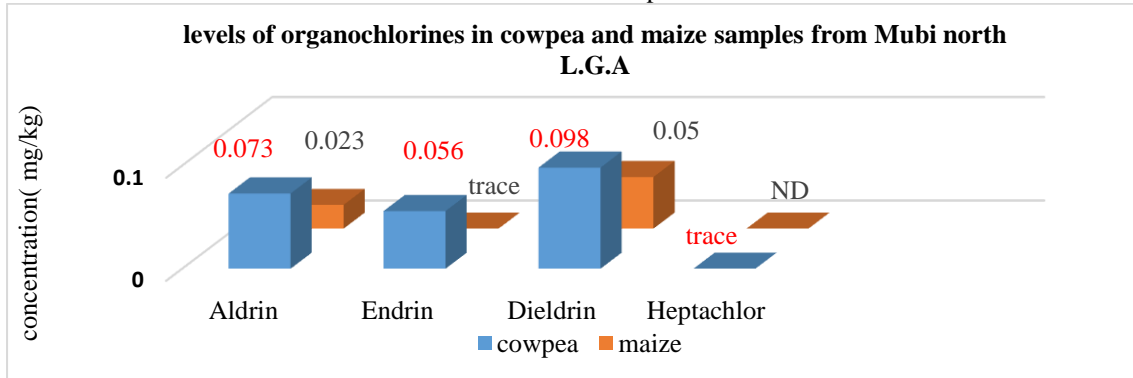
Wts = weight of sample extracted

CF = calibration factor.

The CF of each pesticide was calculated as; Peak Area of Standard /Total Amount of Standard Injected.

**Statistical Analysis**

The analysis was carried out in replicates (n=3) using SPSS software package (2016). Results are presented as mean and standard deviations.



N=3; Trace= Below LOD; LOD=Limit of Detection; BDL=Below Detection Limit

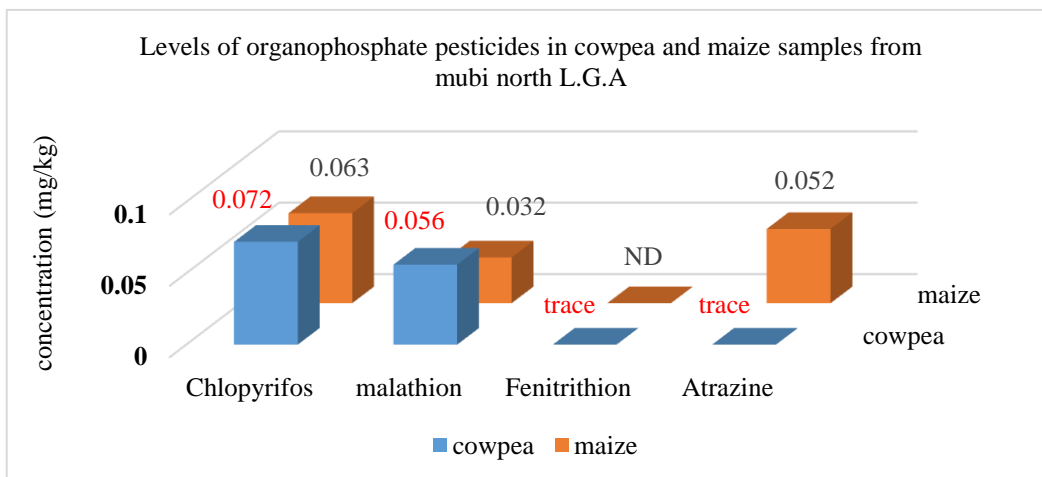
**Figure 1:** Mean concentrations of organochlorines pesticide residues in cowpea and maize crops from Mubi North L.G.A.

**Results and Discussion**

Figure 1 shows the organochlorines pesticides investigated (Aldrin, Dieldrin, Endrin and heptachlor) Aldrin and Dieldrin showed significant levels in both maize and cowpea. Aldrin showed a concentration of  $0.073 \pm 0.002$  (mg/kg) in cowpea and  $0.023 \pm 0.003$  (mg/kg) in maize. The concentration of dieldrin is  $0.098 \pm 0.005$  (mg/kg) in cowpea and  $0.05 \pm 0.004$  (mg/kg) in maize. Endrin showed a concentration of  $0.056 \pm 0.001$  (mg/kg) in cowpea and only traces was seen in the maize. Only traces (below detection limit) of Heptachlor was found in cowpea and completely not detected in the Maize samples, this might be due to potential degradation of the Heptachlor resulting from storage

conditions over time. All organochlorines detected are within safe levels (below their MRLs).

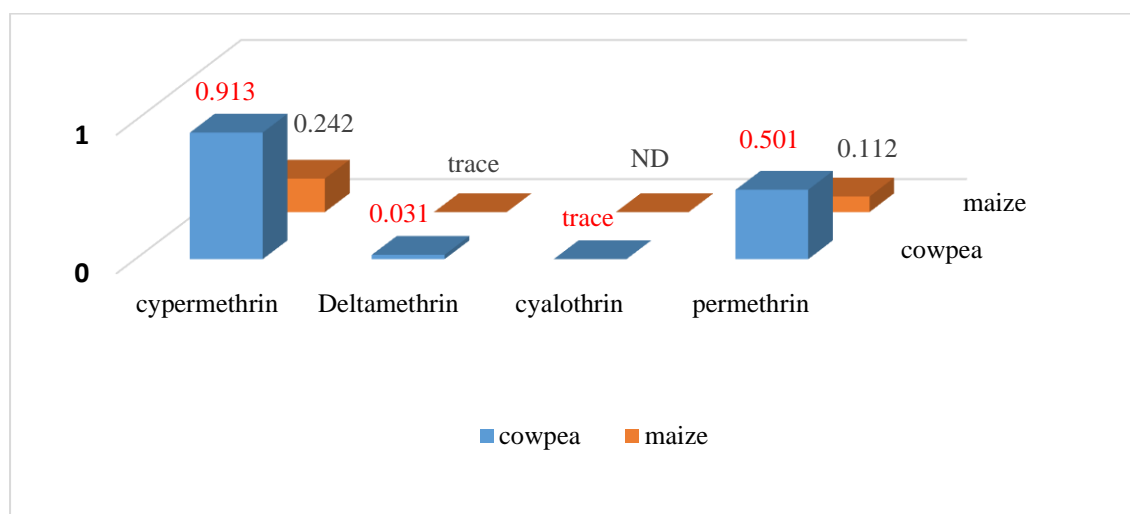
In Nigeria, Organochlorines have been banned by NAFDAC due to their toxicity effects. However, their presence in the study samples suggests that these compounds are still in use, possibly with a different trade name, or in formulations with other unrestricted agrochemicals. The relatively higher amounts of dieldrin relative to aldrin may be an indication that the contamination is not recent. According to Living on Earth (1998), the half-life of dieldrin is about 60 years, as long as it's not exposed to the sun. This is also supported by the fact that aldrin photolyses to dieldrin over time (ATSDR, 2002; Oghar and Coker, 2012).



**Figure 2:** Mean concentration level of organophosphate pesticide residues in maize and cowpea crops sampled at Mubi North L.G.A.

Figure 2 shows the mean concentrations of all organophosphorus pesticides (chlorpyrifos, Malathion and Fenitrothion). From the results, chlorpyrifos showed mean concentrations of  $0.072\pm 0.04\text{mg/kg}$  and  $0.063\pm 0.03\text{mg/kg}$  in cowpea and maize respectively, which are far above the set MRLs. Malathion has mean concentrations of  $0.056\pm 0.03\text{mg/kg}$  and  $0.032\pm 0.07\text{mg/kg}$  in cowpea and maize respectively. Only traces of fenitrothion was seen in cowpea and not completely detected in maize.

Among the organophosphorus pesticides investigated, malathion in beans and maize from Michika showed highest concentration, followed by same malathion in samples from Mubi L.G.A. These high concentrations in both areas suggest they are much in use. Chlorpyrifos is also available above MRL in both maize and beans from the two sample areas. These pesticides are widely used as agricultural insecticides and many uses in households for pest control (Gultekin *et al.*, 2000). This means that the maize and cowpea from these areas may be contaminated beyond safety limits.



**Figure 3:** Mean concentration levels of synthetic pyrethroids in cowpea and maize crops sampled at Mubi North L.G.A

Figure 3 showed that, among the synthetic pyrethroids (cypermethrin, deltamethrin, cyalothrin and permethrin) investigated; cypermethrin shows highest concentration of  $0.913\pm 0.001\text{mg/kg}$  in beans and  $0.242\pm 0.004\text{mg/kg}$  in maize. The amount of cypermethrin in beans was slightly below the set MRL. Such high prevalence may results from post-harvest treatment against insect infestation or absorbed via root uptake from contaminated soil or both. Deltametrin showed  $0.031\pm 0.005\text{mg/kg}$  in cowpea and only trace amounts (below LOQ) in maize. Only traces of cyalothrin was observed in cowpea and completely not detected in maize (below LOD). This low concentration may results from degradation or its application is not pronounced within the area. However, subsequent application over time may lead to bio magnification. Permethrin showed concentration of  $0.501\pm 0.004\text{mg/kg}$  in cowpea and  $0.112\pm 0.003\text{mg/kg}$  in maize. With exception of cypermethrin, all other residues are far below their MRLs. The consistently higher amounts of all the residues in beans compared with maize, suggest that its application on beans is much more prevalent.

### Conclusion

Maize and cowpea grains are highly susceptible to infestation by pest and are consequently subjected to post harvest treatments with pesticides in order to maintain good quality during storage and to avoid economic loss. Apart from the post-harvest treatment, previously treated or contaminated soil can be a source of these pesticides residues in food crops (Ogah & Coker, 2012). The maize and cowpea crops from Mubi north L.G.A may pose a great threat to the health of consumers in terms of POPs contamination (Fernando, 2006). These results indicate that the maize and cowpea samples within the study area are contaminated by organochlorines, organophosphorus and synthetic pyrethroid pesticides. And the concentration of their metabolites most especially dieldrin, chlopyrifos and cypermethrin are above MRLs.

This results show that there is still existence of a variety of organochlorine and organophosphorus pesticides in the maize and cowpea from Mubi north L.G.A. Despite bans and restrictions on the usage of these pesticides in Nigeria, the observed concentrations of the studied organochlorine,

organophosphorus and synthetic pyrethroid pesticides in samples from those areas explain either their persistence in the environment or continued usage. This is supported by the findings of Antonio et al., (2012).

If crops containing pesticides that are above their MRLs are consumed, it may lead to accumulation of the chemicals in the body and eventual threat to human health. In addition, there is this potential ‘‘cocktail effect’’ if various pesticides accumulate in the body. Since combined effect of pesticides mixture may be additive or synergistic. This means that even pesticides that are detected at safe levels may eventually be of great hazard, due to the combined effects of other pesticide that are also accumulated in the body (Antonio et al., 2012).

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