

## Determination of Chlorpyrifos Pesticide Residues in Agricultural Produce by Gas Chromatography-Mass Spectrometry

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### ABSTRACT

Chlorpyrifos pesticide is one of the most commonly used pesticides worldwide. It may cause chronic poisoning in farmers and acute poisoning in homicidal or suicidal cases. This study focused on quantitation of chlorpyrifos in commonly consumed agricultural produce. A total of 100 samples of maize, cassava, yam and fluted pumpkin were collected from a farm where there was extensive use of pesticides for cultivation. The samples were analyzed using a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation, followed by determination of residues using gas chromatography coupled to mass spectrometry (GC-MS). The method was validated for linearity, sensitivity i.e. limits of detection (LOD) and quantification (LOQ), accuracy (recoveries) and precision (relative standard deviation). The method was linear from 10-80  $\mu\text{g/ml}$  with correlation coefficient ( $R^2$ ) of 0.9993. The accuracy of the method ranged from 90.00 to 99.00% while the detection limit and quantification limit were 0.192 and 0.640  $\mu\text{g/mL}$  respectively. The method for the analytes was found to be precise as the percentage relative standard deviation was below 2%. Among the samples analysed, cassava had the highest concentration (29.70 – 75.90  $\text{mg kg}^{-1}$ ) of pesticides followed by yam and maize. The green leafy vegetables (fluted pumpkin) had the lowest concentration (0.05 – 0.07  $\text{mg kg}^{-1}$ ) and therefore, fell within the permissible European Union Maximum Residue Levels (EU MRLs).

**Keywords:** Pesticide, Chlorpyrifos, determination, residue, gas chromatography-mass spectrometry

### INTRODUCTION

Humans have been using pesticides for decades to gain benefits such as increased crop productivity by eliminating pest species like insects, molds, rodents, and weeds [1, 2]. In addition, pesticides are employed in a number of fields, such as forestry, agriculture,

environmental protection, and residential use [3]. The use of these synthetic chemicals has affected not only the health of the human population but also other living species [4, 5]. The effect of pesticides causes illness in multiple systems of the human body for example, in dermatological, neurological, and gastrointestinal functioning [6, 7]. Continuous exposure to pesticides may give rise to carcinogenic, toxic, and mutagenic problems in the victim. Unfortunately, pesticides are being used in homicides and suicides in human society [8-10].

The unchecked utilization of pesticides in crops is creating toxicity in natural foods including fruits, vegetables, grains, milk, and drinking water [11-13]. Pesticide residues may retain in exposed food and eventually reach the body of the consumer [14]. Early studies have proved that these synthetic chemicals may destroy the immune system, urinary system, cardiac system, skin, muscular system, gastric system, and even respiratory system [15-17].

In Nigeria, the rate of pesticides application is increasing but little information is available regarding the environmental impact resulting from their use [18]. The rate at which pesticides are being applied to control weed by farmers is becoming alarming because they have little or no knowledge about the negative impact of the pesticides [19]. It may also be due to the fact that the use of crude implements like cutlasses and hoes is relatively tedious and time-consuming whereas the use of pesticides is fast in action but toxic to the consumers of the food products. Ivbijaro [20] provided ample evidence that insufficient pesticide education in Nigeria has resulted in widespread usage.

Different sample preparation and analytical methods have been used for monitoring pesticide residues in various food products. Sample preparation techniques used for pesticide analysis include liquid-liquid extraction [21], liquid-phase microextraction [22], solid-phase extraction [23], solid-phase microextraction [24], supercritical fluid extraction [25], and microwave-assisted extraction [26]. However, the majority of these techniques are rather time-consuming, labour-intensive, complicated, expensive and produce considerable quantities of waste.

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method developed by Anastassiades et al. [27] has gained significant popularity as the method of choice for agricultural and animal-based matrices within the last two decades due to its simplicity, speed, low cost, high throughput and minimal solvent requirement. Gas chromatography (GC) coupled with mass spectrometry (MS) detectors are among the most powerful techniques and have been used for the accurate simultaneous determination of multiclass pesticide residues in

agricultural matrices [28 - 30]. With these techniques, limits of quantification (LOQs) below  $0.01 \text{ mg kg}^{-1}$  for pesticide residues can be obtained.

Pesticide residue levels in soil and surface water have been documented by numerous researchers worldwide [31-35], with a range of reported residue levels. Similarly, substantial amounts of pesticide residues have been documented in fruits, vegetables, and cereals [36, 37]. In Nigeria, there have been evidences of the presence of pesticides residues in soil [38, 39], crops [40, 41], and surface water [42 – 44]. The residue concentrations are above MRLs in most of these studies. Therefore, there is need for continuous monitoring of the environmental status of these pesticides which can be used as basis for recommendation. Thus, the aim of the present study was to investigate the occurrence and concentrations of chlorpyrifos pesticide residues in maize (*Zea mays*), cassava tuber (*Manihot esculenta*), fluted pumpkin leaves (*Telfairia Occidentalis*) and yellow yam (*Dioscorea alata*) popularly planted in the south west Nigeria.

## MATERIALS AND METHODS

### Description of sampling site

The study area (Figure 1) was in Okenugbo, a village in Ago-Iwoye, Ogun State, south west Nigeria. This area was selected based on frequent application of pesticides to control weeds and pests in the area.

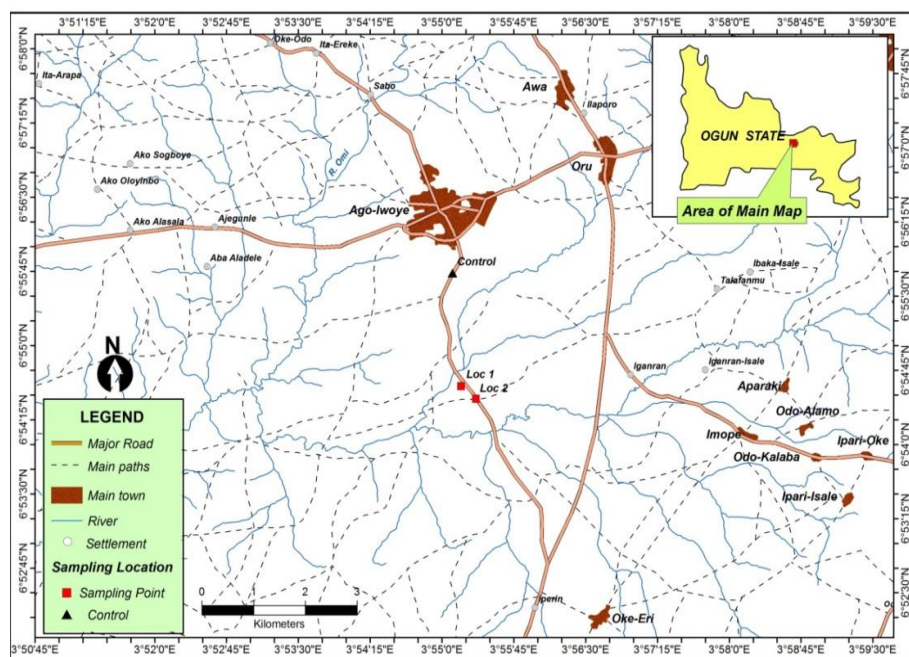


Figure 1: Map of the sampling site

### **Sample collection**

A total of 100 samples consisting of maize fruits (edible part) (n= 40), cassava tuber (n = 20), fluted pumpkin leaves (n = 20) and yellow yam tuber (n = 20) were randomly collected from the selected sampling locations between May and July, 2022. The samples were chopped, homogenized with a home food processor (Phillips HR 7770, Royal Philips Electronics NV, Istanbul, Turkey) and stored in a plastic container in a refrigerator (4 –8 °C) until analysis.

### **Chemicals, reagents and standards**

Acetonitrile and methanol (MeOH) with chromatography grade glacial ethanoic acid (CH<sub>3</sub>COOH), analytical grade ammonium formate (HCOONH<sub>4</sub>, 99% purity) and standard chlorpyrifos powder were purchased from Sigma–Aldrich (UK). MgSO<sub>4</sub> and sodium acetate (CH<sub>3</sub>COONa) were obtained from BDH (USA). One gram of standard chlorpyrifos was dissolved in ethyl acetate to prepare a stock solution, which was then kept at - 20 °C in the dark. Working solutions were prepared by appropriate dilution with ethyl acetate to establish the calibration curve in solvent and matrix.

### **Sample extraction**

Pesticide residues were extracted from green leafy vegetables using the QuEChERS sample preparation technique according to the AOAC official method [45], with minor modifications. A 50 mL extraction tube was filled with 10 g of the homogenized material, followed by the addition of 20 mL of acetonitrile. The tube was rotated (Multi RS-60 rotator, Biosan, Riga, Letonya) vigorously for 15 min and vortexed for 1 min on a vortex mixer (Heidolph, Schwabach, Germany). Then, 6 g of MgSO<sub>4</sub> and 1.5 g of anhydrous sodium acetate were added, and the tube was rotated for another 10 min and vortexed for 1 min. The tube was centrifuged at 3000 rpm for 5 min at room temperature. The supernatant of extract was transferred into glass vials and then injected into the GC-MS instrument.

### **Determination of Chlorpyrifos using Gas Chromatography-Mass spectrometry**

The chlorpyrifos level was determined by using gas chromatography coupled to tandem mass spectrometer (Agilent technologies 7890A) in multiple reaction monitoring (MRM) in EI MS/MS mode. The column used for pesticide residues separation was HP-5 MS (30 m x 250 µm x 0.2 µm). The mass spectrometer was operating in Electron Ionization (EI) mode, the electron energy was 70 eV and the temperature source at 290 °C. The oven condition started at 50 °C for 0.6 minute followed by a temperature increase up to 180 °C at a rate of 15 °C/min

held for one minute. Then the temperature was increased at 230 °C by 7 °C/min and the last step was up to 280 °C by 3 °C/min. The injection volume was 1µl.

### Method Validation

The experiment was carried out according to the official specifications of Global Quality Guidelines 2002 [46] and international conference on harmonization [47]. The method used was validated based on the following parameters: specificity, range and linearity, sensitivity (LOD and LOQ), accuracy and precision.

### Linearity

Linearity was carried out by preparing calibration curves from different concentration range of 10 to 80 µg mL<sup>-1</sup> of chlorpyrifos standard.

### Accuracy

Recovery experiments were carried out by spiking a known amount of chlorpyrifos to pre-analysed samples at three different concentrations and the percentage recovery is calculated using equation (2).

$$\frac{a}{b} \times 100 \quad (2)$$

Where a is amount of analytes (chlorpyrifos) found while b is amount of analytes spiked.

### Precision

Method precision was evaluated by determining the intra-day and inter-day relative standard deviation of the measured concentrations of chlorpyrifos. The reproducibility (intra-day precision) and repeatability of system (inter-day precision) was checked by measuring the area of the chromatogram different concentrations of standard solution on the same day and on different days respectively under the same experimental conditions.

### Sensitivity

The Limit of Detection (LOD) and Quantification (LOQ) were calculated from the calibration lines that defined linearity, using the Long and Winefordner criterion as expressed in equations (3) and (4).

$$LOD = \frac{3 S}{a} \quad (3)$$

$$LOQ = \frac{10 S}{a} \quad (4)$$

Where *a* is the slope of the calibration line and *S* is the standard deviation of response.

### Statistical Analysis

All presented data are means  $\pm$  standard error of the mean of three independent measurements using statistical package for social sciences software, version 18 (SPSS Inc., Chicago, IL, USA). Microsoft excel software (2013) was used for plotting of calibration curves and other graphs.

## RESULTS AND DISCUSSION

### Linearity and Sensitivity

The chromatogram area of standard solution of chlorpyrifos was significantly linear from 10 – 80  $\mu\text{g/mL}$  and therefore, the regression model represents the data correctly. The slope of the standard calibration line for chlorpyrifos standard solution was 0.006. Thus, the method is sensitive enough to detect the analyte at low concentrations of 0.006  $\mu\text{g/ml}$  (Figure 2). The obtained LOD and LOQ values of chlorpyrifos were 0.0155 and 0.0518  $\mu\text{g/ml}$  respectively.

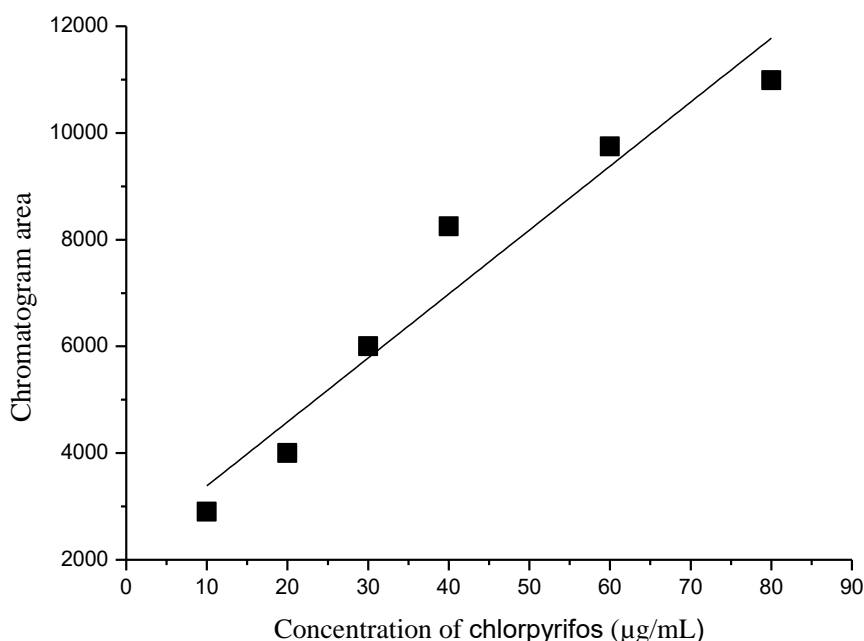


Figure 2: Standard calibration curve obtained from chromatogram area of chlorpyrifos solutions

## Precision

As presented in Figure 3, the intraday and inter-day relative standard deviation (RSD) values of the system were less than 2 for the analyte. This shows the reproducibility of the values obtained from the instrument with little or no interferences.

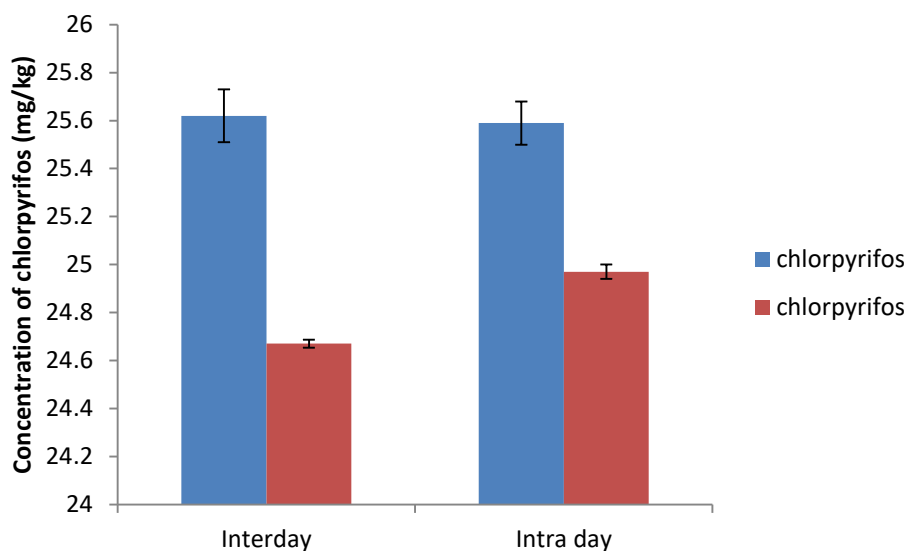


Figure 3: Intra-and inter-day instrument precision validation for GC/MS determination of chlorpyrifos

## Accuracy

The result of accuracy study is presented in Table 1. Data on recovery studies conform to expected values for quantitative estimation of chlorpyrifos in the samples as the statistical parameters are within the acceptance range ( $RSD < 2.0$ ).

Table 1: GC-MS method accuracy for determination of chlorpyrifos extracted from selected samples

s/n	Amount of Sample spiked (mg/kg)	Amount of chlorpyrifos found (mg/kg)	Recovery $\pm$ RSD (%)
1	0.01	0.009	90.00 $\pm$ 0.95
2	0.05	0.047	94.00 $\pm$ 1.64
3	0.1	0.1	99.00 $\pm$ 0.87

In this work, a simple validated and reliable chromatographic technique for determination of chlorpyrifos pesticide in agricultural food products was presented. The technique was fast and easy to perform. Moreover, the method is sensitive enough to detect analytes in the presence of sample matrices even at a low concentration due to good analytical parameters like linearity, sensitivity (LOD and LOQ), and precision with a good recovery studies (accuracy) (Table 1). Figure 4 describes the ion chromatogram of chlorpyrifos component detected without matrix using gas chromatography method.

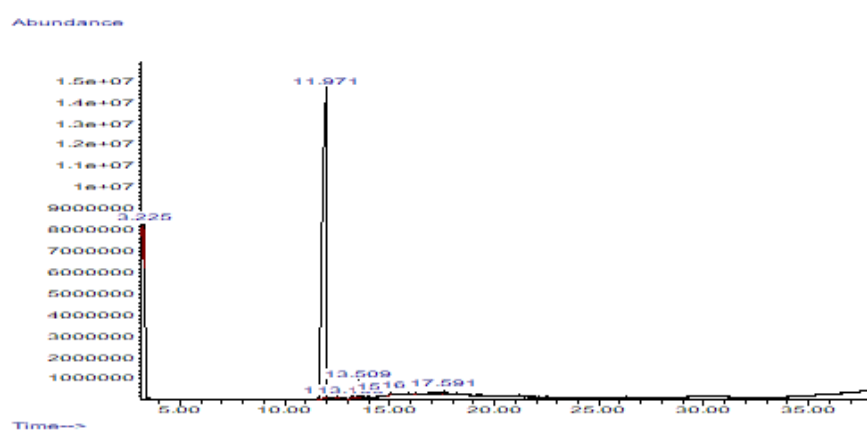


Figure 4. Chlorpyrifos ions chromatogram

Figure 5 showed the concentrations of chlorpyrifos pesticide residues in crop samples analysed. In maize samples, pesticide was detected at a range of 1.9 to 9.8 mg pesticide per kg, although, the pesticide concentration is small when compared to other samples but it exceeded the permissible European Union Maximum Residue Levels (EU MRLs) of pesticides in the range of 0.01–0.09 mg L<sup>-1</sup> for many agricultural products. The reason for the low concentration of the pesticide in maize is that maize is not a tuber crop. The fruit is on the maize and therefore has no direct contact with soil which is a reservoir of toxic chemicals. Also, the time of harvest is short i.e. three months. In cassava tuber samples, the concentration of the pesticides is between the ranges of 29.70 to 75.90 mg pesticide per kg. This is extremely high and exceeded the permissible European Union Maximum Residue Levels (EU MRLs) of pesticides in food products. The reason for the high concentrations may be connected to the nature of the plant i.e. it is a tuber crop and time of maturity i.e. about a year. There were exceedances of the MRL in yam which have the same reason with cassava while the fluted pumpkin is very safe for consumption because the concentration of the pesticide is very low and fall within the permissible European Union Maximum Residue Levels (EU MRLs).



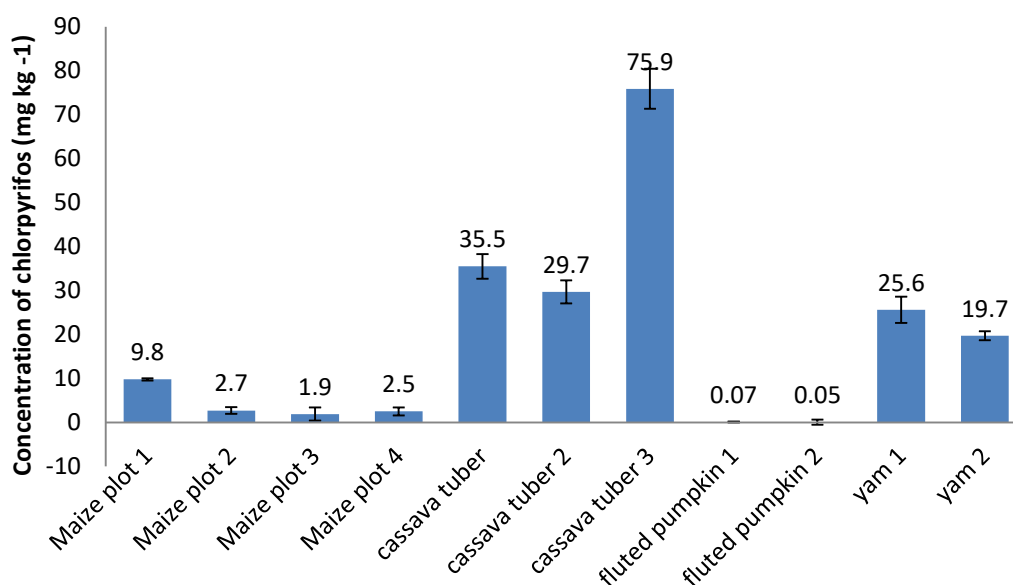


Figure 5: Concentration of chlorpyrifos pesticide residues in crop samples

## CONCLUSION

A method based on the QuEChERS sample preparation procedure and GC-MS was validated for residual analysis of chlorpyrifos pesticide in maize, cassava, yam and green leafy vegetables (fluted pumpkin leaves). The method provided good results for recovery rate, precision, sensitivity (LOD and LOQ) and linearity. The validated method was applied for the analysis of crop samples. Of the samples analysed, fluted pumpkin leaves (green leafy vegetable) are safe for human consumption because its concentration is within the EU MRLs while all other samples studied exceeded the EU MRLs. To protect consumer health, the use of plant protection products must be routinely monitored and the use of pesticides to cultivate should be controlled if not eradicated by the appropriate agencies of the government.

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