LEVELS OF CHLORPYRIFOS RESIDUES IN COWPEA GRAINS GROWN IN KWARA AND NIGER STATES, NIGERIA

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Abstract

Cowpea is intrinsically a crop of many values, but several limitations face its production, especially insect pest attack. Chlorpyrifos is a highly effective insecticide in controlling insect pests of cowpea on field. However, chorpyrifos residue is one of the reasons why Nigerian cowpea was restricted at European Union borders. Information on the residue levels of chlorpyrifos in cowpea grains from Nigerian Southern Guinea Savannah has not been documented particularly in Kwara and Niger states, which is the major receiving hubs of cowpea grain in Nigeria. Therefore, chlorpyrifos residues levels in cowpea grains grown in Kwara and Niger States was investigated.

One kilogram of cowpea grains from each landrace: Danila, Milk, White Agric (WA) and Zobo Red (ZR) were collected from the study areas during harvesting periods of 2020 and 2021. The chorpyrifos residue was extracted from cowpea grains and quantitated (mg/Kg) using GC-MS. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$

The chlorpyrifos residues levels ranged from 0.0172±0.0149 (WA, Niger) to 0.0002±0.0003 (WA, Kwara) in 2020 and 0.0024±0.0013 (WA, Niger) to 0.0002±0.0004 (WA, Niger) in 2021. The presence of chlorpyrifos residues in some of the samples studied, more of which were obtained in 2020 could be attributed to indiscriminate use of insecticides by farmers in the study area. Fewer samples containing chlorpyrifos residue in 2021 than in 2020, could be connected to rise in insecticide prices in Nigeria.

Quantitation of chlorpyrifos residue in the cowpea grains of all landraces obtained showed that not all cowpea grains grown in the study area contained chlorpyrifos residue. Those with chlorpyrifos residue were at levels below legally permissible limit.

Introduction

Cowpea (*Vigna unguiculata*, L. Walp), a member of sub-family Fabiodeae under Fabaceae family (Agbogidi and Egho, 2012) is widely known as beans in Nigeria and *niebe* in all francophone countries (Madode, 2012; Horn *et al*., 2022). It had been grown in many places principally for grains, foliage legume (leafy vegetable, succulent pods and fresh shelled green peas), cover and fodder crop and is largely adapted and highly treasured crop due to its nutritional and medicinal value (Adeola *et al*., 2011). Cowpea could also be termed "vegetable meat" because of more protein content in it, which makes it a preferable substitute for animal protein (Oyewale, 2014; Abdullahi, 2016). On average, cowpea grain contains 26.61% protein content (Owolabi *et al*., 2012; Adino *et al*., 2018).

Cowpea is intrinsically a crop of many values, but several limitations face its production, such as adverse weather, weeds, diseases and insect pests. The main constraint to cowpea production across West African region is insect pests' attacks (Oyewale and Bamaiyi 2013; Abdullahi, 2016; Abudulai *et al*., 2017). Ajeigbe *et al*. (2012) reported approximately 70% yield loss to insect pests alone, both on field and in store.

In attempt to control economic loss of cowpea grains, crop improvement technologies have produced pest-resistant cowpea varieties. However, it had been reported that from most insect-resistant cowpea varieties, no single variety could resist myriad of insects attacking the crop (Kusi *et al*., 2019). The most effective control tactic till date is chemical control using synthetic insecticides (Oyewale, 2014) especially when the insect pest population would cause economic damage. Amare *et al*. (2022) had reported that absolute abstinence from synthetic insecticide in cowpea production would spell economic doom. Among insecticides that had be used successfully for the control of cowpea insect pests are Fenvalerate, profenophos, dischlorvos, endosulfan, dimethoate, monocrophos, imidacloprid, emamecting benzoate, chlorpyrifos, etc (Anusha *et al*. 2016).

Chlorpyrifos is an organophosphate, a broad-spectrum insecticide, acaricide and nematicide (Meriel, 2012; EFSA, 2017). It is a chlorinated organophosphorus insecticide and is among the most widely used insecticide in agriculture (Kaushik *et al*., 2016; Fayinminnu *et al.*, 2017; El-fakharany *et al*., 2017**)**. Chlorpyrifos is the common name of the compound known scientifically as 0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate (Figure 1) (Breslin *et al*. 1996; Hongsibsong*et al*., 2020).

Chlorpyrifos has been found specifically useful in the control of quite a lot of cowpea field insect pests (Egho and Enujeke, 2012; Oyewale *et al*., 2014). However, the health consequences of residues of this chemical in human due to dietary intake of the treated crops remain a big challenge to food safety. This had resulted in the ban of chlorpyrifos in European Union, Canada, Egypt, Indonesia, Morocco, Palestine, Vietnam, Sri Lanka, Saudi Arabia, Switzerland, Thailand, and Turkey (Wołejko *et al*., 2022). Chlorpyrifos insecticides had been reported to instigate behavioural abnormalities, impaired cognitive and psychomotor development in childhood and adolescence (Parrón-Carrillo *et al*., 2024).

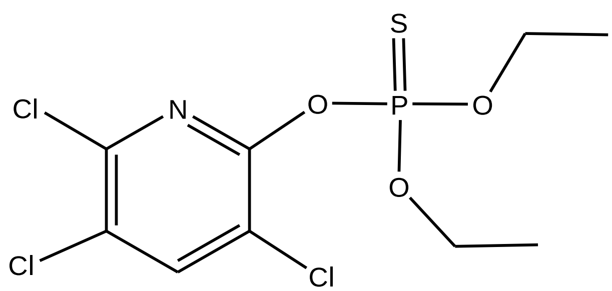

Figure 1. Structure of Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate). (Pawar *et al*., 2015)

Chorpyrifos is one of the reasons why Nigerian cowpea was restricted at European Union borders (Isegbe *et al*, 2016). Hence it is essential that levels of chlorpyrifos residue in cowpea grains are monitored. Information on chlorpyrifos residue in cowpea grains at various cowpea growing regions in Nigeria had not been clearly stated, although pocket of information is available in Kano and Borno States. Nevertheless, information on the residue levels of chlorpyrifos in cowpea grains from Southern Guinea Savannah (one of the foremost regions where cowpea is being cultivated in Nigeria) has not been documented. Kwara and Niger States belonging to Southern Guinea Savannah are the major receiving hubs of cowpea grain in Nigeria for onward distribution to other parts of the nation. Insecticide residue monitoring and regulation will not only reduce crop rejection, stimulate trade but also improve food and agriculture trade, increase GDP and foreign reserves.

Therefore, the objective of this study was to determine chlorpyrifos residues levels in cowpea grains grown in Southern Guinea Savannah, Nigeria.

Scope of the study

162 samples from four cultivars of cowpea were obtained from 54 farmers from 18 communities in 6 local government areas of Niger and Kwara states. Similar cultivar samples from 3 communities in each LGA were bulked to form composite samples representing each local government hence having a total of 54 composite samples. Sampling were done twice at 1 year interval. Chlorpyrifos quantification of each sample was carried out

Materials and methods

Chemicals and standards

Chemicals and solvents used for this study were analytical grade; calibration standards of the target compound (Chlorpyrifos), High Performance Liquid Chromatography grade solvents (n-hexane, acetone and acetonitrile), Sodium chloride and Magnesium sulphate, and distilled water.

All chemicals and reagents used were of high purity (99.99%). The HPLC-grade acetonitrile and n-hexane were procured from VWR PROLABO Chemicals (France), anhydrous Magnesium sulphate and Sodium chloride were procured from BDH Laboratories (UK). Standard of chlorpyrifos were obtained from Sigma-Aldrich (St Louis, MO) and QuEChERS (Quick Easy Cheap Effective Rugged and Safe) dispersive tubes and 2 mL capacity glass vial were purchased from Supelco (USA).

The analysis was carried out in a standard analytical and environmental research laboratory located in University of Ibadan, Nigeria.

Sample collection and preparation

Sample collection was done twice: 2020 and 2021 harvest periods from December to February. Three hundred and twenty four (324) samples (approximately 1000 g each) of four different cowpea landraces were obtained randomly from 54 farmers. One hundred and sixty two (162) samples were obtained from the communities at each sampling period. Three samples (about 1000g each) per landrace were obtained from three different farmers per community. Cowpea landrace samples collected with following alternative identifiers were ‘*Danila*’, ‘Milk’, ‘White Agric’ and ‘Zobo red’.

Cowpea samples of similar landrace obtained from the three cowpea farmers per community were combined and homogenised to obtain a composite sample.

Extraction and Clean-up of Analyte

Supelco (Bellefonte, PA) QuEChERS extraction kits was used for sample extraction and clean-up according to Lehotay (2005), Anastassiades *et al.* (2007) and Kalachov *et al*. (2011) with little amendment. Exactly 2 g each of the homogenised powdered samples were poured in labeled 10 mL centrifuge tubes (which were pre-cleaned with detergent, rinsed with distilled water followed by acetonitrile). A 5 mL acetonitrile solvent was added, and manually and thoroughly shaken for 1 min and centrifuged at 3000 rpm for 5 minutes (using SearchTech 80-2 centrifuge machine). Exactly 0.5 g NaCl and 2 g $MgSO_4$ were added and the mixture was again manually and thoroughly shaken for 1 min. Samples were centrifuged for 3 min at 3500 rpm. The extract was concentrated with gentle stream of nitrogen gas (freeze drying) and 1 mL of the concentrated extracts (top layer/aliquot) was transferred into a Supel™ QuE PSA/C18/ENVI-Carb (AC) dispersive SPE tubes. The SPE tube was vigorously shaken for 1 min and centrifuged for a further 3 mins at 3000 rpm. Clear supernatants were transferred into cleaned labeled 2 mL glass GC-vials. This was concentrated through gentle stream of nitrogen gas and was reconstituted to mark with analytical grade n-hexane for instrumental analysis. The vials, covered with PTFE-lined cap were arranged in a tray and kept in fridge.

Preparation of Standard for Chlorpyrifos Analysis

Calibration was prepared at concentrations levels 1.25 µg/g, 2.5 µg/g and 5 µg/g by serial dilution of the stock solution using n-hexane. The pure standard (stock solution) was kept according to supplier’s instruction (-20^{o}C, dark and water proof condition) in refrigerator.

Quantitation of Chlorpyrifos Residue in Cowpea Grains

Quantitation of chlorpyrifos were executed using an Agilent (Santa Clara, CA) model 6890A Gas Chromatograph equipped with Mass Spectrometry detector (MSD). Column used was Agilent HP-5 ms [30 m (length) × 0.32 mm (internal diameter) × 0.25 µm (film thickness)] (J&W Scientific Inc. Folsom, CA).

The extracts were allowed to equilibrate with room temperature before injection into the analytical instrument. One test of procedural blank was carried out and solvent blanks were run at every 10 samples interval.

Recovery Study

Sample of low level of concentration previously analysed was spiked with 1 µg/g and 2 µg/g standard chlorpyrifos. The recovery of the chlorpyrifos after instrumental analysis was determined by comparing the concentrations of chlorpyrifos in the spiked sample with the unspike sample (Babalola and Adeyi, 2018). Before instrumental analysis, each spiked sample was manually and thoroughly shaken, and kept overnight in fridge to allow uniform distribution of chlorpyrifos in the spiked samples. Thereafter, the sample was equilibrated to room temperature for about 1 hour before extraction and clean-up of the analyte. Extraction and instrumental analysis were done just as previously described. Percentage recoveries were calculated as follows:

$$\% \text{ Recovery} = \frac{A - B}{Z} \times 100$$

A= Concentrations of Chlorpyrifos in spiked samples

B= Concentrations of Chlorpyrifos in unspiked samples

Z= Concentration of Chlorpyrifos standard added.

RESULTS

Chlorpyrifos Residue Levels in Cowpea Grains Grown in Kwara State

Chlorpyrifos residue in Danila was below 0.01 mg/kg [the maximum residue limit of chlorpyrifos in cowpea grain (EFSA, 2017)] in 2020 and 2021 at Ilorin East LGA. In this LGA, chlorpyrifos residue in Milk was below maximum residue limit in 2020 and was even below detectable level in 2021. The residue of chlorpyrifos was below detectable level in White Agric in both years in Ilorin East. Zobo Red was not grown in Ilorin East during the study period (Table 1). In Ilorin South, Danila was not grown during the study period, and chlorpyrifos residue in Milk was below detectable level in 2020 but was detectable in 2021, although with value below MRL. On the other hand, chlorpyrifos residue was detected in White Agric in Ilorin South in 2020 and was below detectable level in 2021. It was detected at levels below MRL in Zobo Red in both years (Table 1).

Chlorpyrifos residue was detected in Danila in 2020 at a level (0.0129 mg/kg) above MRL in Moro LGA and below detectable level in 2021. Though at a level below MRL, chlorpyrifos was also detected in Milk in 2020 and was below detectable level in 2021 in Moro LGA. Chlorpyrifos was detected in both years in White Agric at levels below MRL in Moro LGA. Zobo Red was not grown in Moro during the study (Table 1).

Table 1: Chlorpyrifos Residue Levels (mg/kg) in Cowpea Grains in Kwara State

	Danila		Milk		White Agric		Zobo red	
LGA	2020	2021	2020	2021	2020	2021	2020	2021
Ilorin East	0.0028± 0.0043	0.0012± 0.0011	0.0056± 0.004	Bdl	Bdl	Bdl	NA	NA
Ilorin South	NA	NA	Bdl	0.0016± 0.0004	0.0014± 0.0024	Bdl	0.0014± 0.0018	0.0012± 0.001
Moro	0.0129± 0.0223*	Bdl	0.0014± 0.0152	Bdl	0.0002± 0.0003	0.0006± 0.001	NA	NA

#The asterisks (*) signifies the cowpea landraces and LGA with the identified chlorpyrifos level above EFSA's Maximum residues limit. NB: EFSA's MRL of chlorpyrifos in cowpea grains =0.01mg/kg. Bdl: Below detectable limit. Detectable limit = 0.1µg/kg. NA: Not Available

Chlorpyrifos Residue Levels in Cowpea Grains Grown in Niger State

During the study period, the residue of chlorpyrifos among different cowpea landraces and across LGA varies in Niger State. Danila was not grown in Niger State during this study. In Bosso LGA, chlorpyrifos was below detectable level in Milk in year 2020 and 2021. Chlorpyrifos in White Agric was also below detectable level in 2020 but was detected in 2021, though at a level below MRL. On the other hand, chlorpyrifos in Zobo Red was detected at a level below MRL in 2020 and below detectable level in 2021 (Table 2)

Chlorpyrifos was detected in Milk (0.0171 mg/Kg) and White Agric (0.0172 mg/kg) in Mokwa LGA in 2020 at levels above MRL. In 2021, chlorpyrifos in Milk and Zobo Red was below detectable level but was detected in White Agric in 2021 and in Zobo Red in 2020, both at levels below MRL (Table 2). In Kotangora LGA, chlorpyrifos was detected at levels below MRL in Milk, White Agric and Zobo Red in 2020. Its level was below detectable limit in 2021 in White Agric and Zobo Red but was detected at a level below MRL in Milk in 2021 (Table 2).

Table 2: Chlorpyrifos Residue Levels (mg/kg) in Cowpea Grains in Niger State

	Danila		Milk		White Agric		Zobo red	
LGA	2020	2021	2020	2021	2020	2021	2020	2021
Bosso	NA	NA	Bdl	Bdl	Bdl	0.0018± 0.000	0.0042± -0.0006	Bdl
Mokwa	NA	NA	0.0171± 0.0084*	Bdl	0.0172± 0.0149*	0.0002± 0.0004	0.0008± 0.0121	Bdl
Kotangora	NA	NA	0.0010± 0.0012	0.0005± 0.0004	Bdl	0.0024± 0.0013	0.0084± 0.0049	Bdl

#The asterisks (*) signifies the cowpea landraces and LGA with the identified chlorpyrifos level above EFSA's Maximum residues limit. NB: EFSA's MRL of chlorpyrifos in cowpea grains =0.01mg/kg. Bdl: Below detectable limit. Detectable limit = 0.1µg/kg. NA: Not Available

DISCUSSION

Chlorpyrifos Residue Levels in Cowpea Grains Grown in Southern Guinea Savannah, Nigeria

There are two things that should be considered whenever chlorpyrifos residues in cowpea grains are at levels above the legally permissible limit. Firstly, trade constraint is inevitable. This is especially due to The European Union (EU) ban on Nigerian white and brown cowpea grains and their products/produce which is yet to be lifted since 2015 (Isegbe *et al.*, 2016; The Guardian 8 June, 2023). In this study, the results revealed 44.4% - 77.8% cowpea grains samples from Kwara and Niger States in 2020 and 2021 having different degrees of chlorpyrifos residue concentrations. This is similar to a study by Salihu *et al*. (2023) in Niger State, where chlorpyrifos was detected in maize and millet samples at levels below health risk. The results in this study however might be a red flag to Nigerian trade image in international market, especially places where chlorpyrifos insecticide is being considered banned such as Vietnam, Morocco, Sri Lanka, Saudi Arabia, Indonesia, Palestine, Switzerland, Thailand, Egypt and Turkey (Wołejko *et al*., 2022), in addition to The EU nations.

The presence of chlorpyrifos residues in some of the samples studied, more of which were obtained in 2020 could be attributed to indiscriminate use of insecticides by farmers in the study area. This might be due to non-reading of insecticide labels by most farmers (as observed earlier in this study), which could make them not to follow manufacturers' instruction on insecticide usage. Fewer samples containing chlorpyrifos residue in 2021 than in 2020, could be connected to rise in insecticide prices in Nigeria at this period (Sanchi *et al*., 2022). This made fewer farmers able to procure the farm input. Also, insurgence from herders in the study area especially Niger State (Joab-Peterside, 2020; Anaemene and Fadupin, 2022) might have prevented farmers' frequent visits to their farms. Hence there would be reduction in the frequency of insecticide application in 2021 planting season compared with 2020.

In the second instance, food safety should be prioritised especially due to the presence of insecticide residue in food, which is a major global concern in the recent times (Wołejko *et al*., 2022). For instance, 22.1% and 11.1% of studied cowpea samples in this study had chlorpyrifos residue above legally permissible levels in year 2020 and 2021, respectively. This is similar to findings of Ogah and Coker (2012) and Osesua *et al*. (2018) who observed chlorpyrifos residue in watermelon and maize, respectively at levels above the legally permitted limit. Likewise, chlorpyrifos residue had been observed at a toxic level in Nigerian cowpea grains exported to Accra, Ghana in a study conducted by Donkor *et al*. (2015). Though, Okoye *et al*. (2021) in a study did not observe detectable quantity of chlorpyrifos residue in cowpea grains obtained from Nsuka, Nigeria. This contradiction might be attributed to possible cultural variation in farmers' insecticide application practices.

Presence of chlorpyrifos above recommended level in cowpea would cause adverse health effects (salivation, dizziness, neurotoxicity, etc) in consumers of such cowpea grains, especially children and adolescent due to their relative lower body weights compared with adults (Feldsine *et al.*, 2015). If children are exposed to more chlorpyrifos residue, it could lead to cognitive deficit in such children (Rauh *et al*., 2011). The highest chlorpyrifos residue levels in Kwara State (0.0129±0.0223 mg/kg) and in Niger state (0.0172±0.0149 mg/kg) were recorded in Moro and

Mokwa LGAs, respectively. These are two LGAs sharing geographical boundary, although from different States. This is an implication that the farmers especially cowpea farmers around this area might have haphazard insecticides usage culture. So, this should be a pointer for intervention to ensure food safety on crops, especially cowpea grains emanating from this region.

CONCLUSSION

Quantitation of chlorpyrifos residue in the cowpea grains of all landraces obtained in the study areas showed that not all cowpea grains grown in the study area contained chlorpyrifos at a level detectable by the instrument of analysis (GC-MS). Between 44.4% and 77.8% cowpea grains samples from Kwara and Niger states in 2020 and 2021were having varying concentrations of chlorpyrifos residue. Exactly 16.7% and 0% of analysed cowpea samples had chlorpyrifos above legally permissible levels (0.01mg/kg) as recommended by EFSA (EFSA 2017) in 2020 and 2021, respectively. The highest chlorpyrifos residue levels in cowpea from Kwara and Niger States were observed in Danila from Moro (0.0129±0.0223 mg/kg) and in white Agric from Mokwa (0.0172±0.0149 mg/kg) LGA, respectively

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