academicJournals

Vol. 7(5), pp. 92-102, May 2013
DOI: 10.5897/AJFS2013.1013
ISSN 1996-0794 ©2013 Academic Journals
http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Contamination of cowpea and by-products by organophosphorous pesticide residues in Ngaoundere markets: Dietary risk estimation and degradation study

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Accepted 24 April, 2013

The study was carried out to monitor some organophosphorous (OP) pesticide residues in stored cowpea in Ngaoundere's markets and two by-products (koki and fritter), to assess the dietary risk exposure through quotient risk (RQ) and to degrade some pesticides using kanwa (rock salt) solution. The levels of nine OP pesticides were determined by gas chromatography equipped with nitrogen photometric detector (GC-NPD) and GC-MS for confirmation. Six OP pesticides (dichlorvos, methyl-parathion, malathion, profenofos, diazinon and chlorpyrifos) were found in concentrations ranging from 0.02 to 5.4 mg/kg in peripheral zone while only five OP pesticides (dichlorvos, methyl-parathion, malathion, profenofos and chlorpyrifos) were found in the urban area (0.02 to 4.62 mg/kg). High amounts of these compounds were found in koki and fritter. Malathion, methyl-parathion and dichlorvos were the most frequent (27 to 89%) and some levels exceeded the maximum residue limits (MRLs) or the acceptable daily intake (ADI) per FAO; high values of RQ were found between February and May for all foodstuffs showing high risk for consumer at this period; malathion, dichlorvos, and profenofos were denatured by kanwa solution at the rate of reduction varying from 65% (malathion) to 98% (dichlorvos) with the production of malaoxon while methyl-parathion was not.

Key words: Organophosphorous, pesticide residues, monitoring, cowpea, dietary-risk, degradation.

INTRODUCTION

The use of pesticides in agriculture becomes more and more intensive in the Northern part of Cameroon. Small scale farmers, who are the main users, abusively spray those chemicals to protect crop and harvested products from pests. Since 1989, because of the financial crisis that hit the country, the government decided to set up a program of liberalizing and privatizing the pesticide sub-sector (Souop, 2005). The objective was to make farmers responsible for the purchase and use of pesticides. This decision fundamentally changed the policy of crop protection and increases risk of poisoning in Cameroon since users were not trained for this purpose. Therefore, selling and use became most rampant among the population and users. Their ignorance about pesticide toxicity led to misuse: abusive application, inadequate usage, etc. (Nguegang et al., 2005). Organochlorine

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pesticides are continuously banned (NRCPPCS, 2007). In the opposite, an addition of organophosphorous compounds is increasing and their association with fungicides is rising. Then organophosphorous pesticides are widely applied in agriculture because their degradation elsewhere is known to be rapid (Kavi and Reetha, 2012).

The general public is mainly exposed to organophosphorous pesticides through the intake of contaminated foods (such as grains, vegetables, and fruits), which are directly used with during cropping and storage (Matthews et al., 2003 and Gimou et al., 2008). These OP pesticides cause two major toxic effects. The first is the well known acute toxicity initiated by inhibition of acetylcholinesterase with subsequent accumulation of acetylcholine at nerve endings.

The second effect is organophosphate-induced delayed polyneuropathy (OPIDP; ataxia and paralysis) associated with inhibition of at least 70% neuropathy target esterase activity sometimes followed by the irreversible transformation of inhibited enzyme to its non-reactivatable form (Milan , 2001). They act by binding to the enzyme acetylcholinesterase, disrupting nerve function, resulting in paralysis and death (Liu et al., 1999). Both of these effects are related to chemical structure of OP that can be largely influenced by metabolism of these compounds that may form potent inhibitors of the enzymes.

Kicth and Ntoukam (1990) show that cowpea is the most threatened grain produced among maize and millet in the Northern part of Cameroon. The production of the cowpea in this area represents 98.5% of the total national production and estimated at 130101 tons in 2010, produced in 115 374 ha (AGRI-STAT, 2011). This production is locally consumed, sold in the southern Cameroon and about 40% is exported to Gabon and Nigeria. The unit price per kilogramme is estimated at 1.1 USD (MINADER, 2012). The protection of this cowpea using white powder organophosphorous pesticides is much popularized as; to differentiate it from red powder (organochlorine and some carbamates) supposed to be used only for seed treatment. However, the misuse of those compounds is still harmful for consumers and users. Since cowpea is produced once a year, the protection during storage becomes necessary to preserve grain quality throughout the year.

In Ngaoundere, cowpea is consumed in two main forms (koki and fitters) sold in restaurant and popular places (schools, markets, etc.). The use of some ingredients such as rock-salt during cooking is very common to avoid flatulence, to tenderize or to improve aroma (Efiuvwevwere and Chidi, 1996). The commercial transaction between producers and consumers is generally done by sellers (wholesalers and retailers). They store grains to make most economic benefits within the duration since the price depends on the period of the year (Langyintuo, 1999). Therefore, contamination of this foodstuff by pesticide residues becomes a dramatically topical issue in the area because of multiple treatments.

In developed countries, pesticide residues control in market food is routinous, but in developing countries like Cameroon, few databases are available (Sonchieu et al., 2010 and Yanhong et al., 2006). Many intoxication cases due to OP pesticides through food contamination have been reported by Apa (2010) in Ngaoundere and elsewhere in the country. Many methods of degradation have been developed to reduce amounts in water. This advanced oxidative process (AOP) has been used by authors to degrade pesticide residues in food (Randwan et al., 2005 and Alvaro et al., 2011), but products used for this degradation are laboratory chemicals (acetic acid and bicarbonate of sodium) or microorganisms, which can also damage some functions of the human body (Luisa, 2012). However, those products are found in some house materials used to cook or to flavor: such as the rock-salt commonly known as kanwa.

Objectives of this study were foremost to carry out the monitoring of the organophosphorous pesticide residues in whole cowpea grains and into two by-products (koki and fritters) during twelve months (November 2010 to October 2011) both in Ngaoundere and peripheral areas; and secondly to assess dietary risk and a method of degradation.

MATERIALS AND METHODS

Sampling

Samples of cowpea grains were collected from 10 sites during 12 months in the Ngaoundere central markets: Petit Marché [(&ll= 7.322372, 13.586655), (4 sites)] and Grand Marché [(&ll= 7.326661, 13.582073), (1 site)]; and Ngaoundere peripheral markets: Bamyanga [(&ll= 7.293068, 13.576682), (2 sites), Bantaï [(&ll= 7.310454, 13.597641), (2 sites)], and Dang [(&ll= 7.42273, 13.551979), (1 site)], following the guidance given by Sharma et al. (2007). While those of koki and fritters were collected from restaurants during the same period. Approximately 1 kg of samples were separately collected, put in pharmaceutical bags and directly transported to the laboratory for immediate preparation and extraction. The extract was stored at -20°C until final processing.

Source of materials and sample preparation

Cowpea grains, koki and fritter samples were collected directly from ten local identified sellers who were randomly chosen, according to the availability of foods from them.

Samples of cowpea grains were properly homogenized and 500 g picked for comminution using electrical blinder (ZENPLIN®) or were triturated using laboratory mortar (koki and fritters). After each operation, all the materials were washed and dried before the next grinding. No sample was sieved. The comminuted samples were mixed and homogenized before withdrawing 25 g for analysis. The rock-salt (kanwa) was purchased at the Ngaoundere market, ground and sieved (Ø=01 mm).

Test of matrix quality

The matrices (cowpea, *koki* and fritter) used for recovery were

provided from Mokolo (Northern Cameroon area) on November 2010. Five blanks were taken for each type of food and collected directly from farmers or restaurants during harvest, before any spraying. Analysis was initially carried out for each blank to determine if there were any detectable contaminants. All contaminated samples were discarded. Any interference chromatographic peaks with investigated insecticides were assessed.

Materials and reagents

All organic solvents were products of Spectrochem or Fisher Scientific, high performance liquid chromatography (HPLC) grade, supplied by Supleco of Lucknow, India. Standard OP pesticides of purity 99.0 to 99.9% were provided by the Bureau of Indian Standards (BIS) and supplied by the Core Laboratory, Certified Reference Materials (CRMs) from BCKV (Bidhan Chandra Krishi Vishridhyalya) Centre of India. Stock standard solutions of OP pesticides were prepared in n-hexane at about 100 mg/L. An intermediate solution of each pesticide at 10 mg/L was prepared in the same solvent. The mixture of all pesticides was also made up in n-hexane at 0.5 mg/L and used as working standard. This working solution contained 8 OP pesticides which are all OP pesticides authorized and commonly used in the country: dichlorvos, dimethoate, diazinon, methyl-parathion, edifenfos, malathion, chlorpyrifos and profenofos.

Extraction

Extraction was carried with 100 mL of mixed HLPC grade solvents (acetone and n-hexane) (1:1, v/v) in 250 mL flasks and were mechanically shaken for 1 h using a reciprocating shaker (Sambros®) at 180 translations per min. After filtration, 250 mL of distilled water and 20 ml of saturated sodium chloride solution were added. After 5 min of vigorously shaking of the mixture and 15 min of rest, the two phases were separated. The aqueous phase was retrieved and 75 mL of dichloromethane (DCM) added and mechanically shaken for 10 min. The two organic phases were then mixed and dehydrated with a convenient quantity of activated anhydrous sodium sulphate (anhy. Na_2SO_4). The samples were then concentrated in a rotary evaporator until dryness at 35±10°C. The residue was dissolved in 10 mL of an iso-octane/acetone mixture (9:1, v/v).

Clean-up

A 2.5 mL aliquot of the above extract was loaded on the cartridge and eluted with 60 mL of n-hexane saturated with acetonitrile through a chromatographic column containing 2 g of activated florisil. Elution took about 1 hour. The cartridge provided by Supelco (Lucknow, India) was the Florisil-Sigma, F9127-5006 mesh: 60-100/PR (pesticide residues) with 1 cm of activated anhydrous Na_2SO_4 loaded on top. The eluate was concentrated under the same conditions as described above and the residue dissolved in 5mL of an iso-octane/acetone mixture (9:1, v/v). GC determination was either carried out immediately or the extract was kept refrigerated at 4°C for a maximum of 48 h.

Removal of fat/oil

Since fat is detrimental to the column, extract obtained above was treated to remove fat from samples of koki and fritter. 30 mL of petroleum ether were added to extract and mixed with acetonitrile (20 mL) and shake vigorously: repeated three times. The acetonitrile phase was kept while the other was discarded. 20 mL of petroleum ether was also added to the volume of acetonitrile phase to remove other trace of fat. 30 mL of n-hexane were added to the acetonitrile phase and the mixture was vigorously shaken: three times repeated. The n-hexane phase was washed with 250 mL of bi-distilled water and 20 mL of saturated NaCl. The operation has been repeated once and all n-hexane phases were mixed. Dehydration of this mixture was done using a convenient quantity of activated anhydrous sodium sulphate (anhy. Na_2SO_4) and following by filtration. The filtrate was concentrated in a rotary evaporator until dryness at 35±10°C. The residue was dissolved in 10 mL of pure hexane for clean-up.

Risk estimation

The dietary risk was calculated using the quotient method based on the Risk Quotient (RQ) for each combination of contaminants found in a given foodstuff. The assessment of this risk parameter was calculated using the formula described below (EAWAG, 2003 and EC, 2003).

$$RQ = \frac{Concentration}{\mathrm{TR}V / NOAEL} \quad \text{and } RQ = RQ_1 + RQ_2 + \ldots\ldots RQ_n$$

Where, 1, 2, ….n represent a specific pesticide found in the same sample; TRV is the toxicity reference value which is the no observed adverse effect level (NOAEL). The RQ reference value is “1”, any value above describes a situation of risk and vice-versa.

Scheme of Kanwa solution application on cowpea grains

The ground sieved kanwa was dissolved in bi-distilled water and the pH fitted at 9.52. One kilogram of grain of cowpea was separately spiked with formulations of pesticides found to present dietary risks: Dichlorvos (DDVP) 125 g/L + malathion 100 g/L (Digrain 4), methyl-parathion (Paratox) and profenofos (Plantofos 75 EC), and stored for three months to evaluate the effect of kanwa during this period of storage. Analyses were carried-out once a month. The experimentation was first led on water contaminated with studied pesticides. Mixtures are shown in Table 1, where conditions are the following: time (30 min), temperature (25°C), pH (9.52) and no shaking. The weight used during soaking refers to whole grains or flour.

Apparatus and operating conditions

Gas chromatography: conditions in NPD detector

Gas chromatography in this case was performed using a Carlo Erba gas chromatography equipped with a nitrogen phosphorus detector (NPD). An Optima 5-MS60® (30mx25 mm I.D., 0.32 nm film thickness) capillary column was used. Nitrogen was used as carrier gas at a flow-rate of 2 ml min^{-1}. The oven temperature conditions were: initial temperature isothermal at 90°C for 1 min, increased by 10°C.min^{-1} to 340°C and held for 1 min. Injector and detector temperatures were 200 and 300°C, respectively.

Conditions in GC/MS

A Shimadzu GC-MS QP-2010 was used to identify target compounds, equipped with a split/splitless injector and a DB-5MS® (30mx0.25mm I.D., 0.25 mm) J&W column. EI mass spectra were generated using electron energy of 70 eV, monitoring for ions m/z

Table 1. Levels of spiking and concentration in grains and water.

Pesticides	Spiked amount (mg/kg or mg/L)	Water	Concentration	
	Stored grain		Weight/Vol. of *kanwa*	Vol. of *kanwa* /Vol. spiked water
Dichlorvos	0.1	1.4	5 g/10 mL	1 mL/5 mL
Methyl- parathion	0.1	2.1	5 g/10 mL	1 mL/5 mL
Malathion	0.1	1.3	5 g/10 mL	1 mL/5 mL
profenofos	0.1	1.0	5 g/10 mL	1 mL/5 mL

Table 2. Data of the method validation for active ingredients and metabolite.

Pesticide	n	mLOD (mg kg^{-1})	mLOQ (mg kg^{-1})	spiking level (mg Kg^{-1})	Recovery ±RSD (%)		
					koki	fritters	cowpea
Parent products							
Dichlorvos	3	0.002	0.006	B+0.5	97±7	95±6	89±10
Dimethoate	3	0.002	0.003	B+0.5	79±16	77±14	73±3
Diazinon	3	0.002	0.006	B+0.5	100±12	98±14	78±2
Methyl Parathion	3	0.002	0.006	B+0.5	83±7	81±9	103±11
Chlorpyrifos methyl	3	0.001	0.003	B+0.5	71±3	73±2	88±5
Malathion	3	0.002	0.006	B+0.5	85±10	100±16	106±1
Chlorpyrifos	3	0.003	0.009	B+0.5	74±11	75±9	85±4
Profenofos	3	0.002	0.006	B+0.5	109±16	105±10	103±6
Edinofos	3	0.002	0.006	B+0.5	75±12	101±16	78±6
Degradation product							
Malaoxon	3	0.002	0.006	B+0.5	99±16	77±12	102±1

LOD, Limit of detection; LOQ, limit of quantification.

50→450 in full-scan and selected ion recording (SIR) modes. The scan interval was 0.3 s (1425 amu.s^{-1}), injection volume was 2 ml and the source temperature employed for the ionization technique was 200°C. Injector temperature and detector temperature were set at 250°C. The column was held at 80°C for 1 min, then increased at a rate of 6°C min^{-1} to 150°C, followed by heating to 250°C at a rate of 20°C min^{-1}, and finally held constant at 250°C for 2 min.

Standardization of the method and calculation

Quantification was performed by comparing the peak areas to those of the calibration curve standard while identification was carried out by comparing the retention times with the standard. The precision of the methods expressed as repeatability (% RSD), was evaluated by analyzing samples in triplicate fortified at 0.015; 0.15; 0.2 and 0.25 mg kg^{-1}, depending on the sensitivity of the detector to the compound. OJ/EU (2004), determination coefficients were used to estimate linearity. Peak areas were measured relative to standards. Blanks used to estimate recoveries were provided as non-treated grains and directly acquired from local farmers during harvest. They were used as controls and showed no interference from the pesticides being monitored. The method limits of detection (mLOD) and quantification (mLOQ) were calculated experimentally from signal-to-noise ratios of 3.0 and 10.0, respectively, by spiking samples at low concentrations and subjecting them to sample preparation and clean-up. Blank extracts were used for estimation of background noise in chromatographic analysis. Identification of active ingredients was confirmed by GC–MS on more contaminated samples and on some formulations used for powered grains. Following the conditions described above, a standard curve was used to calculate sample concentrations (x) according to the linear equation: $y = ax + b$.

RESULTS AND DISCUSSION

Method validation

Table 2 presents data of the method validation. Data rule the acceptability for applicability performance of analysis, since repeatability is still conformed to the European Commission (EC) directives (EC, 2010).

Rate of contamination of foodstuffs

Analysis of cowpea samples and by-products from Ngaoundere revealed the presence of seven OP pesticides at rate ranging from 66 to 87% (Table 3). Malathion, methyl-parathion and dichlorvos are found in all types of foodstuffs but with predominance of the former two compounds (Malathion and methyl-parathion) in all contaminated samples. Although, grain samples

Table 3. Rate of cowpea and by-products contamination from Ngaoundere compared to the MRL/ADI.

OP pesticide	Grains (Petit Marché)		Grains (peripheral zone)	*Koki*		Fritter	
	n	Rate of contamination (%) [%>MRL]	Rate of contamination (%) [%>MRL]	n	Rate of contamination (%) [%>ADI]	n	Rate of contamination (%) [%>ADI]
	120	66 [40]	86 [50]	60	87 [60]	60	87 [70]
Dichlorvos	120	25 [0]	20 [0]	60	15 [15]	60	20 [20]
Dimethoate	120	-	2 [0]	60	-	60	-
Diazinon	120	-	4 [0]	60	3 [0]	60	-
Methyl Parathion	120	55 [35]	60 [40]	60	55 [55]	60	35 [35]
Chlorpyrifos-methyl	120	-	-	60	-	60	-
∑Malathion	120	75 [0]	90 [5]	60	85 [60]	60	90 [70]
Chlorpyrifos	120	5 [0]	4 [0]	60	-	60	5 [0]
Profenofos	120	5 [5]	10 [5]	60	5 [5]	60	5 [0]
Edinofos	120	-	-	60	-	60	-

n, Total number of samples; MRL, maximal residues limit; ADI, acceptable daily intake; […] = % samples higher than ADI/MRL.

from the peripheral area and by-products are most contaminated (ranging from 15% (dichorvos) to 90% (malathion)) and present levels higher than norms [maximum residue limits (MRL) and acceptable daily intake (ADI)] comprised between 15% (dichlorvos, koki) and 70% (malathion, fritter). However the methyl-parathion in contaminated samples might go up to 100% higher than norms (koki and fritter) despite its low frequency. The edinifos and chlorpyrifos-methyl were not found in any sample.

Levels of contamination

Analysis of cowpea samples and by-products in Ngaoundere showed contamination throughout the year at various levels. The mean of amounts are presented in Figure 1, methyl-parathion presents higher levels than those of other compounds in grains of Petit Marché and fritter (varying from 4.5 to 5.5 mg/kg), while the malathion presents the same profile in koki and grains of peripheral zone (varying between 5.4 and 8.6 mg/kg). Levels of all OP pesticides in each type of food fluctuate and show maxima during February, March, April and May, but remain low during other months.

These concentrations maybe higher than the limit of report (LR) which is MRL (cowpea grains) and ADI (koki and fritter) or not as shown in Table 4; which also presents levels less than the limit of report varying from 40% (methyl-parathion in koki) to 100% (malathion and dichlorvos in grains). Amounts of pesticides found to be greater than the limit of report were 10, 100, 1000 or 10000 times higher than these norms. The methyl-parathion is the only pesticide showing amounts 10000x higher than the Limit of report in samples of koki (5%) and fritter (2%). The majority of amounts of OP pesticide residues in grains were ranging from LR to LRx10 even those of peripheral area were highest, while those in koki and fritter were varying from LRx10 to LRx100. Although, the methyl-parathion presented higher amounts among compounds, following by dichorvos and malathion. Other OP pesticides residues such as diazinon, chorpyrifos and dimethoate were present at lower concentrations.

Dietary risk exposure

This parameter assessed according to the risk quotient (RQ) refers to Table 5 showing variations of calculated RQ values for each type of food throughout the period of study. Risks of intoxication (exposure) are given for 12 months. All calculated RQ values were lower than the critical level (“1”) except koki of the month of May (RQ=1.77). The RQ calculated values grouped two to three pesticides but this combination did not influence the RQ. Higher calculated values of RQ were ranging between months of the February and May, showing high exposure risk.

Degradation study

The use of kanwa solution degrades OP pesticides such as malathion, dichlorvos and pro-

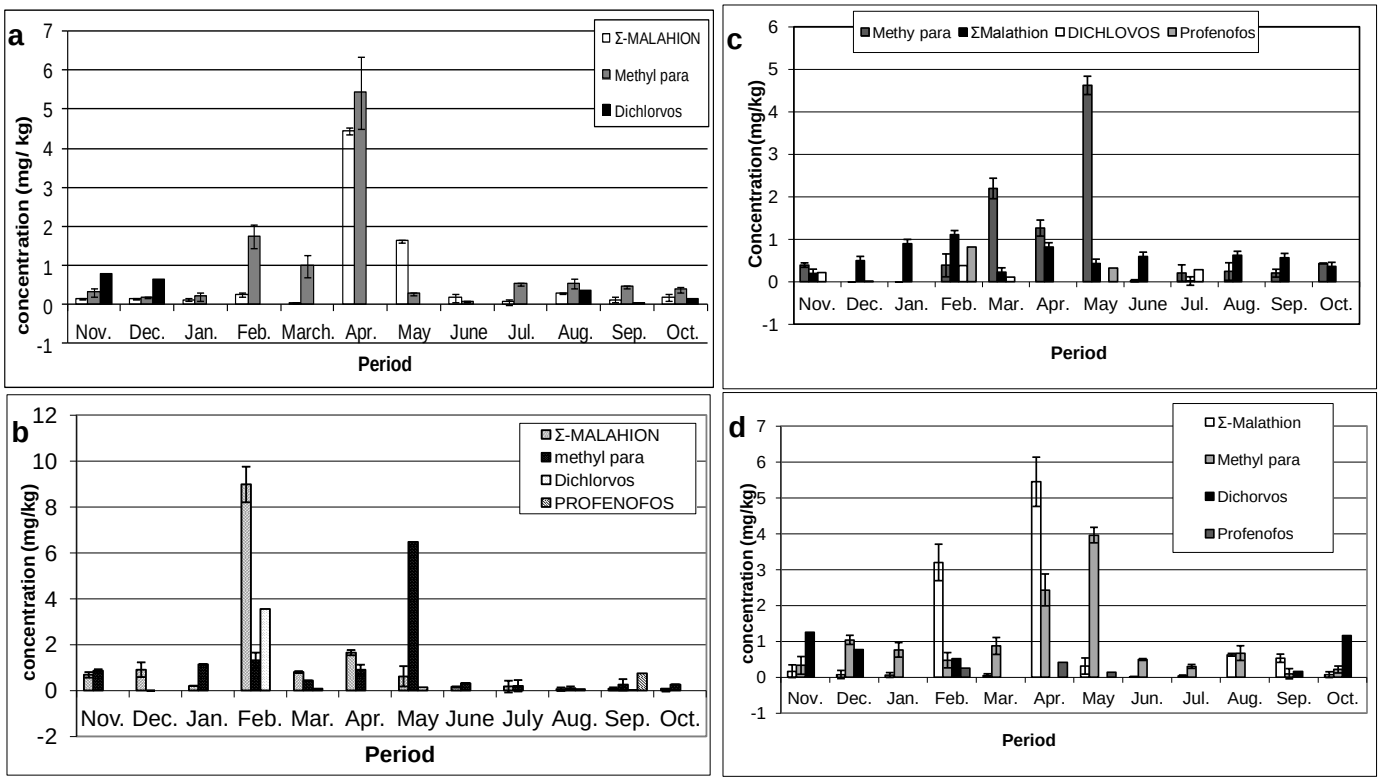

Figure 1. Evolution of OP pesticides amounts in cowpea and by-products from Ngaoundere: November 2010 - October 2011. **a,** Fritter; **b,** *koki*; **c.** grains from Petit Marché (Ngaoundere); **d,** grains from peripheral zone (Ngaoundere).

fenofos (Table 6). After 5 min of mixture, only dichlorvos was degraded, but from 15 to 60 min, all OP pesticides were denaturized; except methyl-parathion. Values of recovery (without treatment) are significantly different from those obtained after application. The reduction of levels of pesticides studied within that time showed significant difference between values (Table 6). The production (formation) of malaoxon followed the same principle but values were not significantly different. Then OP pesticides were reduced at the rate varying from 20 to 98%, starting from 15 min, except methyl-parathion (Figure 2). The duration did not significantly influence the rate of reduction after 15 min.

The results of kanwa solution's application on cowpea grains and flour are detailed in Table 7. The level of recovery are not significantly different ($p<0.01$) between samplings. However, significant differences are observed between each value of recovery and subsequent value after application of kanwa solution. Figure 3 indicates levels of reduction with production of malathion's metabolite (malaoxon). This rate of reduction did not change during the three months of storage. Amounts of malaoxon produced were not significantly different between months.

DISCUSSION

This study is in line with Sonchieu et al. (2010) which confirm the work done on vegetable and fruits by Gimou et al. (2008) because cowpea is a staple food to be eaten with other secondary foods. Then, a dietary risk may be accessed through the complementarity of that data. High concentrations of malathion were also observed in April (Sonchieu et al., 2010). Those high concentrations and frequencies confirm observations of Kicth and Ntoukam (1990) showing that usage of synthetic pesticides is practiced by 73% of those manipulating grains during storage. The methyl-parathion and malathion are still most used since they are most popularized by sellers. Their presence in many formulations sold in the country in many cases as powder, facilitates their accessibility and spraying. The most common formulation is the "poudrox" which had shown it effectiveness on pests, maybe more than "Caiman Rouge" which is a mixture of malathion and dichlorvos. Other OP pesticides (dimethoate, chlorpyrifos, chlorpyrifos-methyl, profenofos and diazinon) determined during this study are commonly used during cropping and mainly in vegetables since they are in liquid form (Apa, 2010 and MINARDE, 2010). Their presence in grains is an indicator of inadequate use or persistence.

Highest amounts observed between February and May are in agreement with observation of Langyintuo et al. (2003), who showed that the main pests (*Callosobruchus maculatus*) damages happen three months after harvest. This period corresponds to that of best exchanges

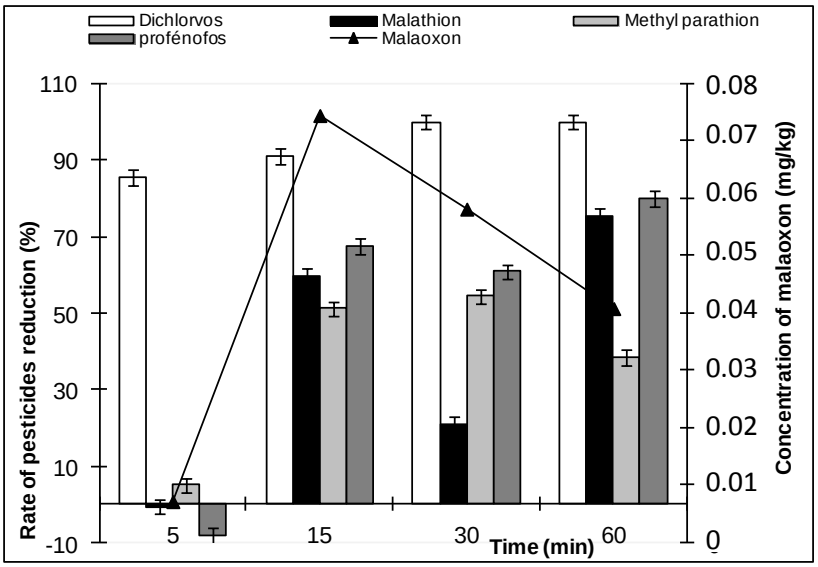

Figure 2. Rate of OP pesticides reduction and metabolite production in distilled water after soaking in *kanwa* solution.

Table 4. Variation of concentrations of main pesticides found in *koki*, fritter and cowpea grains in Ngaoundere's markets.

Foodstuff	Pesticide	Concentration (mg/kg)					Total (n)
		<LR (5)[	[5-50]	[50-500]	[500-5000]	[5000<]	
Petit *Marché* grains	ΣMalathion: n(%)	48 (100)	0(0)	0(0)	0(0)	0(0)	48 (100)
	Dichlorvos: n(%)	48(100)	0(0)	0(0)	0(0)	0(0)	48(100)
	Methyl-Parathion	<LR (0.1)[	[0.1-1.0]	[1.0-10.0]	[10.0-100.0]	[100.0<]	
	n (%)	30 (63)	14(29)	3 (6)	1(2)	0(0)	48(100)
Peripheral area grains	ΣMalathion: n(%)	70 (97)	2 (3)	0(0)	0(0)	0(0)	72(100)
	Dichlorvos: n(%)	72 (100)	0(0)	0(0)	0(0)	0(0)	72(100)
	Profenofos	<LR (0.05)]	[0.05-0.5]	[0.5-5.0]	[5.0-50.0]	[50.0<]	
	n(%)	71 (99)	1 (1)	0(0)	0(0)	0(0)	72(100)
	Methyl-Parathion	<LR (0.1)	[0.1-1.0]	[1.0-10.0]	[10.0-100.0]	[100.0<]	
	n(%)	42 (58)	20 (28)	5 (7)	5(7)	0(0)	72(100)
Koki	ΣMalathion	<LR (0.3)	[0.3-3.0]	[0.3-30.0]	[30.0-300.0]	[300.0<]	
	n(%)	45(75)	14(23)	0(0)	1 (2)	0(0)	60(100)
	Profenofos	<LR (0.03)	[0.03-0.3]	[0.3-3.0]	[3.0-30.0]	[30.0<]	
	n(%)	59(98)	1 (2)	0(0)	0(0)	0(0)	60(100)
	Methyl-Parathion	<LR (0.003)	[0.003-0.03]	[0.03-0.3]	[0.3-3.0]	[3.0<]	
	n(%)	24 (40)	0(0)	18 (28)	16 (27)	2 (5)	60(100)
	Dichlorvos	<LR (0.004)	[0.004-0.04]	[0.04-0.4]	[0.4-4.0]	[4.0<]	
	n(%)	52(87)	1 (2)	6(9)	1(2)	0(0)	60(100)
Fritter	ΣMalathion	<LR (0.3)	[0.3-3.0]	[0.3-30.0]	[30.0-300.0]	[300.0<]	
	n(%)	45(75)	13(22)	2(3)	0(0)	0(0)	60(100)
	Methyl-Parathion	<LR (0.003)	[0.003-0.03]	[0.03-0.3]	[0.3-3.0]	[3.0<]	
	n(%)	38(63)	0(0)	9(15)	13(21)	1(1)	60(100)
	Dichlorvos	<LR (0.004)	[0.004-0.04]	[0.04-0.4]	[0.4-4.0]	[4.0<]	
	n(%)	49(82)	1(2)	6(9)	4(7)	0(0)	60(100)

LR, Limit of report (MRL/ADI); n, number of sample.

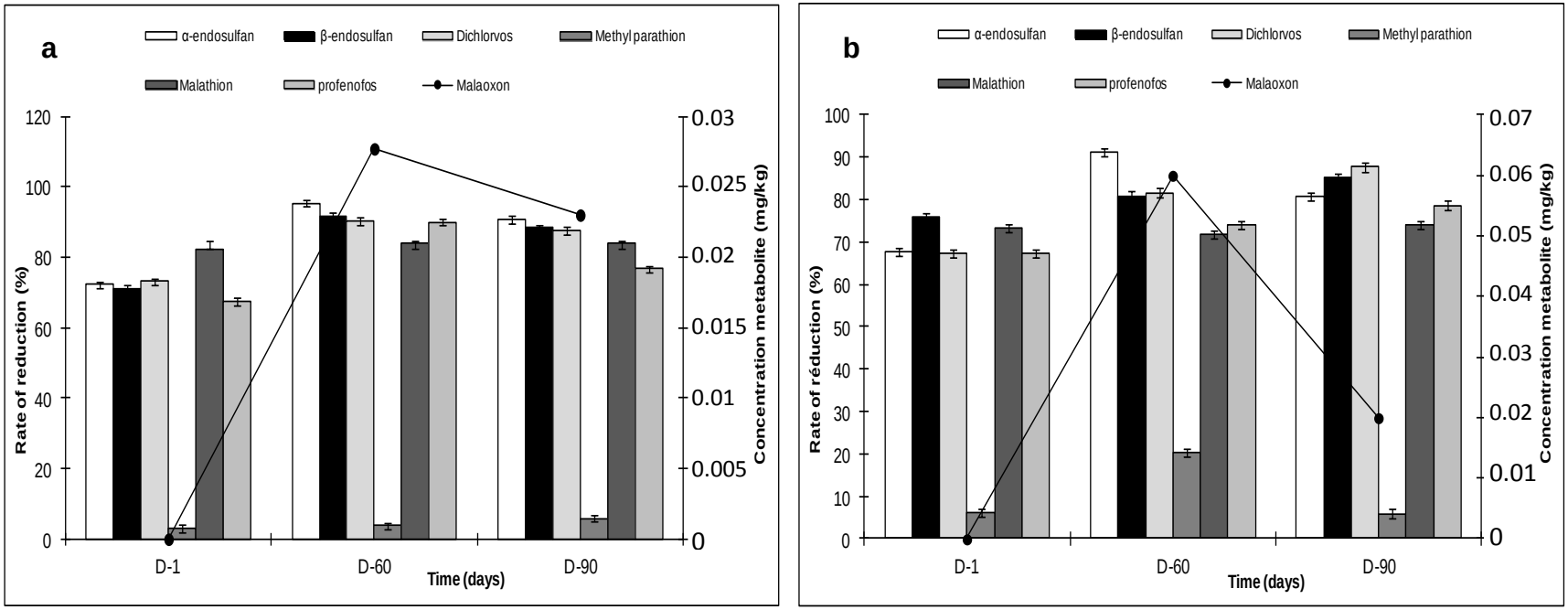

Figure 3. Rate of OP pesticides reduction in stored cowpea and metabolite production after soaking in *kanwa* solution. **a)** Rate of OP pesticides reduction in cowpea flour; b) rate of OP pesticides reduction in cowpea whole grains. D-, Days after spraying.

for cowpea commercialization in Cameroon; subsequently, the appropriate period of multiple treatment for greatest preservation (langyintuo, 1999). This is the main reason why grains from peripheral areas have shown higher amounts than those from Ngaoundere central markets (urban area): wholesalers in the city supply to retailers at the peripheral area, who constitute another step of grain treatment. This observation demands further investigation.

The high pollution of by-products issuing cowpea grains is due to the addition of some ingredients (palm oil, cotton oil, pepper, etc.) which can also contain pesticide residues (Mbofung et al., 1999; Sougnabe et al., 2010). The effect of heat did not effectively affect the amount of these pesticides during cooking (WHO, 2002; Lalah and Shem, 2002).

The level of risk calculated according to a man of 60 kg is relatively low, but may become dangerous for children and young infants who are not neglected consumers (EC, 2003). They are exposed to pesticide daily intake through koki or fritter, since they consume more food per kilogram of body weight than do adults (Noriko et al., 1998). Many symptoms related to pesticides effects maybe found within the local population as controlled in Indian population by Ajith et al. (2006). However, observed effects are correlated to the health of the consumers (EC, 2003). The high risks observed in February, March, April and May are likely related to the levels of contamination observed during this period. Results of this investigation show concordance to those observed by Gimou et al. (2008) in Yaounde between June and August in general food consumption.

The degradation of OP pesticides in the kanwa solution which is an alkaline medium is attributed to the pH effects, since Perihan et al. (2007) demonstrated that the alkalinity affects pesticides. After five minutes of soaking, there was no reduction characterizing the negative chart part. This effect is great as Peggy (2002) advised pesticides users to control the acidity of the water

Table 5. Variation of risk quotient (RQ) in cowpea and by-products from Ngaoundere's markets.

Period	Grain (Petit Marché)		Grains (peripheral zone)		Fritter		*Koki*	
	Number of pesticides	RQ	Number of pesticides	RQ	Number of pesticides	RQ	Number of pesticides	RQ
November	3	0.03	3	0.03	3	0.03	2	0.01
December	3	0.01	2	0.08	3	0.02	2	0.01
January	2	0.01	1	0.05	3	0.03	2	0.03
Febuary	3	0.04	4	0.04	2	0.12	3	0.12
March	2	0.15	3	0.06	2	0.07	3	0.08
April	3	0.1	2	0.18	2	0.37	2	0.06
May	3	0.32	3	0.28	2	0.03	3	1.77
June	2	0.01	2	0.04	2	0.01	2	0.01
July	2	0.02	3	0.03	2	0.04	2	0.03
August	2	0.02	2	0.05	3	0.05	3	0.01
September	3	0.02	2	0.02	3	0.03	3	0.02
October	3	0.08	2	0.03	3	0.03	2	0.02

Table 6. Effects of *kanwa* on distilled water pesticides fortified**.**

Pesticide	Variation of concentration (mg/mL)							
	5 min		15 min		30 min		60 min	
	Recovery	After application	Recovery	After application	Recovery	After application	Recovery	After application
Dichlorvos	0.98^{1}	0.14 ± 0.04^{a2}	1.06^{1}	0.09 ± 0^{b2}	0.94^{1}	ND	1.03^{1}	ND
Malathion	1.46^{1}	1.47 ± 0.1^{a1}	1.11^{1}	0.45 ± 0.1^{b2}	1.2^{1}	0.35 ± 0.01^{c2}	1.16^{1}	0.28 ± 0.1^{d2}
Methyl-parathion	2.02^{1}	1.92 ± 0.04^{a}	2.05^{1}	1.71 ± 0.01^{b1}	2.08^{1}	0.95 ± 0.01^{c2}	2.00^{1}	1.23 ± 0.03^{d2}
Profenofos	0.94^{1}	1.02 ± 0.2^{a}	0.98^{1}	0.32 ± 0.01^{b2}	0.82^{1}	0.32 ± 0.01^{b2}	0.86^{1}	0.17 ± 0.02^{c2}
Malaoxon	0.0	0.01 ± 0^{2}	0.0	0.07 ± 0.05^{2}	0.0	0.06 ± 0.01^{2}	0.0	0.04 ± 0.01^{2}

Values of the same rows with similar digits or letters are not significantly different at P>0.01.

Table 7. Effects of *kanwa* solution on stored cowpea pesticides fortified**.**

Pesticide	Variation of concentration (mg/kg)					
	Cowpea flour					
	Day 1		Day 60		Day 90	
	Recovery	After application	Recovery	After application	Recovery	After application
Dichlorvos	0.74^{1}	0.17 ± 0.02^{a2}	0.64^{1}	0.06 ± 0.01^{a2}	0.72^{1}	0.15 ± 0.02^{a2}
Methy-parathion	0.50^{1}	0.43 ± 0.02^{a1}	0.42^{1}	0.4 ± 0^{a1}	0.50^{1}	0.44 ± 0.02^{a1}
Malathion	0.50^{1}	0.08 ± 0.01^{a2}	0.77^{2}	0.12 ± 0.01^{a2}	0.60^{1}	0.07 ± 0.01^{a2}
profenofos	0.69^{1}	0.22 ± 0.01^{a2}	0.27^{2}	nd	0.34^{1}	0.03 ± 0.01^{a2}
Malaoxon	0.0	nd	0.0	0.03 ± 0.01^{2}	0.0	0.02 ± 0.01^{2}

Values of the same rows with similar digits or letters are not significantly different at P>0.01.

before any dilution for spraying. Radwan et al. (2005) and Alvero et al. (2001) observed this same effect using solutions of sodium carbonate (Na_2CO_3). The prompt reaction was also observed on organochlorine (lindane and heptan) and some organophosphates (diazinon) (Nyakundi et al., 2011 and Xiangyu et al., 2013). This degradation is attributed to oxidation or hydrolysis reactions by desulfuration (P-S→P-O) pathway (Eto, 1974). The production of the malaoxon is then due to the attack of P=S and P-O bonds by the OH^- ions. The formation of this degradation product follows the reaction (NRCPPS, 2007; Eto, 1974) as follows:

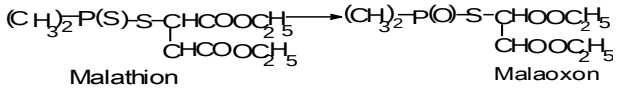

Table 7. Continued.

Pesticide	Variation of concentrations (mg/kg)					
	Whole grain					
	Day 1		Day 60		Day 90	
	Recovery	After application	Recovery	After application	Recovery	After application
Dichlorvos	0.74[1]	0.24±0.02[b2]	0.29[2]	0.05±0.01[a2]	0.29[2]	0.04±0.02[a2]
Methy-parathion	0.39[1]	0.27±0.06[a1]	0.29[1]	0.25±0.1[a1]	0.24[1]	0.24±0.2[a1]
Malathion	0.40[1]	0.1±0.01[a2]	0.71[2]	0.41±0.1[a2]	0.68[2]	0.38±0.2[a2]
profenofos	0.80[1]	0.26±0.01[a2]	0.27[2]	0.06±0 [a2]	0.22[2]	0.04±1[a2]
Malaoxon	0.0	nd	0.0	0.03±0.01[2]	0.0	0.02±0.01[2]

Conclusions

These results provide an important information on the current contamination status of a key agricultural product in Cameroon since cowpea produced in Northern Cameroon is sold in the whole country or in other neighboring countries such as Nigeria and Gabon. This study points to the need for urgent action to control the use and management of some excessively applied OP pesticides; since recent literature reveals that the largest proportion of human acute toxicity data is related to OP pesticide intoxications.

ACKNOWLEDGEMENT

The authors express their thanks to the Academy of Science for the Developing World (TWAS) and the Council of Scientific and Industrial Research (CSIR) for the funding and support provided. They also thank the Director of IITR, Lucknow, for his research interest. IITR manuscript number: 2869

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