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Multi-residue levels of Organophosphorous pesticides in cocoa beans produced from Ghana

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ABSTRACT

Residual levels of organophosphorous pesticides were determined in 44 fermented and dried cocoa beans samples collected from two cocoa beans storage warehouses located in Tema and Takoradi; cities in Ghana from November 2010 to January 2011. The main objective of the study was to monitor and assess the residue levels of 13 organophosphorous pesticides in fermented and dried cocoa beans produced from Ghana. The extraction method uses acetonitrile as the extracting solvent. Two solid phase extraction clean-up cartridges were employed; bond elut C18 cartridge, followed by envi-carp/LC-NH_2 superclean cartridge; using acetonitrile and a mixture of toluene/acetonitrile in the ratio 1:3 as eluting solvents, respectively. The determination was done using gas chromatography with pulse flame photometric detector (GC-PFPD). The targeted compounds were methamidophos, ethoprophos, phorate, fonofos, diazinon, dimethoate, pirimiphos-methyl, chlorpyrifos, malathion, fenitrothion, parathion, chlorfenvinphos and profenofos. The percentage recoveries ranged from 70-95 percent, with instrumental method determination limit of 5.0 µg/kg. The results indicated that ethoprophos is not being used in cocoa production in Ghana. However, there were appreciable amounts of Dimethoate (22.3 µg/kg), Pirimiphos-methyl (29.5 µg/kg), Malathion (20.6 µg/kg), Chlorpyrifos (50.2 µg/kg) and Fenitrothion (93.8 µg/kg). These notwithstanding, residue levels of all organophosphorous pesticides detected were below both the EU and Japanese maximum residue limits; with the exception of Methamidophos, Chlorpyrifos, Malathion and Profenofos which average residue values were comparable with either one of the two international maximum residue limits set.

Introduction

Many farmers believe that pesticides work, at least against some cocoa pest problems, and continue to use them depending on the pest and country (ICCO, 2008). Diazinon is an approved insecticide for the control of capsids in cocoa production in Ghana (EPA Ghana, 2009). Aside the control of cocoa capsids, there are other widespread fungal diseases in cocoa production such as black pod, witches' broom and monilia. The latter two are rather encountered in Latin America and South-East Asia respectively. Of course, there are other pests and disease problems which are of concern in cocoa production in Ghana. These include the cocoa swollen shoot virus, capsids (miridae), insect pests of the cocoa tree (example termites and stem-borers), weeds (especially in young cocoa plant) and insect pests of storage such as beetles and warehouse moths (ICCO, 2008). Therefore, to minimize the economic losses caused by these pests and diseases in the cocoa industry; various insecticides, fungicides, rodenticides and herbicides are used on cocoa on a massive scale (Hayes and Laws, 1991).

Insecticide application techniques on cocoa remain essentially based on experiments that were carried out in the 1960s. After the banned of the organochlorine pesticides due to their human and environmental adverse effects concerns, researchers in the cocoa industry then focused on carbamates and organophosphorous compounds (Entwistle et al., 1959). Organophosphorous pesticides are widely used pesticides that are applied primarily to crops for the control of agricultural pests, as well as in and around residences and offices for the control of urban pests (Vermeire, T. et al., 2011). Organophosphorous pesticides act through inhibition of the cholinesterase enzyme, resulting in the accumulation of acetylcholine, which interferes with the neuromuscular junction producing rapid twitching of voluntary muscles and finally paralysis (Yeboah, P. O., 2009). However, all organophosphorous pesticides are subject to degradation by hydrolysis yielding water-soluble products that are believed to be non-toxic at all practical concentrations. The toxic hazard of organophosphorous pesticide is therefore essentially short-term in contrast to that of the persistent organochlorine pesticides, though the half-life at neutral pH may vary from a few hours for dichlorvos to weeks for parathion (WHO, 1986).

In Ghana, some organophosphorous pesticides are registered for agricultural and public health purposes. These include Pirimiphos-methyl, diazinon, dimethoate, Chlorpyrifos and Fenitrothion (EPA Ghana, 2009). When these pesticides are applied on cocoa, some amount of these pesticides, or indeed

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what they change to in the plant (their metabolites or degradative products), can remain in/on cocoa until after it is harvested (Masud and Hassan, 1992). This is known as pesticide residue. Organophosphorous pesticide residues above allowable limits in cocoa beans have potential detrimental effects on human health, depending on the frequency of exposure and/or the potency or toxicity of the pesticide (US EPA, 2000). Thus to prevent the occurrence of high pesticide residues on cocoa beans, there are instructions in the cocoa producing industry to ensure the correct use of these pesticides (insecticides and fungicides) in cocoa cultivation in Ghana (Amoa-Awua et al., 2006).

The Ghana Standards Authority screens three organophosphorous pesticides on cocoa beans, for shipment to Japan. These are chlorpyrifos, pirimiphos-methyl and fenitrothion (GSA, 2011). There is however, still a paucity of data existing for the current levels of most organophosphorous pesticides in cocoa beans produced from Ghana. There is therefore, the need for surveillance and monitoring of organophosphorous pesticide residues in the Cocoa Industry of Ghana, in order to keep up the high quality standard of Ghana's cocoa beans (COCOBOD Ghana, 2011), food safety and also to bridge the knowledge gap. In the current study 13 organophosphorous pesticides were assessed in cocoa beans produced from Ghana.

Material and methods

Sampling

Within the sampling period of November 2010 to January 2011, fermented dried cocoa beans ready for export were sampled at random from two main cocoa storage warehouses located in Tema in the Greater Accra region and Takoradi in the Western region of Ghana. At Tema, a total of twenty-four (24) bagged samples, each weighing about a kilogram of fermented dried cocoa bean was collected. At Takoradi storage house, a total of twenty (20) samples were collected and bagged in labeled zip lock plastic bags.

In all, forty-four (44) fermented dried cocoa beans samples, ready for export were sampled, labeled accordingly and were transported to the laboratory for analysis.

Chemicals and Reagents

Acetonitrile, Acetone, Ethyl Acetate and Toluene were pesticide grade and obtained from BDH, England. *Acetone, dipotassium hydrogen phosphate and Potassium dihydrogen phosphate* were analytical grade and obtained from BDH, England. *Sodium sulfate* was pesticide grade obtained from Aldrich- Chemie, Germany), *Sodium chloride* (Pesticide grade, Riedel-de Haen), *Envi-carb/LC-NH2* (500mg/500mg/6mL) from Supelco and *Strata C18-E* (55um, 70A, 1000mg/6mL) from Phenomenex.

Individual certified reference standards; methamidophos, phorate, fonofos, diazinon, ethoprophos, dimethoate, pirimiphos-methyl, chlorpyrifos, malathion, fenitrothion, parathion, chlorfenvinphos and profenofos used for the identification and quantification were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Analysis for organophosphorous pesticides

Sample preparation, extraction, cleanup and analysis were carried out according to the procedure described in multi-residue method for agricultural chemicals with slight modifications (Syoku-An (2006); No. 0124001.

Sample extraction and clean-up

The extraction procedure, as stated above, was that of a Japanese analytical method for multi-residues for agricultural chemicals (2006). Cocoa beans samples were thoroughly grinded and homogenized. 20 ml of distilled water was added to approximately 10.0 g of the sample in the batch, stirred to form a homogeneous mixture and allowed to stand for 15 minutes. 50ml acetonitrile was added and macerated for 2 min using the ultra turrax. It was then centrifuge at a speed of 3000 rpm for 3 minutes and decanted through No. 4 filter paper into labeled 100 ml volumetric flasks. 20 ml acetonitrile was added to the residue and further homogenized for 2 minutes, and 5 ml acetonitrile was used to rinse the dispersing element into the container. Then centrifuged at 3000 rpm for 3 minutes and filtered again into each corresponding labeled 100 ml volumetric flask. A further 15ml acetonitrile was used to rinse the jar and residue, filtered and all filtrates adjusted to the 100 ml mark with acetonitrile. An aliquot of 20 ml was pipetted into labeled 250 ml separating funnel, and 10g of NaCl and 20 ml of 0.5mol/L phosphate buffer (pH 7.0) were added. The separating funnel was corked and shaken for 10 minutes using the horizontal shaker and allowed to stand for another 10 minutes. The NaCl and lower aqueous layers in each separating funnel were carefully removed and the organic layer transferred into labeled 50 ml beaker for further clean-up. Two solid phase extraction (SPE) cartridge clean-ups were employed; the first by bond Elut C-18 (1000mg/6ml) and then followed an Envi-carb/LC-NH_2 (500mg/500mg/6ml) cartridge. For the C-18 cartridge, after being conditioned with 10ml acetonitrile and the extracts loaded onto it, 2 ml acetonitrile was used as the eluting solvent; whiles in the Envi-carb/LC-NH_2 cartridge; 20ml mixture of toluene/acetonitrile in the ratio 1:3 was used as the eluting solvent after the extracts had been loaded onto the cartridge previously conditioned with 10ml of the toluene /acetonitrile mixture. All filtrates were concentrated below 40^{o}C to approximately 1 ml on the rotary evaporator, and 10 ml of acetone added to each flask and further concentrated just to dryness. The extracts were re-dissolved in 1ml ethyl acetate and transferred into labeled 15ml screw capped tubes, closed and placed in freezer for about 30 minutes. They were removed and immediately centrifuged at 3000rpm for 5 minutes, and the top layer carefully transferred into labeled 2ml GC standard opening vial for quantitation by gas chromatography analysis using Pulse Flame Photometric Detector (GC-PFPD).

Gas chromatographic determination

The final extracts were analyzed by Gas Chromatograph-Varian CP-3800 (Varian Association Inc. USA) equipped with combiPal autosampler and pulse flame photometric detector (PFPD) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/g range) from the matrix to which other detectors do not respond. The GC conditions and the detector response were adjusted so as to match the relative retention times and response as spelt out by Japanese analytical methods for agricultural chemicals. The GC conditions used for the analysis were capillary column coated with VF-1701 (30 m x 0.25 mm i.d, 0.25 µm film thickness). The injector and detector temperature were set at 270^{o}C and 280^{o}C respectively. The oven temperature was programmed as follows: 70^{o}C held for 2 min, ramp at 25^{o}Cmin^{-1} to 200^{o}C, held for 1 min, and finally ramp at 20^{o}Cmin^{-1} to 250^{o}C maintained for 3.3 min. Nitrogen was used as carrier gas at a flow rate of 2.0 mLmin^{-1} and detector make-up gases (17.0, 14.0 and 10.0 mLmin^{-1}) for hydrogen, air-1 and air-

2, respectively. The injection volume of the GC was 2.0 µL. The total run time for a sample was 14 min. Additionally, Varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with 1177 type injector, Saturn 2200 Mass Spectrometer (MS) as detector and 8400 Varian autosampler was used for confirmation of detected organophosphorous pesticides. Sample extract of 2 µL aliquots was injected and the separation was performed on a fused silica gel capillary column (VF- 5ms, 30 m + 10 m column guard x 0.25 mm id., 0.25 um film thickness). The carrier gas was ultra pure helium at flow rate of 1.2 $mLmin^{-1}$. The temperature of the injector operating in splitless mode was 270^{o}C and the MS detector with an Ion trap analyzer was set to scan mass range of 40 m/z – 450 m/z at auto EI. The column oven temperature was programmed as follows; 70^{o}C for 1 min, then at 30^{o}C min^{-1} up to 240^{o}C and finally at 5^{o}Cmin^{-1} to 300^{o}C held for 2.3 min. The total run time for a sample was 30 min.

Quantification of Organophosphorous Pesticide Residues

The residue levels of organophosphorous pesticides were quantitatively determined by the external standard method using peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times coincided with the standards were extrapolated on their corresponding calibration curves to obtain the concentration.

Quality control and quality assurance

The quality of organophosphorous pesticides was assured through the analysis of solvent blanks, matrix blanks and duplicate samples. All reagents used during the analysis were exposed to same extraction procedures and subsequently run to check for interfering substances. In the blank for each extraction procedure, no organophosphorous pesticide was detected. Sample of each series was analyzed in duplicates. The method was optimized and validated by fortifying the grinded and homogenized cocoa beans sample with 500 µL of 1.0 μgmL^{-1} organophosphorous pesticide standards mixture before analysis to evaluate the recovery of compounds. The recoveries of internal standards ranged between 70% and 95% for most of the organophosphorous pesticides analyzed.

Data Analysis

The mean of samples, maximum values, corresponding standard deviation and statistical significant test were performed using XLSTAT 2011 software and SPSS version 16 software for windows. All other calculations were performed using Microsoft excel. Statistical analyses incorporated in the work include mean of samples, minimum and maximum values, corresponding standard deviation and statistical significant test. All test were regarded as statistically significant when $p < 0.05$. Ranges were compiled from minimum and maximum values for levels detected in each individual organophosphorous pesticide residues detected in the study.

Results and Discussion

The study involves the analysis of organophosphorous pesticides in fermented dried cocoa beans produced in Ghana. The organophosphorous pesticides selected for this study are Methamidophos, Ethoprophos, Phorate, Fonofos, Diazinon, Dimethoate, Pirimiphos-methyl, Chlorpyrifos, Malathion, Fenitrothion, Parathion, Chlorfenvinphos and Profenofos. Concentrations of the various organophosphorous pesticide residues detected in each sample were calculated (in ug/kg sample). Residue results for organophosphorous pesticides were as indicated in Table 1.

Table 1: Summary of analysis of variance results for Organophosphorous Pesticides Residues (µg/kg) in Fermented Dried Cocoa Beans Sampled from Tema and Takoradi Warehouses.

Organophosphorous	TEMA, N=24		TAKORADI, N=20			
Pesticides	Range	x ± sd	Range	x ± sd	Df	p-value
Methamidophos	ND - 10.0	7.0 ± 1.4	8.0 - 15.0	12.0 ± 2.0	6	0.097
Ethoprophos	ND	ND	ND	ND	NA	NA
Phorate	ND - 6.0	5.2 ± 0.8	ND	ND	NA	NA
Fonofos	ND - 11.0	8.1 ± 5.8	ND - 14.0	10.4 ± 8.7	17	0.538
Diazinon	ND - 5.0	5.0 ± 0.1	ND - 5.0	5.0 ± 0.1	3	0.001
Dimethoate	ND - 48.0	15.6 ± 3.9	ND - 58.0	28.9 ± 7.1	34	0.044
Pirimiphos-methyl	ND - 64.0	32.7 ± 2.5	10.0 - 30.0	22.5 ± 8.7	11	0.758
Chlorpyrifos	ND - 40.0	35.1 ± 8.8	17.0 - 62.0	52.3 ± 13.2	35	0.211
Malathion	ND - 44.0	22.2 ± 11.1	11.0 - 21.0	17.5 ± 5.4	13	0.043
Fenitrothion	19.0 - 133.0	95.9 ± 11.2	16.0 - 96.0	77.3 ± 5.3	20	0.175
Parathion	ND - 11.0	9.8 ± 2.3	ND - 5.0	5.7 ± 1.6	7	0.25
Chlorfenvinphos	ND - 14.0	11.8 ± 3.8	ND - 10.0	8.0 ± 0.1	7	0.103
Profenofos	5.0 - 8.0	7.3 ± 1.6	ND - 17.0	12.0 ± 0.1	9	<0.0001

ND=Not Detected, NA=Not Applicable, x=Mean, sd=Standard Deviation, LOD =5.0 µg/kg, Df=Degree of freedom and P=Probabilty

Table 2: Results of Organophosphorous pesticides residues against EU and Japanese MRLs

PESTICIDES	RANGE	MEAN	EU, MRL	JAPAN, MRL
Organophosphorous:	µg/kg			
Methamidophos	ND - 15.0	10.4	20	10
Ethoprophos	ND	ND	20	10
Phorate	ND - 6.0	5.2	100	10
Fonofos	ND - 14.0	9.4	10	10
Diazinon	ND - 5.0	5.0	20	50
Dimethoate	ND - 58.0	22.3	50	50
Pirimiphos-methyl	ND - 64.0	29.5	50	50
Chlorpyrifos	ND - 62.0	50.2	100	50
Malathion	ND - 44.0	20.6	20	500
Fenitrothion	16.0 - 133.0	93.8	200	100
Parathion	ND - 11.0	8.0	100	10
Chlorfenvinphos	ND - 14.0	9.8	10	50
Profenofos	ND - 17.0	10.5	100	10

N = 44, ND = Not Detected

Variation of Organophosphorous Pesticides in Fermented Dried Cocoa Beans

Among the organophosphorous pesticides screened, Ethoprophos was not detected in all 44 samples analysed from both Tema and Takoradi warehouses. However, 4 of out 24 (17%) of samples from Tema warehouse had Methamidophos with mean residue concentration of 7.0 ± 1.4 µg/kg. This mean residue concentration was not significantly different from that recorded in cocoa beans samples analysed from Takoradi warehouse 12.0 ± 2.0 µg/kg (p=0.097). Phorate residue was not detected from all 20 cocoa beans samples analysed from Takoradi warehouse. However, 5 out of 24 (21%) of the samples from Tema warehouse contained Phorate, with mean residue concentration of 5.2 ± 0.8 µg/kg. The maximum residue level for Fonofos detected was 14.0 µg/kg. Fonofos recorded mean residue concentrations of 8.1 ± 5.8 µg/kg and 10.4 ± 8.7 µg/kg for Tema and Takoradi warehouses, respectively. These mean residue levels were however not significantly different (p=0.538). From both warehouses, only one sample each from Tema and Takoradi were found to contain Diazinon. The residue concentration ranges from not detected to 5.0 µg/kg. Dimethoate was detected in 18 out of 24 (75%) of samples from Tema warehouse with mean residue concentration of 15.6 ± 3.9 µg/kg. Dimethoate occurred in 18 out of 20 (90%) of the cocoa beans samples analysed from Takoradi warehouse with mean residue concentration of 28.9 ± 7.1 µg/kg. However the mean residue concentrations of Dimethoate in cocoa beans from both Tema and Takoradi were significantly different (p=0.044).

Pirimiphos-methyl, one of the three organophosphorous pesticides screened routinely for shipment to Japan (GSA, 2011) was detected in the cocoa beans samples analysed from both warehouses (Table 1). This may possibly be that Pirimiphos-methyl is being used extensively in cocoa production in Ghana. The mean residue concentration for samples from Tema warehouse was 32.7 ± 2.5 µg/kg, while samples from Takoradi warehouse had Pirimiphos-methyl mean residue concentration of 22.5 ± 8.7 µg/kg. Statistically, the mean residue concentrations of pirimiphos-methyl in cocoa beans from the warehouses were not significantly different (p=0.758). The highest residue level of Chlorpyrifos detected was 62.0 µg/kg. This was detected among samples from Takoradi warehouse with mean residue concentration of 52.3 ± 13.2 µg/kg. From Tema warehouse, 35.1 ± 8.8 µg/kg was recorded as the mean residue concentration of Chlorpyrifos in cocoa beans, but this was not significantly different from Takoradi samples (p=0.211). Malathion was detected in 11 out of 24 (46%) of samples from Tema warehouse, with mean residue concentration of 22.2 ± 11.1 µg/kg. Out of 20 cocoa beans sampled from Takoradi warehouse, Malathion occurred in 4 (20%) with mean residue concentration of 17.5 ± 5.4 µg/kg. However, these mean residue levels of Malathion were significantly different (p=0.044). Among the organophosphorous pesticides, Fenitrothion recorded the highest mean residue concentration of 95.9 ± 11.2 µg/kg with the widest spread of residue concentration, 16.0 µg/kg to 133.0 µg/kg (Table 1). This highest mean residue concentration was recorded for samples from Tema, which was not statistically significantly different from mean residue concentration of Fenitrothion in cocoa beans from Takoradi warehouse, 77.3 ± 5.3 µg/kg. Parathion was detected in 5 out of 24 (21%) of cocoa beans samples analyzed from Tema warehouse. Meanwhile, 25% of cocoa beans samples analyzed from Takoradi warehouse contained Parathion residue, with mean residue concentration of 5.7 ± 1.6 µg/kg. This mean residue concentration was less than but not significantly different from mean residue concentration of 9.8 ± 2.3 µg/kg for samples from Tema warehouse (p=0.250). Chlorfenvinphos mean residue concentration for samples from Takoradi warehouse of 8.0 ± 0.1 µg/kg was less than mean residue concentration of 11.8 ± 3.8 µg/kg for samples from Tema. However, statistically the mean values were not significantly different from each other (p=0.103). Wider spread of Profenofos residue in the fermented dried cocoa beans samples was recorded from both warehouses (Table 1). And thus, the mean residue concentrations of 7.3 ± 1.6 µg/kg and 12.0 ± 0.1 µg/kg for samples from Tema and Takoradi warehouses, respectively were significantly different ($p < 0.0001$).

Comparison of pesticide residues in fermented dried cocoa beans Against International standard levels

Among the thirteen organophosphorous screened in the fermented dried cocoa beans, Ethoprophos was not detected in all samples analyzed. Again all of the organophosphorous pesticides recorded average residue concentrations below both the EU and Japanese MRLs, with exception of Methamidophos, Malathion, Chlorpyrifos and Profenofos which average residues were comparable to at least one of the two countries MRLs (Table 2).

As shown in Table 2, Methamidophos average residue concentration was 10.4 µg/kg, and it is below the EU MRL of 20 µg/kg but comparable with the Japanese maximum residue limit of 10 µg/kg. Phorate recorded 5.2 µg/kg as average residue concentration in cocoa beans. This residue level is below both EU and Japanese MRLs of 100 µg/kg and 10 µg/kg, respectively. Fonofos average residue concentration in the cocoa beans samples analyzed was comparable to the MRLs of both EU and Japan. The concentration was 9.4 µg/kg, whereas both EU and Japanese MRL is 10 µg/kg. Diazinon, an organophosphorous pesticide approved for use on cocoa production in Ghana, recorded an average residue concentration of 5.0 µg/kg. This residue level is below both the EU MRL of 20 µg/kg and the Japanese MRL of 50 µg/kg (Table 2). The average residue concentration of Dimethoate was 22.3 µg/kg. This residue level is below both the EU and Japanese limits of 50 µg/kg. Pirimiphos-methyl, Chlorpyrifos and Fenitrothion are the organophosphorous pesticides screened for shipment to Japan (GSA, 2011). Their average residue concentrations were 29.5 µg/kg, 50.2 µg/kg and 93.8 µg/kg, respectively. All are below the EU MRLs; however Chlorpyrifos residue is comparable to the Japanese MRL (Table 2). Malathion and Chlorfenvinphos with average residue concentrations of 20.6 µg/kg and 9.8 µg/kg, respectively were comparable to their various EU MRLs, but were below the Japanese MRLs of 500 µg/kg and 50 µg/kg for Malathion and Chlorfenvinphos respectively (Table 2). On the contrary, Profenofos average residue concentration of 10.5 µg/kg was comparable to the Japanese MRL of 10 µg/kg, but below the EU MRL of 100 µg/kg. However, Parathion average residue concentration of 8.0 µg/kg is below both the EU and Japanese MRLs of 100 µg/kg and 10 µg/kg, respectively.

Conclusion

Organophosphorous residue levels of pesticides in fermented dried cocoa beans produced in Ghana as determined by Gas chromatography coupled with Pulse Flame Photometric Detector shows significantly varying results.

The results prove that Ethoprophos is not being used in cocoa production in Ghana. However, there were appreciable

amounts of Dimethoate (22.3 µg/kg), Pirimiphos-methyl (29.5 µg/kg), Malathion (20.6 µg/kg), Chlorpyrifos (50.2 µg/kg) and Fenitrothion (93.8 µg/kg). These indicate the extensive use of these pesticides in Ghana, with Fenitrothion recording the widest range among them (16.0 – 133.0 µg/kg).

Considering levels of organophosphorous pesticides residues in fermented dried cocoa beans against the European Commission and Japanese regulations on pesticide residues, cocoa beans produced in Ghana will not pose any significant threat. All organophosphorous pesticides residues detected were below both international maximum residue limits; with the exception of Methamidophos, Chlorpyrifos, Malathion and Profenofos which were comparable with either one of the two international maximum residue limits set.

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