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Distribution pattern of pesticide residues in cocoa beans across the cocoa growing regions of Ghana

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ABSTRACT

Pesticides have been used on cocoa for more than 50 years in Ghana. Notable among these pesticides were the organochlorines lindane, DDT and aldrin. However, after the banned of the organochlorine pesticides came other pesticide groups like the organophosphorous, carbamates and synthetic pyrethroids in cocoa production in Ghana. With cocoa being a major cash crop in Ghana, the purpose of this study was to investigate the distribution pattern of pesticide residues in cocoa beans produced in Ghana in order to advise producers and buyers of the possible residual trend in cocoa production in Ghana. A total of 44 distinct samples of fermented dried cocoa beans were randomly collected from two main cocoa beans warehouses, identified from the six cocoa growing regions in Ghana. The extracting solvent was acetonitrile, followed by two solid phase extraction cartridges; C18 and Envi-carb/LC-NH_2 for extract clean-up. The investigating pesticides consist of 15 organochlorines, 12 organophosphorous and 9 synthetic pyrethroids pesticides. The results showed traces of organochlorine pesticides in cocoa beans from all six cocoa growing regions of Ghana, with Ashanti region recording the highest occurring frequency of 54 percent and the Volta region with 1.5 percent frequency of occurrence as the least.
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KEYWORDS

Pesticides;
Organochlorines;
Organophosphorous;
Synthetic pyrethroids;
Solid phase extraction;
Fermented cocoa beans.

INTRODUCTION

Pesticides have been used in cocoa production in Ghana for more than 50 years[1]. These pesticides can be grouped into organic or inorganic compounds. The organic pesticides can further be classified into organochlorine, organophosphorous, carbamates and synthetic pyrethroids pesticides[2]. Organochlorine pesticides such as lindane, DDT, aldrin, and so on were once actively used in cocoa production in Ghana, however, due to their environmental persistent and accumulation in food chain, they have been banned (or restricted use) in agricultural cultivation in many countries including Ghana[3,4]. The less persistent organophosphorous, carbamates and synthetic pyrethroids pesticides were the immediate alternatives after the banned of the organochlorines. For instance, the carbamates: propoxur and promecarb; the organophosphorous: chlorpyrifos, diazinon and pirimiphos-methyl; and the pyrethroids: bifenthrin, cypermethrin, deltamethrin and lambda-cyhalothrin were the early screening active ingredients in cocoa production after the organochlorine pesticides banned in Ghana[1,5]. Many cocoa farmers believe that pesticides work, at least against some cocoa pest problems, and continue to use them depending on the pest and country[5]. These pesticides, whichever class they may belong are formulated for use to aid in cocoa production, and are normally allowed under specified conditions and timings during production in order to minimize residual concentrations in cocoa produce. Factually, the use of pesticides in agriculture is the most common way of controlling pests, killing weeds, and to preserve the crops from insect attack. Pesticides are also applied to prevent or minimise the level of pest outbreaks in farming[6].

However, when crops are treated with pesticide, some amount of the pesticide, or indeed what it changes to in the plant (its metabolites or degradative products), can remain in or on the crop until after it is harvested[7].

Contamination of cocoa beans by pesticides can occur directly or indirectly. Directly, either by treating the crop with pesticides before harvest, storage and/or distribution. It can also occur indirectly by uptake from the soil of residual pesticides by the subsequent cocoa farming, from the atmosphere or drifting from neighbouring fields, or from a storage space pretreated with pesticides[8]. Thus, the contamination of the environment and cocoa beans by pesticides has become an issue of considerable concern in many parts of the world[9]. In addition, there exist differences in pesticide residues levels in produce grown from one place to the other, with reasons such as the different modes of pesticides application, variability in farms and storage practices, access to extension officers, and others such as soil organic matter, pH, and some meteorological factors like temperature, rainfall and solar radiation patterns have been attributed[10]. Due to these concerns, many researchers have been and are investigating pesticide residues occurrence, their distribution and concentrations in various media[11-16,19]. And thus, the purpose of this study was to investigate the distribution pattern of pesticide residues in cocoa beans produced in Ghana in order to advise producers and buyers of the possible residual trend in cocoa production in Ghana. This is essential since cocoa is a major cash crop for Ghana[17].

MATERIALS AND METHOD

Sampling

Fermented dried cocoa beans were sampled at random from the two main cocoa warehouse stations in Ghana (Tema and Takoradi). From Tema, a total of twenty-four (24) distinct bagged samples each weighing a kilogram dried cocoa beans, were sampled from November, 2010 to January, 2011. These sampled cocoa beans were identified from seven (7) registered cocoa buying companies in Ghana labeled A to G (representing Produce Buying Company, Adwumapa Buyers, Cocoa Merchants Buyers, Olam, Akuafo Adamfo, Transroyal and Armajaro, but not necessarily in that order), and were also identified with the region of origin in Ghana (Western, Eastern, Ashanti, Brong Ahafo, Central and Volta regions). The same cocoa buying companies and regions of origin were identified at Takoradi station, with a total of twenty (20) distinct samples collected from November to December of 2010, and bagged in labeled zip lock plastic bags.

In all a total of forty-four (44) cocoa beans samples were sampled, labeled accordingly and transported to the laboratory in five different batches.

Chemicals and reagents

Reagents used in the study comprised the following: Acetonitrile (Pesticide grade, BDH, England), Acetone (Pesticide grade, BDH, England), Acetone (Analytical grade, BDH, England), Ethyl Acetate (Pesticide grade, BDH, England), Toluene (Pesticide grade, BDH, England), Sodium sulfate (Pesticide grade, Aldrich-Chemie, Germany), Sodium chloride (Pesticide grade, Riedel-de Haen), dipotassium hydrogen phosphate (Analytical grade, BDH, England), Potassium dihydrogen phosphate (Analytical grade, BDH, England), Envi-carb/LC-NH_2 (500mg/500mg/6mL – Supelco), Strata C18-E (55um, 70A, 1000mg/6mL – Phenomenex) and distilled water.

The individual certified reference standards, lindane, beta-HCH, delta-HCH, aldrin, heptachlor, gamma-chlordane, alpha-endosulfan, p,p'-DDE, dieldrin, endrin, beta-endosulfan, p,p'-DDT, p,p'-DDD, endosulfan sulfate, methoxychlor, methamidophos, phorate, fonofos, diazinon, dimethoate, pirimiphos-methyl, chlorpyrifos, malathion, fenitrothion, parathion, chlorfenvinphos, profenofos, allethrin, fenpropathrin, bifenthrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate and deltamethrin used for the identification and quantification were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Sample processing and preparation

Stones and other foreign objects found in the sample were removed by hand picking. Using the hammer mill, each labeled fermented dried cocoa beans sampled was ground into fine powder and collected into new sample plastic bag and re-labeled accordingly to form the analytical samples, each about 500 grams weight. These individual homogenized cocoa beans samples were kept frozen in a freezer at a temperature of -18°C or less until the time for the residue determination.

Sample extraction and clean-up

Extraction and clean-up of cocoa beans samples were carried out according to procedures described by multi-residue method for agricultural chemicals by GC/MS from the Department of Food Safety, Ministry of Health, Labour and Welfare, Japan with slight modifications[18].

Extraction

20 mL of distilled water was added to 10.0 g of a ground fine cocoa beans powder sample and allowed to stand for 15 minutes. 50 mL acetonitrile was added and the sample homogenized using the ultra turax macerator. It was then centrifuged at 3000 rpm and filtered into 100 mL volumetric flask. Additional 20 mL of acetonitrile was added to the residue, and homogenized, centrifuged and filtered. Both filtrates were combined, and acetonitrile was added to make up a 100 mL solution. 20 mL aliquot of the extracted solution was then measured, and 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0) were added. It was then shook vigorously for 10 minutes on a horizontal shaker and allowed to stand for 10 minutes until the solution was clearly separated into layers. The aqueous layer was discarded.

An octadecylsilanized silica gel mini column (1000mg/6mL) was conditioned with 10 mL of acetonitrile. The acetonitrile layer from the above was then loaded onto the column, and the column eluted with 2 mL of acetonitrile afterwards. The entire volume of effluent was dry over anhydrous sodium sulfate, and filtered. The filtrate was concentrated to dryness at 40°C or lower and the residue re-dissolved in 2 mL of acetonitrile/toluene (3:1) mixture.

Extract clean-up by a graphite carbon/aminopropylsilanized silica gel

A graphite carbon/aminopropylsilanized silica gel layered mini column (500mg/500mg/6mL) was conditioned with 10 mL of acetonitrile/toluene (3:1) mixture. The solution obtained from above extraction step was then loaded onto this column, and the column eluted with 20 mL of acetonitrile/toluene (3:1) mixture afterwards. The entire volume of effluent was then concentrated to 1 mL or less at 40°C or lower. 10 mL of acetone was added to the concentrated solution and further concentrated to 1 mL or less at 40°C or lower. A further 5 mL of acetone was added to the concentrated solution and then concentrated to dryness. The residue was re-dissolved in ethyl acetate to make a 1 mL solution, and was made ready for residue determination by GC-MS.

Instrumental analysis

This was as described in[16,19]. A Varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with 1177 type injector, Saturn 2200 Mass Spectrometer (MS) as detector and a Varian 8400 autosampler were used for gas chromatography analysis. Sample extract of 2 μL aliquot 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 mL/min. The temperature of the injector operating in splitless mode was 270°C and the MS detector with an Ion trap mass analyzer was set to scan mass range between 40 m/z – 450 m/z at auto EI. The temperature of the manifold, ion trap and transferline were set at 80°C, 210°C and 260°C, respectively. The column oven temperature was programmed as follows; 70°C for 1 min, then at 30°C/min up to 240°C and finally at 5°C/min to 300°C held for 2.3 min. The total run time for a sample was 30 minutes. The residue levels of all detected pesticides were quantitatively determined by the external standard method using their peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times and spectra coincided with the reference standards were extrapolated on their corresponding calibration curves to obtain their respective concentrations.

Quality control

All reagents used for the analysis were exposed to the same extraction procedures, and solvents used were run to verify for any interfering substances within the GC runtime. In all batches of organochlorine, organophosphorous and synthetic pyrethroids pesticides residues analysis, reagent blanks, procedural matrix blanks and triplicate samples were included. For the reagent blanks in each extraction and clean up procedure, none of the pesticides were detected. All extracts were kept frozen until quantification was achieved. Recalibration curves were run with each batch of samples to check that the correlation coefficient was kept around $r2=0.999$. A fortification level of 0.05mg/kg of standard mixtures was chosen before analysis to evaluate the recovery of compounds in the cocoa beans samples analysed. The recoveries of the pesticide residues ranged between 70% and 120% for most of the pesticides analyzed. The limit of quantification for organochlorine pesticides was 0.005 mg/kg, while it was 0.01 mg/kg for both organophosphorous and synthetic pyrethroids pesticides.

Data analysis

All calculations and graphical representations were performed using Microsoft excel 2010. Statistical analyses incorporated in the work include mean of residue concentrations, minimum and maximum values, tallies and percentage occurrence frequency of pesticide residues count. Ranges were compiled from minimum and maximum values for levels detected in each individual organochlorine, organophosphorous and synthetic pyrethroids pesticide residues detected in the study.

RESULTS AND DISCUSSION

The distribution of pesticide residues in cocoa beans produced from Ghana show significantly varying results

Distribution of Pesticide Residues in Cocoa Beans from across Cocoa Buying Companies in Ghana

As shown from Figure 1, and from the seven selected cocoa buying companies in Ghana, cocoa beans samples from Buying Companies C and D co-recorded the highest organochlorine pesticide residues occurrences (17% of the total organochlorines detected in the cocoa beans). This same organochlorine pesticide residue frequency of occurrence between Buying Companies C and D could be attributed to the fact that, these Buying Companies bought cocoa beans from the same sources/origins (Central, Western and Ashanti regions of Ghana). Cocoa beans samples from Buying Companies A and F also co-shared the same levels of organochlorine pesticide residues frequency of occurrence (14% each). Thus, with Buying Companies A and F, even though the produce originated from different sources, the cocoa beans might have been treated by same agricultural and crop storage practices, and hence same organochlorine pesticide residue frequency of occurrence between them. However, the least organochlorine pesticide residues occurrence frequency (11%) was recorded by cocoa beans samples from Buying Company B.

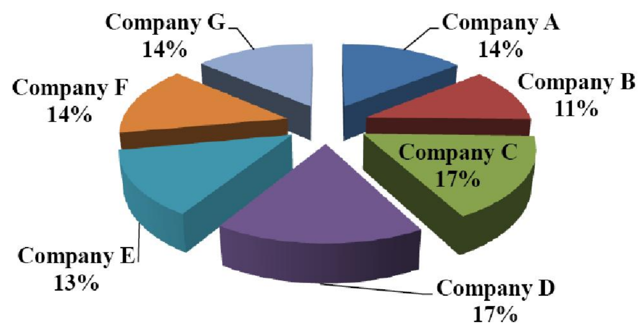

Figure 1 : Distribution of organochlorine pesticide residues across selected buying companies in Ghana

For organophosphorous pesticide residues in the cocoa beans (Figure 2), samples from Buying Companies C and D, again shared the same level (15% each) of the total organophosphorous pesticide residues detected. Cocoa beans samples from Buying Company G recorded the highest organophosphorous pesticide residues frequency of occurrence (18%). Again Buying Company B samples recorded the least organophosphorous pesticide residues frequency of occurrence (10%) of total organophosphorous pesticide residues detected. This may suggest that, Buying Company B may be applying good agricultural and crop storage practices to its produce.

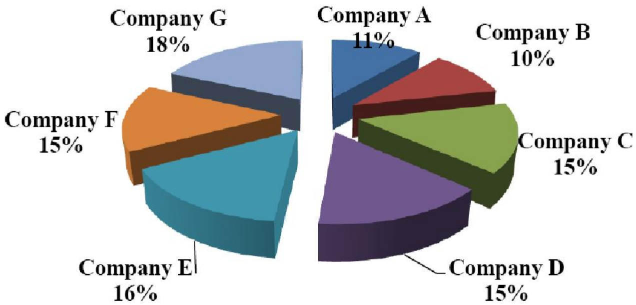

Figure 2 : Distribution of organophosphorous pesticide residues across selected buying companies in Ghana

In the case of synthetic pyrethroids residues (Figure 3), samples from Buying Company E recorded 18% of the total synthetic pyrethroids detected as the highest frequency of occurrence. Samples from Buying Company B again had the least occurring frequency among the synthetic pyrethroids pesticide residues with (12%) residue occurrence.

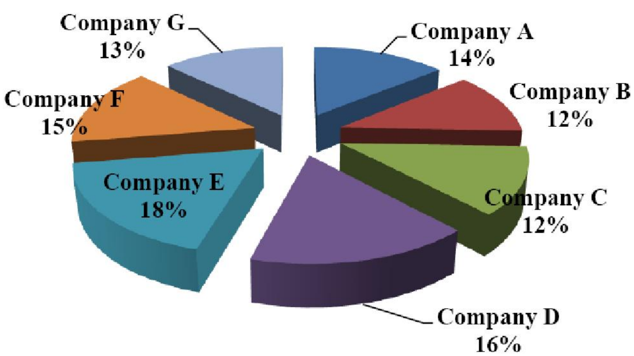

Figure 3 : Distribution of synthetic pyrethroids pesticide residues across selected buying companies in Ghana

This confirms the earlier submission that, cocoa beans samples from Buying Company B were treated with best produce practices.

In all, apart from Buying Companies C, D and G, it appears that synthetic pyrethroids pesticide residue frequencies of occurrences were always among the highest between Companies. This suggests current use of synthetic pyrethroids pesticides in cocoa beans production in Ghana.

Distribution of pesticide residues in cocoa beans across the six cocoa growing regions in ghana

As shown from Figure 4, there were significant differences in the distribution of pesticide residues in cocoa beans samples in the various regions of Ghana. Ashanti region recorded the highest frequency of occurrence for organochlorine pesticide residues (54% of total organochlorines detected), organophosphorous 37% and 41% of occurrence in synthetic pyrethroids pesticide residues. This was followed by the Western region with 24%, 30% and 33%, then Central region with 12%, 15% and 13% of organochlorine, organophosphorous and synthetic pyrethroids pesticide residues frequency of occurrence, respectively. Brong Ahafo region recorded 6% of organochlorine detected, 9% of organophosphorous and 11% of synthetic pyrethroids detected while Eastern region recorded 2.5% of organochlorine pesticides, 6% of organophosphorous detected and 1.6% of synthetic pyrethroids pesticides detected. Volta region had 1.5% of organochlorine occurrence, 3% of organophosphorous residue occurrence and 0.4% of synthetic pyrethroids pesticide residues occurrence in the cocoa beans samples analysed.

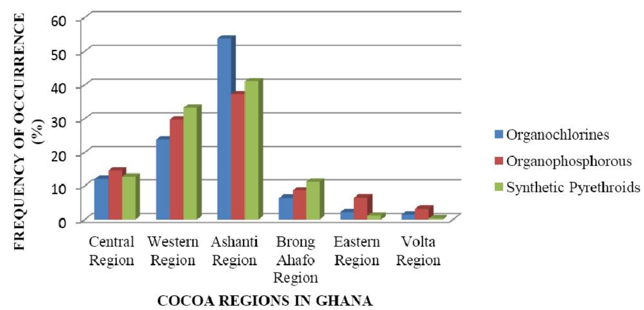

Figure 4 : Frequency of pesticide residues in cocoa beans across the six cocoa growing regions in Ghana

Thus, Volta region recorded the lowest occurrence of the pesticide residues contamination in cocoa beans among the cocoa growing regions in Ghana. The differences in the pesticide residues levels from one region to the other may be attributed to the different modes of pesticides application, variability in farms and storage practices, access to extension officers, and others such as soil organic matter, pH, and some meteorological factors like temperature, rainfall and solar radiation patterns[10].

Within cocoa growing regions, apart from Ashanti region with organochlorine pesticide residues occurrence frequency level of 54% being higher occurring than both organophosphorous (37%) and synthetic pyrethroids pesticide residues (41%), all with the exception of Eastern and Volta regions recorded lower pesticide residues frequency of occurrence for organochlorines compared to their organophosphorous and synthetic pyrethroids pesticide residues occurrence frequencies (Figure 4). This suggests greater use of organochlorine pesticides in the Ashanti region either now or in previous times compared to the five other cocoa growing regions.

Even though all regions recorded different frequencies of occurrence percentages, samples from Central region, Eastern region and Volta region share similar distribution pattern, with organophosphorous pesticide residues being the highest occurring pesticide residues among the regions (Figure 4). Similar distribution pattern also existed between samples from Western region and Brong Ahafo region, with synthetic pyrethroids pesticide residues recording the highest levels than both organochlorine and organophosphorous pesticides residues (Figure 4). These similarities may be due to same use of agrochemicals in those regions, either in the past or present[10].

TABLE 1 : Occurrence frequency of pesticide residues in cocoa beans across the selected cocoa buying companies

COCOA BUYING COMPANIES	OCCURRENCE FREQUENCY OF RESIDUES, DETECTS (%)		
	ORGANO CHLORINES	ORGANOPH OSPHOROUS	SYNTHETIC PYRETHROIDS
A	28 (14)	20 (11)	35 (14)
B	22 (11)	18 (10)	30 (12)
C	33 (17)	28 (15)	30 (12)
D	33 (17)	28 (15)	40 (16)
E	25 (13)	30 (16)	45 (18)
F	28 (14)	28 (15)	37 (15)
G	28 (14)	34 (18)	33 (13)
TOTAL	197 (100)	186 (100)	250 (100)

TABLE 2 : Occurrence frequency of pesticide residues in cocoa beans across the six cocoa growing regions of Ghana

COCOA GROWING REGIONS	OCCURRENCE FREQUENCY OF RESIDUES, DETECTS (%)		
	ORGANO CHLORINES	ORGANOPH OSPHOROUS	SYNTHETIC PYRETHROIDS
Ashanti Region	106 (54)	68 (37)	102 (41)
Western Region	47 (24)	56 (30)	82 (33)
Central Region	24 (12)	28 (15)	33 (13)
Brong Ahafo Region	12 (6)	17 (9)	28 (11)
Eastern Region	5 (2.5)	11 (6)	4 (1.6)
Volta Region	3 (1.5)	6 (3)	1 (0.4)
TOTAL	197 (100)	186 (100)	250 (100)

CONCLUSION

Although organochlorine pesticides had been banned for agricultural purposes in Ghana, they were detected in cocoa beans from the six cocoa growing regions in Ghana. Ashanti region recorded the highest frequency of organochlorine pesticides occurrence (54% of total organochlorine detected), followed by Western region (24%), Central region (12%), Brong Ahafo region (6%) and Eastern region (2.5%), in that order. Volta region recorded the least use of organochlorine pesticide on cocoa beans in Ghana with 1.5% of the total organochlorine pesticides detected in the cocoa beans.

It can also be concluded, that apart from Ashanti region, cocoa farmers from the five other cocoa growing regions in Ghana preferred organophosphorous and synthetic pyrethroids pesticides over the very persistent organochlorine pesticides.

It was also evident that one particular accredited cocoa buying company in Ghana (labeled B for identification sake) was applying good crop storage practices to its produce.

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