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Assessment of synthetic pyrethroids pesticides residues in cocoa beans from Ghana

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ABSTRACT

One group of pesticides that play an important role after the ban of the persistent organochlorine pesticides in agriculture is synthetic pyrethroids pesticide. Synthetic pyrethroids mimic the broad efficacy of the botanical, but they contain only one of the six groups of esters of the natural pyrethrums, and insect species tend to develop resistance to them. However, the latest groups of synthetic pyrethroids are photo-stable, as well as extremely toxic to insects. The objective of this study is to determine residue levels of selected synthetic pyrethroids pesticides in cocoa beans produced in Ghana, and to assess these levels against the European Union and Japanese residue regulation limits. The determination was done by gas chromatography coupled with an electron capture detector, and confirmed with Saturn 2200 Mass Spectrometer using ion trap mass analyzer. The study revealed the presence of all nine selected synthetic pyrethroids pesticides at significantly varying concentrations, with Permethrin recording the widest range of residue concentration from not detected to 105.0 micrograms per kilogram. The percentage recovery for most of the pesticides ranges from 75 to 120 percent, with method determination limit of 5.0 micrograms per kilogram. None of the detected synthetic pyrethroids pesticides' average residue concentrations did exceed the European Union or Japanese Maximum Residue Limits in cocoa beans produced in Ghana. However, Allethrin, Cypermethrin and Fenvalerate average residues concentrations were at the borderline of the Japanese MRLs.

Introduction

In the quest to reduce proliferation of insects that attack humans and crops in order to control disease causing vectors and increase food production, the use of pesticide is inevitable (Kuranchie, H. et al. 2009). There are different types of pesticides, and these may be classified as insecticides, herbicides, fungicides and antibiotics (Nollet, 2000). One group of pesticides that play an important role after the ban of the persistent organochlorine pesticides is synthetic pyrethroids pesticide. The synthetic pyrethroids mimic the broad efficacy of the botanical, but they contain only one of the groups of esters, and insect species tend to develop resistance to them. Compared with the botanical pyrethrum, which contains all six ester properties, no resistance has yet been observed as it becomes very difficult for insects to develop the requisite combination of alternative pathways to these six properties. Generally, pyrethroids show low mammalian toxicity, but are highly toxic to fish and bees. They are anoxic nerve poisons; however, their residues in crops do not cause problems.

The natural insecticide pyrethrum has seldom been used for agricultural purposes because of its cost and instability in sunlight. However, recently, several synthetic pyrethrin-like materials have become available and are referred to as synthetic pyrethroids. These materials are very stable in sunlight and are generally effective against most agricultural pests when used at the low rate of 0.11 to 0.23 kg/ha (P. O. Yeboah, 2009).

Pyrethrum and the older synthetic pyrethroids are very sensitive to sunlight, because their molecules split under UV light. Therefore, they are not suited to agricultural use, but some of them have good volatility and are very effective against indoor insect pests. For household use they are formulated as aerosols together with piperonyl butoxide for synergistic action. This group includes allethrin, bioallethrin, bioresmethrin, phenothrin, resmethrin, and tetramethrin. The next generations of pyrethroids were produced with better photo-stability and low volatility; therefore, they are used for agricultural purposes. These include deltamethrin, fenverate and permethrin. The latest groups of synthetic pyrethroids are photo-stable, as well as extremely toxic to insects. Their efficacy is also good that a dose of only 10 - 40 g active ingredient per hectare is just required. These new pyrethroids are not mixed with synergists. They include bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, fenpropathrin, flucythrinate, fluvalinate and tralomethrin.

In Ghana, synthetic pyrethroids are the most preferred pesticides on the market for agricultural and household purposes. Most of their formulations exist in combination with other pesticides, such as organophosphorous or nitroguanidine compound. Some of these synthetic pyrethroids are registered in Ghana for cocoa production. This includes deltamethrin, cypermethrin and pyrethrums (EPA Ghana, 2009).

Cocoa is a cash crop in Ghana. And as such there are diseases and pests that attack the plant or the fruits known as

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pod. Hence, pesticides are therefore applied on cocoa to prevent, control, monitor or kill the pest. Thus a higher productivity of cocoa is achieved, which has direct correlation with foreign exchange earnings for the Nation. However, since these pesticides are toxic and can cause severe adverse health effects when contaminated foods are consumed; and also has the potential to negatively affect the image of Ghana's cocoa (The Statesman News Paper, Nov. 2006), their residue levels in cocoa beans produced in Ghana should be ascertained. Thus, the objective of this study is to determine residue levels of synthetic pyrethroids in cocoa beans produced in Ghana, and to assess these levels against some selected international regulation limits.

Material and methods

Study Area

Fig. 1: Map of Ghana showing sampling Locations Cities (Tema and Takoradi)

Sampling

Cocoa beans samples were collected from two main cocoa storage warehouses located in Tema, a city in the Greater Accra region and Takoradi, one of the twin capital cities in the Western region of Ghana. The sampling was performed randomly in each warehouse. In Tema warehouse, a total of twenty-four (24) bagged samples each weighing a kilogram of cocoa beans was collected. Whiles from the Takoradi warehouse, a total of twenty (20) samples were collected and bagged in labeled zip lock plastic bags.

In all, a total of 44 different cocoa beans samples were collected within the sampling period of November 2010 to January 2011, and were dispatched to the pesticide residue laboratory for sample preparation and subsequent residue analysis.

Chemicals and Reagents

Certified pesticide reference standards (*Allethrin, bifenthrin, fenpropathrin, lambda-cyhalothrin, cis and trans permethrin, Cyfluthrin – all four isomers, Cypermethrin – all four isomers, Fenvalerate – both isomers and deltamethrin)*, with certified purity of at least 99% were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Pesticide grade *Acetonitrile, Acetone, Ethyl Acetate and Toluene,* and analytical grade *dipotassium hydrogen phosphate and Potassium dihydrogen phosphate* were supplied by BDH, England. Anhydrous sodium sulphate analytical grade were purchased from Aldrich- Chemie, Germany. *Sodium chloride* (Pesticide grade, from Riedel-de Haen), *Envi-carb/LC-NH2* (500mg/500mg/6mL) from Supelco and *Strata C18-E* (55um, 70A, 1000mg/6mL) from Phenomenex.

Analysis for synthetic pyrethroids pesticides

Sample preparation, extraction, clean-up 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

Prepared fermented dried cocoa beans were weighed in triplicate sub samples into batches of twenty samples including reagent blanks and fortified samples. Within each batch of twenty samples, 10 g analytical portions were weighed into 250 ml nalgene jars and labeled accordingly. And 20 ml of distilled water was added to each jar in the batch, stirred to form a homogeneous mixture and allowed to stand for 15 minutes. A 50 ml acetonitrile was added and homogenized using the ultra turrax for 2 minute. Four samples were centrifuged at a time, at a speed of 3000 rpm for 3 minutes and decanted through filter paper into labeled 100 ml volumetric flasks. To the residue, 20 ml acetonitrile was added and further homogenized for 2 minutes, and 5 ml acetonitrile was used to rinse the dispersing element into the jar. The four were centrifuged at 3000 rpm for 3 minutes and filtered again into each corresponding labeled 100 ml volumetric flask. A further 15 ml acetonitrile was used to rinse the jar and residue, filtered and all filtrates adjusted to the 100 ml mark with acetonitrile. From each filtrate, 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 to each. The separating funnels were 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 layers transferred into labeled 50 ml beakers for further clean-up.

Sample Extracts Clean-up

Bond elutes C-18 (1000mg/6ml) cartridges were conditioned using 10 ml each of acetonitrile. Labeled 30 ml tubes were placed under the columns to collect elutes. Sample extracts from the extraction stage above were loaded onto each corresponding columns, and 2 ml acetonitrile was used to elute each column.

Anhydrous Na_2SO_4, 5 g were placed on filter paper in funnels and the extracts from the tubes dried over them. Each filtrate was collected into labeled 50 ml round bottom flask and was concentrated to dryness using the rotary evaporator set below 40^oC. The residue was re-dissolved in 2 ml of a mixture of toluene/acetonitrile in a ratio of 1:3 prior to the next clean-up step.

ENVI-Carb/LC-NH_2 (500mg/500mg/6ml) cartridges were conditioned using 10 ml of 1:3 toluene-acetonitrile mixture. Labeled 50 ml pear shape flasks were placed under the columns, and the extracts from the previous clean-up step loaded onto the corresponding cartridges. The extracts were allowed to filter and the cartridges eluted with 20 ml of the toluene-acetonitrile mixture in four portions with intermittent vacuum use. All filtrates were concentrated below 40^oC 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 15 ml screw capped tube, closed and placed in freezer at -18^oC for at least 30 minutes. The extracts were removed from the freezer and immediately centrifuged at 3000 rpm for 5 minutes, and the top layer carefully transferred into labeled 2 ml GC standard opening vial prior to running on GC-MS and quantification by GC-ECD. Extracts were kept frozen until quantification was achieved.

Gas chromatographic determination

The final extracts were analyzed by Gas Chromatograph-Varian CP-3800 (Varian Association Inc. USA) equipped with combiPal autosampler and ^{63}Ni electron capture detector (ECD) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/kg 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-5 (30 m + 10 m guard column x 0.25 mm i.d, 0.25 µm film thickness). The injector and detector temperature were set at 270^{o}C and 300^{o}C respectively. The oven temperature was programmed as follows: 70^{o}C held for 2 min, ramp at 25^{o}Cmin^{-1} to 180^{o}C, held for 1 min, and finally ramp at 5^{o}Cmin^{-1} to 300^{o}C. Nitrogen was used as carrier gas at a flow rate of 1.0 mLmin^{-1} and detector make-up gas of 29mLmin^{-1}. The injection volume of the GC was 1.0 µL. The total run time for a sample was 31.4 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 synthetic pyrethroids 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 EI auto mode. The column oven temperature was programmed as follows; 70^{o}C for 1 min, then at 30^{o}Cmin^{-1} 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 Synthetic Pyrethroids Pesticide Residues

The residue levels of synthetic pyrethroids 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 analysis of synthetic pyrethroids pesticides residues were 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 synthetic pyrethroids pesticide was detected. Sample of each series was analyzed in duplicates. The method was optimized and validated by fortifying the cocoa beans samples with 500 µL of 1 µgmL^{-1} standards mixture of synthetic pyrethroids before analysis to evaluate the recovery of compounds. The recoveries of internal standards ranged between 75% and 120% for most of the synthetic pyrethroids pesticides analyzed.

Results and discussion

The study involves the analysis of synthetic pyrethroids pesticides in cocoa beans from Ghana. Synthetic pyrethroids pesticides selected for this study are Allethrin, Bifenthrin, Fenpropathrin, Lambda-cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate and Deltamethrin. However, residue concentrations of cis- and trans-permethrin were expressed as total permethrin and that of fenvalerate expressed as total fenvalerate, whiles residue concentrations of the four isomers of cyfluthrin and cypermethrin were also expressed as total cyfluthrin and total cypermethrin respectively, in order to conform to standard requirements of residue limits internationally. Concentrations of the various synthetic pyrethroids pesticide residues in each sample were calculated in µg/kg sample. The results for the types and residue levels of synthetic pyrethroids pesticides in the cocoa beans samples are shown in Table 1. The values in the table indicate the range, mean and standard deviation of residue concentrations in µg/kg, degrees of freedom and probability of mean residue values for cocoa beans samples from Tema and Takoradi warehouses.

Variation of Synthetic Pyrethroids Pesticide Residues in Cocoa Beans

Across the pesticide residues screened in the cocoa beans, all nine synthetic pyrethroids pesticides were detected at significantly varying concentrations (Table 1). Allethrin was detected in 18 out of 24 (75%) of samples from Tema warehouse. The mean residue concentration of Allethrin from Tema samples was 16.0 ± 1.3 µg/kg. This was higher than the mean residue concentration of 5.2 ± 0.9 µg/kg for cocoa beans samples analysed from Takoradi warehouse, and was statistically different in mean residues (p=0.028).

Bifenthrin occurred in 20 out of 24 (83%) cocoa beans samples analysed from Tema, with mean residue concentration of 12.6 ± 9.2 µg/kg. While all 20 cocoa beans samples analyed from Takoradi warehouse contained Bifenthrin, with mean residue concentration of 11.5 ± 8.8 µg/kg. However, Bifenthrin mean residues were not significantly different statistically (p=0.689).

Fenpropathrin was detected in cocoa beans samples analysed from both Tema and Takoradi warehouses with mean residue concentrations of 12.8 ± 11.8 µg/kg and 6.1 ± 2.9 µg/kg respectively. However, these mean residue levels of Fenpropathrin were statistically and significantly different (p=0.017).

Table 1: Summary of analysis of variance results for Synthetic Pyrethroids Pesticides Residue (µg/kg) in Cocoa Beans from Tema and Takoradi Warehouses.

Synthetic	TEMA		TAKORADI			
Pyrethroids	Range	x ± sd	Range	x ± sd	Df	p-value
Allethrin	ND - 28.0	16.0 ± 1.3	5.0 - 9.0	5.2 ± 0.9	34	0.028
Bifenthrin	ND - 31.0	12.6 ± 9.2	5.0 - 32.0	11.5 ± 8.8	38	0.689
Fenpropathrin	5.0 - 47.0	12.8 ± 11.8	5.0 - 16.0	6.1 ± 2.9	37	0.017
Lambda-cyhalothrin	ND - 38.0	9.6 ± 1.1	5.0 - 21.0	9.3 ± 7.0	17	0.952
Permethrin	ND - 75.0	28.3 ± 8.5	5.0 - 105.0	49.6 ± 8.6	15	0.329
Cyfluthrin	ND - 42.0	19.3 ± 8.4	ND - 28.0	12.2 ± 8.7	34	0.190
Cypermethrin	ND - 47.0	23.5 ± 2.3	6.0 - 58.0	40.9 ± 22.1	37	0.020
Fenvalerate	ND - 19.0	11.9 ± 2.6	7.0 - 32.0	21.3 ± 15.3	12	0.258
Deltamethrin	ND - 68.0	29.4 ± 7.5	48.0 - 60.0	54.0 ± 36.8	7	0.267
ND=Not Detected, NA=Not Applicable, x=Mean, sd=Standard Deviation, LOD = 5.0 µg/kg,						
Df=Degree of freedom and *p*=Probability						

Lambda-cyhalothrin was detected in 10 out of 24 (42%) samples from Tema warehouse, and occurred in 9 out of 20 (45%) of cocoa beans samples analysed from Takoradi. The mean residue concentrations of Lambda-cyhalothrin were 9.6 ± 1.1 µg/kg and 9.3 ± 7.0 µg/kg for samples from Tema and Takoradi warehouses respectively were not significantly different (p=0.952).

The widest range of synthetic pyrethroids pesticides occurred with Permethrin. This was from not detected to 105.0 µg/kg, with the highest residue level recorded in cocoa beans sample from Takoradi warehouse (Table 1).

Cyfluthrin was detected in 22 out of 24 (92%) of cocoa beans samples from Tema warehouse, with mean residue concentration of 19.3 ± 8.4 µg/kg. And out of 20 samples analyzed from Takoradi warehouse, 14 (70%) of the samples contained Cyfluthrin with mean residue concentration of 12.2 ± 8.7. However there was no significant differences statistically in mean values (p=0.190).

Cypermethrin, a very popular synthetic pyrethroids in Ghana, and also registered for cocoa production (EPA Ghana, 2009) was detected in 20 out of 24 (83%) of the cocoa beans samples from Tema warehouse. Mean residue concentrations of 23.5± 2.3 µg/kg and 40.9 ± 22.1 µg/kg were recorded for cocoa beans samples analyzed from Tema and Takoradi warehouses respectively, for Cypermethrin. These mean residue concentrations of Cypermethrin, however are statistically and significantly different from each other (p=0.020).

Fenvalerate, one of the problematic pesticides as far as shipment to Japan was concerned (Chocolate and Cocoa Association of Japan, 2007) was detected in cocoa beans from both warehouses. It was detected in 20 out of 24 (83%) of cocoa beans samples analyzed from Tema warehouse with mean residue concentration of 11.9 ± 2.6 µg/kg. For samples analyzed from Takoradi warehouse, 4 out of 20 (20%) occurred with Fenvalerate, mean residue concentration of 21.3 ± 15.3 µg/kg. However the mean residue concentrations of Fenvalerate were not significantly different (p=0.258).

Lastly, among the synthetic pyrethroids pesticides, Deltamethrin was detected in 7 out of 24 (29%) of cocoa beans samples analyzed from Tema warehouse. The mean residue concentrations of Deltamethrin were 29.4 ± 7.5 µg/kg and 54.0 ± 36.8 µg/kg for samples from Tema and Takoradi warehouses respectively; however the difference in mean was not statistically significant (p=0.267).

The high frequency of occurrence of the synthetic pyrethroids screened could be attributed to the fact that most of these pesticides are registered for use in Ghana for either agricultural or household purposes (EPA Ghana, 2009).

Comparison of Synthetic Pyrethroids Pesticide Residues in Cocoa Beans to Different International Standard Levels

In Ghana there is no established maximum pesticide residue limits for cocoa beans. The levels of synthetic pyrethroids pesticides residues recorded in the study were however compared to standard regulation limits set by the European Union (EU) and Japan; Ghana's major trading partners as far as export of cocoa beans are concerned (CMC COCOBOD, 2010).

Synthetic pyrethroids pesticides appear to be the most widely preferred pesticides among cocoa farmers in Ghana. Thus, all nine selected synthetic pyrethroids pesticides screened in the cocoa beans samples were detected with significantly varying average residue concentrations.

Considering cocoa beans shipment to any of the European Union countries, and based on average residue concentrations recorded (Table 2), there may be no threats of trade sanctions as far as synthetic pyrethroids pesticides in cocoa beans are concerned. This is true, since all nine selected synthetic pyrethroids pesticides recorded average residue concentration levels below the EU's maximum residue levels (MRLs), and as such the study considered residue levels of synthetic pyrethroids pesticides in the whole dried cocoa beans, whiles for EU regulations, MRLs of pesticides apply to only the cocoa beans after the shells have been removed (that is the nib only).

Allethrin, Cypermethrin and Fenvalerate with average residue concentrations of 10.5 µg/kg, 30.0 µg/kg and 10.4 µg/kg respectively, are comparable with their various Japanese MRLs (Table 2). Even though these pesticides average residue concentrations did not exceed their various MRLs, care must be taken in their applications on cocoa, since they are more of borderline levels as far as shipment to Japan is concern. However, the other six synthetic pyrethroids pesticides detected; Bifenthrin, Fenpropathrin, Lambda-cyhalothrin, Permethrin, Cyfluthrin and Deltamethrin with average residue concentrations of 12.0 µg/kg, 9.4 µg/kg, 9.5 µg/kg, 37.1 µg/kg, 16.5 µg/kg and 39.3 µg/kg respectively, were below both the EU and Japanese maximum residue limits for the various pesticides (Table 2).

Table 2: Average Residue Results of Synthetic Pyrethroids pesticides against EU and Japanese MRLs

PESTICIDES:	RANGE	MEAN	EU	JAPAN
Synthetic Pyrethroids:	**ug/kg**			
Allethrin	ND - 28.0	10.5	10	10
Bifenthrin	ND - 32.0	12.0	100	100
Fenpropathrin	5.0 - 47.0	9.4	20	10
Lambda-cyhalothrin	ND - 38.0	9.5	50	10
Permethrin	ND - 105.0	37.1	100	50
Cyfluthrin	ND - 42.0	16.5	100	20
Cypermethrin	ND - 58.0	30.0	100	30
Fenvalerate	ND - 32.0	10.4	50	10
Deltamethrin	ND - 68.0	39.3	50	50

ND = Not Detected, LOD = 5.0 µg/kg

Conclusion

The study revealed the presence of all nine selected synthetic pyrethroids pesticides at significantly varying concentrations, with Permethrin recording the widest range of residue concentration (Not detected – 105.0 µg/kg).

Generally, there was high frequency of occurrence for all nine selected synthetic pyrethroids pesticides screened in the cocoa beans produced in Ghana. This indicates a higher preference to synthetic pyrethroids pesticides among cocoa farmers in Ghana. And that could be attributed to the fact that most of these pesticides are registered for use in Ghana for either agricultural or household purposes (EPA Ghana, 2009).

Finally, in all 44 sampled cocoa beans, none of the detected synthetic pyrethroids pesticides' average residue concentrations did exceed the European Union or Japanese Maximum Residue Limits in cocoa beans produced in Ghana. However, Allethrin, Cypermethrin and Fenvalerate average residues concentrations were at the borderline of the Japanese MRLs. Thus, the application rate, nozzle size and the pre-harvest interval of these pesticides should be taken into consideration when applying those pesticides on cocoa, in order not to exceed their maximum limits for regulatory purposes.

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