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Residual levels of some pesticides in cocoa beans (*Theobroma cocoa*) from Ashanti and Brong Ahafo regions of Ghana

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ABSTRACT

A study was carried out to determine pesticide residue levels in cocoa beans (*Theobroma cocoa* L.) samples, collected from Ashanti and Brong Ahafo regions of Ghana. The pesticides were extracted from cocoa beans using liquid-liquid extraction followed by clean up with C-18 and Envi – Carb NH_2 solid-phase extraction cartridges. Final extracts were dissolved in ethyl acetate and analysis was carried out by gas chromatography with electron capture detector. Pesticides were identified by their retention times and quantification using an external calibration method. The investigated pesticides were DDT, DDD, Endosulfan I and II, Aldrin, Chloropyrifos, Permethrin I and II, Cypermethrin I and II. From the results it could be deduced that 60% of the pesticides residues detected in samples collected from both regions were below the EU and Japanese allowable limits. Endosulfan I had high residual concentrations for cocoa beans found in Berekum, Hwidiem, Brofoyedru and Ampenim samples. Endosulfan II exceeded permissible levels for Goaso and Ampenim samples. However, DDT and DDD were found to be below maximum residue limits (MRL) established by the EU and Japanese organizations in all samples. Aldrin was below the detection limit for all the samples within the regions. Chloropyrifos recorded the highest residual concentration for cocoa beans from Mim. Permethrin I and II were found to be above MRL values established by the EU and Japan.

Key words: Cocoa beans, Gas chromatograph, Maximum residue limits, Pesticide residues, Retention times

Cocoa (*Theobroma cocoa* L.) is susceptible to attack by black pod disease, cocoa swollen shoot virus (CSSV), and insect pests such as cocoa capsids. Cocoa farmers use a wide range of pesticides to minimize the economic losses caused by these pests and diseases in the cocoa industry. Botwe et al. (2006) reported that two-thirds of crops in the field would be lost without usage of pesticides.

The import, sale and use of organochlorine pesticides (OCPs) have been banned in Ghana. However, there is evidence of their presence in the ecosystem (EPA Ghana, 2009; Bempah *et al*., 2011; Kuranchie-Mensah et al., 2011). The OCPs such as aldrin / dieldrin had reportedly been used on cocoa in Ghana (Owusu-Ansah et al., 2010; Kuranchie-Mensah et al., 2011). The use of pesticides and their mode of application including their abuse especially in agriculture have been of much concern to environmental scientists (Essumang et al., 2009). Contamination of cocoa beans can occur directly by treating the crop with pesticides before harvesting, storage and distribution. It can 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 (Belitz et al., 2004).

The hazardous nature of OCPs led to introduction of organophosphates (OPs) and pyrethroid pesticides. In 1999, the US Environmental Protection Agency (USEPA) initiated a complete review of all pesticides. The OPs were evaluated because of their threat to human and all mammalian life (EPA, 2003a). As

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a result of the evaluation, the EPA eliminated OPs production in the US and began a program to phase out OPs (EPA, 1999). In the absence of OPs, synthetic pyrethroid pesticides, which were derived from the chrysanthemum in the 1970's, are quickly increasing in popularity because of their high efficiency against insects, greater stability in the environment and a greater safety to human and wildlife (Al-Makkawy and Modbouly, 1999). Although pyrethroids are generally safer for the environment than OPs, it is more difficult to determine if they are causing problems in the environment than OPs because they are toxic at such small concentrations.

In recent times, emphasis has been placed on the use of pesticides in cocoa production (Photius, 2004). The focus of repetitive pesticides use in cocoa production is for economic gains. The fate of the environment with respect to potential pollution by agrochemical residues of the ecosystems needs to be assessed. This has led to establishment of Maximum Residue Limits (MRLs) in cocoa beans by organizations such as, European Union, Food and Agricultural Organization and Japan. The MRLs encourage food safety by restricting the concentration of a residue permitted on a commodity the (European Communities, 2005; FAO/UNEP/WHO, 1991; FDA, 2005). Ghana is one of the leading cocoa exporters worldwide and therefore it is necessary to monitor the levels of pesticide residues in cocoa beans to determine whether the quality of cocoa beans produced in Ghana conforms to international standards.

MATERIALS AND METHODS

Sampling

Dry cocoa beans were sampled from Tema ports. Cocoa beans from different districts of the Ashanti and Brong Ahafo Regions of Ghana were received at the port and the method adopted by Quality Control Company Limited (QCC) in Ghana was used in obtaining samples. The samples were sent to the laboratory for analysis (MS, 2007).

Reagents and apparatus

Reagents and apparatus used were acetonitrile, acetone, ethyl acetate and toluene (pesticide grade, BDH, England); acetone, dipotassium hydrogen phosphate and potassium dihydrogen phosphate (analyte grade, BDH, England); Sodium sulfate (pesticide grade, Aldrich-Chemie, Germany); sodium chloride (Pesticide grade, Riedel-de Haen) and Envi-carb/LC-NH_2(500 mg/500 mg/6 mL) from Supelco C-18 (USA).

Experimental and chromatographic conditions

Shimadzu Gas Chromatograph – 2010 with AOC 20i Autoinjector and AOC 20S Auto sampler and Electron Capture Detector was used. The analytical column was 30 m × 0.25 mm internal diameter fused silica capillary column coated with VF-5ms (0.25µm film). Before analysing the samples all glass wares were acid washed and cleansed with distilled water and dried in the oven at 200^{o}C for about four hours. Nitrogen was used as carrier gas and flow rate of 1 ml/min. The injection and detector temperatures of 225°C and 300°C were used for analysis.

Analysis for pesticides

Sample preparation, extraction, clean-up and analysis were carried out according to the procedure described in multi-residue method for agricultural chemicals (Syoku-An, 2006).

Preparation of organochlorine standards: All the pesticides standard stock solutions were prepared in ethyl acetate with the aid of an ultrasonic bath, by dissolving a weight of the pesticides which when corrected for purity will be equivalent to 1000 µg/ml. One ml of each of the prepared stock solutions were pipette into a 50 ml volumetric flask resulting in a mixed standard (MIX 1) of concentration 20 µg ml^{-1}. The MIX 1 was diluted to produce new concentrations of 2.0 µg ml^{-1} (MIX 2) and 0.2 µg ml^{-1}(MIX 3).

Quality control and quality assurance: Quality of pesticides was assured through the analysis of blanks and replicate 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 pesticide was detected. Sample of each series was analyzed in replicates. The method was optimized and validated by fortifying the ground and homogenized cocoa beans sample with MIX 1 before analysis to evaluate the recovery of compounds. This is equivalent to a fortification level of 1 mg kg^{-1}. Extraction and clean up procedure as in the methodology were carried out before its injection into the GC. Same chromatographic conditions were used. This was repeated for fortification levels of 0.1 mg kg^{-1} and 0.01 mg kg^{-1}. The recoveries of internal standards ranged between 70% and 120% for most of the pesticides analyzed. The percentage recovery was calculated as:

$$\text{Recovery (\%)} = \frac{\text{Amount of analyte recovered}}{\text{Amount of analyte spiked}} \times 100$$

RESULTS AND DISCUSSION

Pesticide residues identified in the cocoa beans

includes DDT, DDD, endosulfan I and II, cypermethrin I and II and permethrin I and II. The retention times of individual pesticides obtained from the chromatogram are shown in Table 1.

Pesticide DDT and its metabolites were detected in 100% of the analysed samples. The mean concentration for DDT and DDD were 0.13 mg kg^{-1} and 0.03 mg kg^{-1} (Table 2). The DDT and its metabolite DDD have been banned in Ghana since 1985 (EPA Ghana, 2008). Their occurrence in the cocoa beans could be attributed to the soil and climatic conditions favouring persistence and long range transport of DDT and DDD (Kanan et al., 1994; Ritter et al., 1995; Parimi et al., 2006). The concentrations of DDT residues in all the cocoa beans from the sampling areas in Brong Ahafo were higher than that of Ashanti region (Fig. 1). It could be inferred from the trend that there is a continuous use of DDT in the area due to its lower cost and effectiveness as well as its broad spectrum activity despite ban on its use (Amoah et al., 2006). In the Ashanti region, DDD residues were not detected in cocoa beans sampled from Ampenim, Nyinahini and Juaso. The mean concentration for DDD residues was 0.03 mg kg^{-1} (Table 2). All the samples were within the EU and Japanese MRL value of 0.50 mg kg^{-1} and 0.05 mg kg^{-1} respectively. The mean concentration of DDT was 0.13 mg kg^{-1} (Table 2). Out of the number of samples analysed for DDT residues in the Ashanti region, 80% were above the Japanese MRL of 0.05 mg kg^{-1} except cocoa beans from Agona and Brofoyedru. Conversely all the samples analysed were below the EU permissible level of 0.50 mg kg^{-1}.

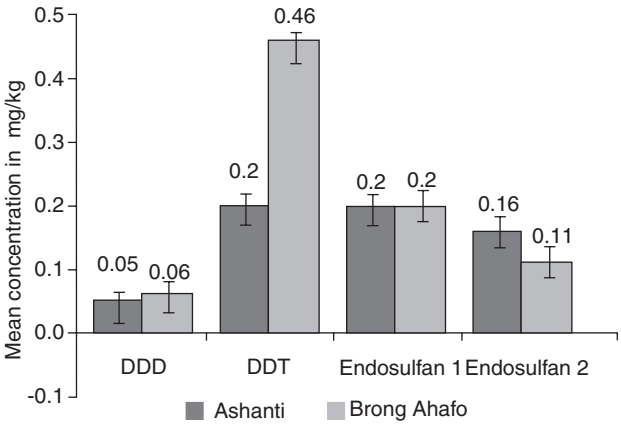

Fig. 1. Regional comparison of mean concentrations of some pesticide residues in cocoa beans

Table 1 Retention times of organochlorine pesticide standards

Standards	Retention time (Min)
Chloropyrifos	16.924
Aldrin	17.563
Endosulfan I	19.621
Endosulfan II	21.535
DDD	21.990
DDT	23.348
Permethrin I	25.292
Permethrin II	25.296
Cypermethrin I	30.566
Cypermethrin II	30.690

Endosulfan I and II were present in all the cocoa beans sampled from the different locations in the Brong Ahafo region. Concentrations of the endosulfan II residues in cocoa beans in all the sampling areas were lower than endosulfan I.The mean concentration of endosulfan I was 0.07 mg kg^{-1} (Table 2). All the cocoa beans sampled from most of the locations in the Brong Ahafo region had concentrations lower than

Table 2 Pesticide residues in cocoa beans from Brong Ahafo and Ashanti region

Pesticide	Mean concentration for Brong Ahafo ±SD (mg kg^{-1})	EU (mg kg^{-1})	Japan (mg kg^{-1})	Mean concentration for Ashanti ±SD (mg kg^{-1})
Aldrin	0.01 ± 0.00	0.05	0.10	0.01 ± 0.00
Chloripyrifos	7.89 ± 2.30	0.19	0.05	7.06 ± 1.50
Permethrin I	0.33 ± 0.13	0.10	0.05	0.37 ± 0.21
Permethrin II	0.61 ± 0.60	0.10	0.05	0.47 ± 0.42
Cypermethrin I	0.07 ± 0.05	0.10	0.03	0.06 ± 0.22
Cypermethrin II	0.16 ± 0.07	0.10	0.03	0.02 ± 0.16
Endosulfan I	0.07 ± 0.03	0.10	0.10	0.07 ± 0.06
Endosulfan II	0.07 ± 0.03	0.10	0.10	0.08 ± 0.03
DDT	0.28 ± 0.30	0.50	0.05	0.13 ± 0.04
DDD	0.03 ± 0.01	0.50	0.05	0.03± 0.02

EU and Japan MRL values of 0.10 mg kg^{-1} with the exception of samples from Berekum and Hwediem. These findings confirm the results of Frimpong et al. (2012). However, endosulfan I has been considered for restrictive use in Ghana (EPA, 2008). All samples analysed for endosulfan II for Brong Ahafo region were within the EU and Japanese MRL values of 0.10 mg kg^{-1} with the exception of samples from Goaso which had concentration of 0.11 mg kg^{-1} (Table 2). In the Ashanti region, the mean concentration for endosulfan I was 0.07 mg kg^{-1} (Table 2). There were non-detectable levels of endosulfan I in cocoa beans from Juaso and Apagya. The concentration of endosulfan I was below the EU and Japanese MRL of 0.10 mg kg^{-1} except cocoa beans from Ampenin and Brofoyedru. For endosulfan II, all the samples were below the EU, Japanese and Codex MRL value of 0.10 mg kg^{-1} except cocoa beans from Ampenim. Generally, the concentration of endosulfan I was higher than endosulfan II. A similar trend was observed in both regions and the levels of endosulfan II in the Brong Ahafo region were higher than samples from the Ashanti region.

Aldrin was below detection limit in all the cocoa beans sampled from the Brong Ahafo and Ashanti region (Table 2).This may be attributed to its volatility and instability in environment. Aldrin is one of the OCPs that has been banned under the Stockholm Convention for POPs. Its absence could therefore be due to the fact that its use has been discontinued (UNEP, 2001).

Concentrations of chloropyrifos in cocoa beans from the Brong Ahafo recorded the highest mean concentration of 7.89 mg kg^{-1} and 7.06 mg kg^{-1} for Ashanti region (Table 2). All the cocoa beans sampled in the Brong Ahafo region had concentrations above MRL values established by EU (0.10 mg kg^{-1}), Japan (0.05 mg kg^{-1}) and Codex Alimentarius. A similar trend was observed for the cocoa beans sampled from Ashanti region. Generally, the concentrations of chloropyrifos in cocoa beans sampled from the Brong Ahafo region were higher than in the Ashanti region.

Mean concentrations of permethrin I and II in cocoa beans from the Brong Ahafo region were 0.33 mg $kg^{-1}$and 0.61 mg kg^{-1}(Table 2). On the other hand, mean concentration for permethrin I and II in Ashanti region were 0.37 and 0.47 mg kg^{-1}(Table 2). For permethrin I, all the samples had levels above the Japanese and EU permissible limit of 0.05 and 0.10 mg kg^{-1}, respectively, for cocoa beans. For permethrin I in Ashanti region all the samples recorded levels above the Japanese and EU MRL of 0.05 and 0.10 mg kg^{-1}respectively. However, the concentrations of permethrin II and I in the Brong Ahafo region were lower than in the Ashanti region.

Mean concentration of cypermethrin I and II in cocoa beans sampled from the Brong Ahafo region were 0.07 and 0.16 mg kg^{-1}(Table 2). In the Ashanti region, mean concentrations of cypermethrin I and II in cocoa beans were 0.06 mg $kg^{-1}$and 0.02 mg kg^{-1}(Table 2). Levels of cypermethrin I in all cocoa bean samples from Ashanti region were below the MRL value of 0.10 mg $kg^{-1}$for EU, Japanese and Codex Alimentarius with the exception of cocoa beans from Brekum. In the Ashanti region, 90% of the cocoa beans were below the EU, Japanese and Codex Alimentarius permissible limits with the exception of samples from Nyinahini that was slightly above the set limit. It can be deduced that the concentrations of the cypermethrin I in cocoa beans from the Brong Ahafo region were lower than the cocoa beans from the Ashanti region. For cypermethrin II in Brong Ahafo region, all the locations in the region had concentrations in the samples above the MRL values established by EU of 0.10 mg kg^{-1}except Brekum samples and limits established by Japanese with MRL of 0.03 mg kg^{-1}except Sankore samples. For Ashanti region, none of the samples were below the EU, Japanese and Codex MRL for cypermethrin II. Generally, the concentrations of cypermethrin II were higher for cocoa beans from Ashanti region than Brong Ahafo region.

CONCLUSION

It can be concluded that most of the pesticides residues analysed in the Brong Ahafo and Ashanti regions were below the EU and Japanese pesticide residue permissible levels for cocoa beans. Among the pesticides detected, chloropyrifos showed the highest residual concentrations for samples from in the Brong Ahafo region. The lowest pesticide residual concentration was recorded for Endosulfan I for Sankore in the Ashanti region. Aldrin was below detection limit for all samples analysed in the two regions.

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