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# Assessment of systemic insecticide residues in cocoa beans from some farms in the Western North region, Ghana

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**Abstract**
Residue concentrations of systemic insecticides were analysed in cocoa beans sampled from the Western North Region. The objectives of the study were to assess the residue concentrations of 11 systemic insecticides in the sampled cocoa beans, and compare the residue concentrations detected with the European Union's Maximum Residue Limit (MRL) of cocoa beans to ascertain whether or not it is safe to consume cocoa beans' products from the Western North region. Purposive sampling and simple random sampling were used to choose 10 cocoa farms and 10 communities respectively across five Municipal and District Assemblies (MDAs) in the Western North Region. The sampled cocoa beans were analysed by a partially modified QuEChERS Method MRM by LC-MS/MS. A total of 11 active ingredients were analysed, and out of these only one was found in the cocoa beans sampled from WF2. This was pirimiphos-methyl with a residue concentration of 0.01 mg/kg, which falls within the EUs MRL set for cocoa beans. The other 10 systemic insecticide residues were not detected in all the samples analysed. This implies that it is either the insecticide residues have diminished from the cocoa beans or the farmers have been following the prescribed insecticide application procedures, hence their non detection in cocoa beans sampled. Therefore, in terms of food safety as it relates to insecticide residue concentrations, cocoa beans from the Western North Region can be considered to be of good quality, and safe to consume as it poses no health risk to its consumers.

**Keywords:** Systemic, insecticide, residue concentration, cocoa beans

## Introduction

Agriculture is among the largest sectors of the Ghanaian economy and one of the highest contributors to Ghana's Gross Domestic Product (GDP), employing about 60 % of the country's labour force (ISSER, 2010, as cited in Denkyirah, *et al*., 2016) [14]. The agricultural sector in Ghana is dominated by cash crops such as cocoa, coffee, oil palm and rubber. Among these cash crops, cocoa is of particular interest for Ghana and for the global chocolate industry (Danso-Abbeam, *et al*., 2014) [13]. Cocoa is grown in 10 out of the 16 regions in Ghana cultivated on about 1.5 million hectares of land (COCOBOD Ghana, 2010) [10]. These cocoa farms owned by some 800,000 farm families which provide food, employment, tax revenue and foreign exchange earnings for the country (Appiah, 2004; Anang, 2011; Danso-Abbeam *et al*., 2014) [5, 3, 13].

The dark brown well fermented cocoa beans from Ghana have good flavour and moisture content between 6 and 8%, as a result continues to enjoy high premium on the World's Commodities Markets because of its unsurpassable high quality (Frimpong *et al*., 2012a) [20]. A study by Denkyirah, *et al*. (2016) [14] indicated that despite the high quality of Ghanaian cocoa beans and its economic importance to the country, its production is threatened by insect, pests and diseases, a situation which has resulted in the decline in cocoa production, with adverse impact on the Ghanaian economy.

According to the International Cocoa Organization (ICCO), up to 40% of global annual cocoa production is lost to insect, pests and diseases (ICCO, 2013) [23]. Similarly, declining cocoa production in West Africa in recent years has been attributed to mainly pest and disease infestation (Kongor, *et al*., 2016) [25]. In Ghana, cocoa yields per hectare are considerably lower than other top cocoa producing countries like Ivory Coast, Cameroon and Brazil (Aneani and Ofori-Frimpong 2013) [4]. Pest and diseases are said to contribute significantly to the low yields in Ghana (Dankyi, et al. 2014) [12].

Over the years, the use of insecticides to control cocoa pest and diseases have been the surest pest control method adopted by farmers in Ghana. This is in congruence with the reports of (Baig, 2009) [6] who opined that the surest way of protecting crops including cocoa from pests' attack, and also to maximize productivity in agriculture is through insecticide application. However, a drawback to the mass application of insecticide (and pesticides in general) programme is the tendency for crop and environmental contamination, due to the extensive use and multiple application rates. Previous studies have reported that, applied insecticides in cocoa farms may enter and persist in soils, water bodies and cocoa beans for several months or years following application (Frimpong, *et al*., 2012a; Dankyi, *et al*., 2014; Okoffo, 2015; Fialor, 2017; Boadu & Boadu, 2021) [20, 12, 28, 17].

Therefore, insecticides in general are seeing as necessary evil due to the health and economical treats they pose when found to contain residue levels above the maximum residue limits (Frimpong, Yeboah & Fletcher, 2011) [18]. Similarly, Gill and Garg (2014) [22] aver that insecticide residues also have the potential to cause toxicity to plants, their product and contaminate the food chain when taken up by plant roots and leaves from soils, air and other nutrient solutions. Previously studies have reported the effect of insecticide residues on humans. According to Akan, *et al*. (2013) [2] and Agbeve, *et al*. (2014) [1], increased accumulation of these insecticide residues in the food chain may pose serious health hazards when they are not metabolized by the body and accumulate in the soft tissues. Additionally, exposure to insecticides compounds through food and drinking water are reported to affect thyroid function, and cause low sperm count in males, birth defects, increased in testicular cancer, reproductive and immune malfunction, endocrine disruptions, cancers, immunotoxicity, neurobehavioral and developmental disorders (Gill & Garg, 2014) [22].

This has led to the prescription of tolerance Maximum Residue Limits (MRLs), and Acceptable Daily Intake (ADI) as well as No Observable Adverse Effect Level (NOAEL) for various insecticides (and all other pesticides) in food (including cocoa beans), soil and drinking water, especially by the Codex Alimentarius Commission (CODEX), the World Health Organisation (WHO) (Sosan *et al* as cited in Okoffo, 2015) [7] and other designated authorities (Bateman, 2015) [7]. In view of the strict enforcement of the MRLs harmonization, export of cocoa beans from Ghana in particular to its main partners in Europe and Japan are liable to possible rejections if prohibited substances are found in the product or at levels above the MRLs (Okoffo, 2015) [28]. The rejection of cocoa beans as a result of high level of prohibited substances will threaten the livelihood of smallholder farmers, thereby worsening unemployment and poverty as well as the foreign income earning of the country.

Unfortunately, the fate of systemic insecticide residue in cocoa beans has received low scholarly attention. In the literature, much of the current knowledge on residues of systemic insecticides has been restricted to honey and honey bees, avocados, and other fatty fruits/vegetables. (Blacquie`re, *et al*., 2012; Laycock, *et al*., 2012; Jovanov, *et al*., 2015) [8, 26, 24].

However, knowledge in tropical foods, such as cocoa beans, and of actual sources of contamination remains low. Although, insecticides like the neonicotinoids, some organophosphorous, and some pyrethroids, with systemic action have been used extensively in the production of cocoa and other crops in Ghana and other West African countries for several years, their levels in cocoa beans are not yet known. Whereas some research exists on insecticide residues in cocoa beans, they have been restricted to other classes of insecticides with contact action such as the organochlorines, some organophosphates and carbamates, which are less readily used in cocoa production in recent years, particularly in Ghana (Frimpong, *et al*., 2012a, 2012b; Dankyi, *et al*., 2014) [20, 19, 12].

In view of findings from literature, an assessment of systemic insecticide residue concentrations in cocoa beans from the Western North Region was therefore imperative in order to ascertain whether or not the levels of the insecticide residues in the cocoa beans are above the maximum contamination levels, and whether or not it was safe to consume products of cocoa beans from these cocoa farms per WHO standard.

Unlike most previous studies in Ghana that sampled cocoa beans from the harbour and the depot offices, this current study sampled fresh cocoa beans from the farms, fermented and dried them to assess the levels of systemic insecticides in them. This has helped to trace the source of the contamination of the cocoa beans and ascertain whether or not the cocoa farmers are indiscriminate in insecticide application. Additionally, the Western North region was selected for this study as it is one of the highest cocoa producing regions in the country (COCOBOD, 2010; COCOBOD, 2012) [10, 11], but has limited data on residue concentrations from the region (Oppong & Attuah, 2016; Boadu & Boadu, 2021) [29, 9].

Also, in literature, conventional methods that are widely used for the extraction, purification and quantification of insecticides (pesticides in general) from fatty matrixes such as cocoa beans include solid-phase micro-extraction (SPME), matrix solid-phase extraction (MSPD, gel permeation chromatography (GPC), and supercritical fluid extraction (SFE) by Gas Chromatography (GC)- Mass Spectra (MS) method (Frimpong, *et al*., 2011; Frimpong, *et al*, 2012; Okoffo, 2015, Fialor, 2017) [18, 19, 28, 17]. Contrariwise, this current study employed a partially modified QuEChERS Method MRM by LC-MS/MS procedure in the extraction, purification/clean-up and quantification of the 11 residues of systemic insecticides. The QuEChERS procedure was simple, less expensive techniques and reliable for quantitative and qualitative assessment of residues in food. Additionally, partially modified QuEChERS Method MRM by LC-MS/MS procedure has a high flexibility for application to a wide range of analytes and matrixes (as cited in Dankyi *et al*., 2014) [12].

## Materials and Methods

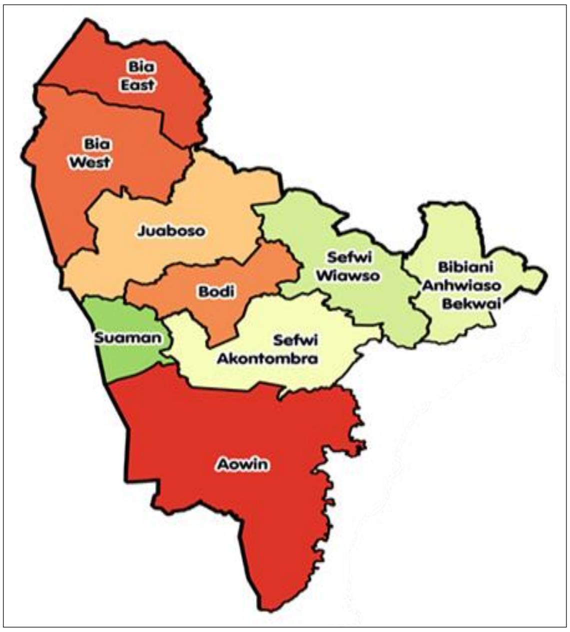
### Study Area

The study was carried out in the Western North Region. The region is one of the six new regions of Ghana created in February 15, 2019. This new region was carved out of the existing Western Region of Ghana. It lies between latitude 6.227937° or 6° 13' 41" north and longitude -2.823489° or 2° 49' 25" west. The land area of the region is 10,079 km$^2$ area. The region shares boundaries with the Comoe District of the republic of Ivory Coast on the west, the central region in the southeast, the western region in the southwest, and the Ashanti, Ahafo, Bono East and Bono regions in the north.

The region is divided into three Municipal and six District Assemblies (MDAs), namely: Aowin, Wiawso, Bibiani/Anhwiawso/Bekwai, Bia East, Bia West, Bodi, Juaboso, Sefwi Akontombra and Suaman respectively. Sefwi Wiawso is the administrative capital of the region.

The Western North Region, falls within the tropical rainforest climatic zone with high temperatures throughout the year between 25°C and 30°C and moderate to heavy rainfall pattern between 1200 mm and 1780 mm per annum (National Commission on Culture, 2020). It comes with double maxima characteristics in June-July and September-October as its peaks. Humidity is relatively high, which is about 90% at night falling to 75% during the day. The National Commission on Culture further reports that the rainfall pattern in the region is unique and suitable for agricultural activities in the Region. It has two long wet seasons separated by relatively short dry season.

Agriculture is the main source of employment in the region. The major economic activities in the region include the cultivation of food (such as plantain, cassava, cocoyam, rice, maize, fruits, vegetables, etc.) and cash crops (including cocoa), livestock farming, mining and subsistence fishing (Ghana Statistical Service, 2021) [21]. The main exportable produce are cocoa, timber, gold, bauxite and crops. Figure 1 shows the map of Western North region carved out of the map of Ghana. All the nine Municipal and District Assemblies in the Western North region are indicated in Figure 1.




**Source:** National Commission of Culture

**Fig 1:** Map of Western North Region showing the Administrative Divisions

**Sampling Technique and Sample Size**

Five out of the nine Municipal District Assemblies (MDAs) in the region were purposively selected for sample collection. They were Bia East, Bia West, Bodi, Juaboso, Wiawso. These MDAs were selected because of their high production of cocoa in the region and also proximity of these assemblies to each other. Two communities (totalling 10 communities) were randomly selected from each of the districts listed chosen for the study. A farm was then purposively selected from the sampled communities.

The eligibility criteria for the selection of the farms in the communities were:

1. Age of cocoa farm (farms not less than 8 years and not more than 20 years with a history of at least five years of insecticides application).
2. The willingness of the farmers to sample their cocoa beans for the study. cocoa bean samples were collected within a period of three months (March to May, 2022).

The cocoa farms selected from the ten communities in the region are listed in Table 1 below.

**Table 1:** List of farms sampled from the Western North region for this study

| Code | MDAs | Communities | Farm No. designated |
|---|---|---|---|
| BEF1 | Bia East district | Adabokrom | Farm one |
| BEF2 | | Fosukrom | Farm two |
| BWF1 | Bia West district | Adwuofua | Farm one |
| BWF2 | | Eluokrom | Farm two |
| BF1 | Bodi district | Kwafukaa | Farm one |
| BF2 | | Ahebenso | Farm two |
| JF1 | Juaboso district | Bonsu-Nkwanta | Farm one |
| JF2 | | Mafia | Farm two |
| WF1 | Wiawso municipality | Asafo | Farm one |
| WF2 | | Boako | Farm two |

**Sampling Design and Preparation of Samples of Cocoa Beans**

In each of the ten cocoa farms, two quadrats of 80 x 80 metres were marked. For each quadrat, about five cocoa trees were randomly selected. about10 to 20 fresh, mature and ripped cocoa pods were randomly taken and kept in labelled bags. The pods were broken and the cocoa beans fermented for seven days and sun-dried for another seven days. Foreign objects found in each sample were removed by hand picking when the beans were air dried. The dried cocoa beans from each quadrat were bulked together to form a composite sample and one kilogramme samples were taken into a clean labelled transparent zip-lock bags and transported to the Ghana Standards Authority Pesticide Residue Laboratory in Accra for the analysis of insecticides residues.

Using the hammer mill at the Ghana Standards Authority Pesticide Residue Laboratory, Accra, each of the labelled fermented dried cocoa bean samples of each farm were ground into fine powder (homogenized) and collected into a new sample plastic bag and re-labelled accordingly to form each individual analytical sample for each farm, weighing 1 kg. After each sample was ground, the mill was thoroughly cleaned with a brush. To avoid cross contamination, a few grams of the next sample to be prepared were ground and discarded before the analytical sample was collected into a new labelled sample plastic bag.

**Sample Extraction and Clean-up**

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) SPE (Solid-Phase Extraction) methodology was used in the analysis of the sampled cocoa beans. The QuEChERS procedure is usually a two-stage process consisting of sample extraction followed by dispersive SPE as enumerated below:

**Extraction:** The cocoa samples were homogenized to maximize the available surface area of the samples for better extraction efficiencies. About 10g±0.1g of the comminuted homogenous sample was weighed into a 50 mL centrifuge tube. After this, 15 mL acetonitrile was added and vortex for 1 minute. The sample was then centrifuged for 5 minutes at 3500 rpm.

**Extract purification:** Dispersive Solid Phase Extraction (SPE) - The second stage of the QuEChERS method uses dispersive SPE, which involves transferring a portion of the acetonitrile extract to a clean-up tube containing a combination of sorbents for removal of unwanted sample components.

Approximately 6 mL aliquot of the extract was transferred into a 15 mL centrifuge tube which contains 150 mg PSA, 150 mg C-18 and 1 g magnesium sulphate. Magnesium sulphate ensures that, upon addition of acetonitrile, a phase separation is induced between water and organic solvent, with the insecticides of interest being extracted into the organic phase. The tube was closed and vortexed for 1 minute and then centrifuged for 5 min. at 3500 rpm. 1 mL of the purified extract was transferred into a 2 mL standard opening vial for quantitation by LC MS/MS.

## Laboratory Requirements

The individual certified reference standards, acetamiprid, clothianidin, imidacloprid, thiamethoxam, acephate, allethrin, dimethoate, methamidophos, monocrotophos, pirimiphos-methyl, and sulfoxaflor used for the identification and quantification were provided by the Pesticide Residue Laboratory of the Ghana Standard Authority (GSA). These were obtained from the appropriate chemical stores and stored in the freezer to minimize degradation. The laboratory apparatus and materials used for the analysis are outlined in Table 2.

**Table 2:** Descriptions of laboratory apparatus and materials used for analysis at GSA

| Apparatus | | | |
|---|---|---|---|
| **Instrument** | **Description** | | |
| Liquid Chromatograph | Agilent Technologies-Infinity 1290 LC System with a 4226A Autosampler, 4204A Quaternary Pump, G1316C Temperature Control Compartment. | | |
| Analytical column | Agilent-Zorbax Eclipse Plus C-18 Rapid Resolution HD Column with specifications 2.1x 50mm 1.8-Micron | | |
| Injection Volume | 6ul | | |
| Solvent Program | Time | Solvent A – 0.1% formic acid in water | Solvent B – 0.1% formic acid in acetonitrile |
| | 1 min | 95 | 5 |
| | 2 min | 85 | 15 |
| | 2.5 min | 70 | 30 |
| | 6 min | 55 | 45 |
| | 12 min | 20 | 80 |
| | 14 min | 5 | 95 |
| | 16 min | 5 | 95 |
| | 18 min | 95 | 5 |
| Mass Spectrometer: | | | |
| Item | Conditions | | |
| Source Gas Temperature | 300 °C | | |
| Gas Flow | 13l/min | | |
| Nebulizer | 30 psi | | |
| Capillary | Positive 4000 V | | |
| Scan Type | Dynamic MRM | | |
| Delta EMV | 350 V | | |

## LC–MS/MS Instrumentation

Chromatographic separation of analytes was performed on an Agilent Technologies-Infinity 1290 LC System with a 4226A Autosampler, 4204A Quaternary Pump, G1316C Temperature Control Compartment. Mobile phases A and B consisted of 5 mM ammonium acetate and 95% acetonitrile in 5 mM ammonium acetate, respectively. The gradient was run at 300 mL min 1 for 16 min as follows: 10% B increased linearly to 100% in 8 min; held constant for 2 min; decreased back to 10% B in 1 min; and maintained for an equilibration time of 5 min. The injection volume was 5 mL.

The mass spectrometer used equipped with electrospray ionization (ESI). The MS determination of all analytes was performed in positive mode with multiple reaction monitoring (MRM) of the two most intense precursor-product ion transitions for each analyte, one used for quantification and the other for confirmation. The source parameters employed were: Source Gas Temperature of 300 °C, Gas Flow: 13l/min, Nebulizer: 30 psi, Capillary Positive 4000V, Scan Type: Dynamic MRM, Delta EMV: 350V. Data obtained were processed using the Analyst software (version 1.6.2). Analytes were quantified from matrix-matched standard curves using peak area and a weighting of 1/x.

## Quality Control and Quality Assurance

Proper quality assurance procedures and precautions were taken to ensure the reliability of the results. During the drying of the cocoa beans, all foreign materials were removed by handpicking. This was done to ensure that the 1kg bagged was pure cocoa beans free any foreign objects that may interfere with the sample weight and the subsequent analysis. The zip-lock bags used were clean, dried and free from any dirt, impurities or contaminations.

Additionally, the samples were carefully handled to avoid any external influences that could interfere with the integrity of the sample and hence contaminate it. Glasswares were properly cleaned, and reagents used were of analytical grades. All the glassware to be used for insecticides residue analysis (extraction and cleaning) were rigorously washed with detergent and tap water. They were then rinsed with distilled water and thoroughly rinsed with analytical grade acetone and dried overnight in an oven at 150ºC. The glass wares were then removed from the oven and allowed to cool down and stored in dust free cabinets.

Deionized water was used throughout the study. For validation of the analytical procedure, repeated analysis of the samples against internationally certified/standard reference material (SRM-1570) of National Institute of Standard and Technology were used. Additionally, the quality of insecticides residues was assured through the analysis of solvent blanks, procedural matrix blanks duplicate samples and proper calibrations of all laboratory materials. All reagents used during the analysis were exposed to same extraction procedures and subsequently run to check for interfering substances. Sample of each series were analysed in duplicates.

## Ethical Consideration

An ethical consideration on this study refers to protecting the rights of cocoa farmers in which the study was conducted as well as maintaining scientific integrity. To ensure ethics in this study, the consent of all the cocoa farmers were sought before taking the cocoa beans samples from their farms. Most importantly, confidentiality and anonymity of these farmers were ensured. Also, the participants were not coerced to participate in this study and could opt out at any time.

**Data Analysis**

Microsoft Excel 2019 was used to generate the mean scores for the 11 systemic insecticide residue concentrations. The mean score was computed for the two farms selected from each Municipal or District Assembly in the Western North region. Insecticides whose concentrations were not detected in the analysed cocoa beans samples were given a mean of ND, which meant Not Detected.

**Results and Discussion**

This study involved the analysis of 11 systemic insecticides, namely; acetamiprid, clothianidin, imidacloprid, thiamethoxam, acephate, allethrin, dimethoate, methamidophos, monocrotophos, pirimiphos-methyl, and sulfoxaflor in cocoa beans from ten selected cocoa farms from five Municipal and District Assemblies (MDAs) within the Western North Region, Ghana.

The insecticide residue concentrations in each sample were calculated in mg/kg sample and compared with the European Union (EU) Maximum Residue Limits (MRLs) of cocoa beans. In Ghana there is no established maximum insecticides (pesticide in general) residue limits for cocoa beans. That is why the levels of systemic insecticides recorded in the study were compared to standard limits set by the European Union. The results for the types and residue levels of systemic insecticides, in the cocoa beans samples are shown in Table 3. The values in Table 3 indicate the mean of residue concentrations, European Union maximum residue limits (MRLs) for cocoa beans all in mg/kg.

**Table 3:** Mean concentrations of cocoa beans samples from the selected MDAs in the Western North Region

| Systemic Insecticide | Mean concentrations of cocoa beans samples from the selected MDAs in the Western North region | | | | | | | | | | EU MRL (mg/Kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | BIA East | | BIA West | | BODI | | JUABOSO | | WIAWSO | | |
| | BEF1 | BEF2 | BWF1 | BWF2 | BF1 | BF2 | JF1 | JF2 | WF1 | WF2 | |
| Acetamiprid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |
| Clothianidin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.02 |
| Imidacloprid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |
| Thiamethoxam | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.02 |
| Acephate | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |
| Allethrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 |
| Dimethoate | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |
| Methamidophos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |
| Monocrotophos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |
| Pirimiphos-Methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | **0.01** | 0.05 |
| Sulfoxaflor | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |

The 11 systemic insecticides were analysed in the cocoa beans from the selected farms from the Western North Region, Ghana. Out of the 11, five of the insecticides belonged to the neonicotinoids class, namely; acetamiprid, clothianidin, imidacloprid, thiamethoxam and sulfoxaflor. Currently, the neonicotinoids are the approved insecticides for cocoa in Ghana by the COCOBOD (COCOBOD, 2012; Dankyi, *et al*., 2014; Okoffo, 2015; Fialor, 2017) [12, 28, 17]. Five out of the 11 systemic insecticides analysed belonged to the organophosphorous class, and they were acephate, dimethoate, methamidophos, monocrotophos, and pirimiphos-methyl. Though these organophosphorous are not currently approved by the COCOBOD to control cocoa insects, previous studies still found their use and existence in cocoa beans from Ghana (Frimpong, *et al*., 2012b; Okoffo, 2015; Fialor, 2017) [20, 28, 17]. Allethrin belongs to the synthetic pyrethroid chemical class of insecticides and its use is unapproved in Ghana for the control of cocoa pests (COCOBOD, 2012) [11].

**Levels of systemic insecticide residue concentrations in the cocoa beans**

The levels of these systemic insecticide residue concentrations analysed in the sampled cocoa beans are discussed below:

**Acetamiprid** was not detected in all the 10 samples of cocoa beans from the selected farms in the Western North Region. Acetamiprid is the main or one of the active ingredients in insecticides with trade names such as Buffalo super, Califan super, Viper super and Acetastar. Though these insecticides are readily available in the agrochemical markets in Ghana, they are not endorsed by the quality control division of the Ghana COCOBOD (COCOBOS, 2012) [11]. An earlier study reported that cocoa farmers use them to control cocoa insects (Okoffo, 2016). Since there was no acetamiprid detected in all the 10 samples of whole cocoa beans from the five districts in the Western North Region, it can be argued that it is either the farmers have stopped using it to control insects in their farms or have been following the prescribed procedures for its use, hence having a mean value of not detected in mg/kg of cocoa beans sampled from the region.

**Clothianidin** was not detected in the cocoa beans sampled from the farms in all the five districts in the region. Clothianidin is a neonicotinoid insecticide with similar action as imidacloprid and thiamethoxam sold by Bayer Crop Science under the brand names Poncho, Prosper and Votivo. These brands of insecticides are not common in the agrochemical markets in Ghana and not known much by cocoa famers in the country. Clothianidin is not part of the approved neonicotinoids insecticides for cocoa in the country (Dankyira, *et al*., 2016; Fialor, 2017) [17]. These could account for its non-existence in all the cocoa beans sampled for residue analysis in this current and previous study (Fialor, 2017) [17].

**Imidacloprid:** From Table 3, imidacloprid had a mean score of not detected (ND). It means imidacloprid was not detected in all the 10 samples of cocoa beans from the farms in the five MDAs in the region. In the EU, the approved maximum residue level for imidacloprid in fermented cocoa beans is 0.05 mg/kg. Imidacloprid still remains one of the insecticides approved by COCOBOD for use by cocoa farmers. Imidacloprid is the active ingredient in Confidor, the insecticide provided by the government of Ghana through the COCOBOD for the cocoa mass spraying programme (Dankyi, *et al*., 2015) [28]. Previous studies in Ghana that sampled fermented cocoa beans from the depots was found to contain some levels of imidacloprid residues ranging from not detected to 0.005 mg/kg residue levels (Dankyi, *et al*., 2014; Okoffo, 2016; Fialor, 2017) [12, 28, 17].

**Thiamethoxam** was analysed in all the 10 samples of cocoa selected from the five MDAs. It was not detected in all the samples of cocoa beans taken for this study. Actara (Thiamethoxam) is also one of the insecticides approved by COCOBOD for use by cocoa farmers under the mass spraying exercise. Other insecticides in the market that contains thiamethoxam as the active ingredient are LUFU, Acati Power, D lion Akate global, among others. Though these ones are not endorsed by the Ghana COCOBOD, they are still used by cocoa farmers for the control of aphids and mirids. The EU approved maximum residue limit for thiamethoxam in fermented cocoa beans is 0.02 mg/kg. Although this current study did not detect the presence of thiamethoxam in all the cocoa beans sampled and analysed, some previous studies detected its presence in varying degrees in cocoa beans sampled from the cocoa shells, depots and ports (Dankyi, *et al*., 2014; Okoffo, 2016; Fialor, 2017) [12, 28, 7]. This could be attributed to the fact that sampled cocoa beans from the depots and ports in the previous studies comprised beans from multiple farms and multiple farmers in the various towns and villages.

**Sulfoxaflor**, like the other neonicotinoids was also not detected in all the 10 samples of cocoa beans analysed. Sulfoxaflor, which is one of the latest neonicotinoid compounds (Zhu, *et al*., 2010), has recently come onto the market in China (Shao, Swenson, & Casida, 2013), the USA (US Environmental Protection Agency (USEPA), 2013), and in Ghana sold under the trade name XDE, and has been reviewed by the European Food Safety Authority (EFSA) for approval in the European Union (European Food Safety Authority (EFSA), 2014) [16]. However, its usage by farmer in Ghana is not found in previous researches.

**Acephate** was not detected in all the cocoa samples analysed. From Table 3, it has a mean sample of no detection (ND). acephate belongs to the class of organophosphorous insecticides with systemic action for the control of insects in seeds, food crops and cash crops like cocoa. Acephate is not approved insecticide for cocoa farmers (COCOBOD, 2012) [10] and its use is not known among cocoa farmers in Ghana (EPA, 2009), and this proves that acephate had not been used on cocoa production in Ghana.

**Allethrin** is the first member of the synthetic pyrethroids that was produced. Pyrethroids are environmentally stable and very effective in the control of beetles, aphids, mites, mealy bugs, white flies and caterpillar infestations in food and cash crops. When used in cocoa production, pyrethroids are mostly used against insects such as mirids and the cocoa pod borer (Bateman, 2015) [7]. Allethrin is not part of the approved cocoa insecticides in Ghana. Allethrin was not detected in all the cocoa beans analysed from the first districts in the Western North Region. It suggests that allethrin has not been used by cocoa farms or better still its use has been diminished from the environment.

**Dimethoate** was another class of organophosphorous insecticide with systemic action that was analysed in the 10 samples of cocoa beans from the Western North Region. Dimethoate was not detected in any of the cocoa bean samples collected from the farms. Though dimethoate was not detected in any of the cocoa beans from the farms, it is available for sale in agrochemical shops under the trade names such as Rainlambda Plus, Dimeking, for the control of certain insects including aphids, lucerne flea, red-legged earthmite, leaf hoppers and wingless grasshopper. This suggests that it is either this insecticide is not used by farmers in the Western North Region or its residue level is still not detectable in the cocoa samples collected.

**Methamidophos** residue was not detected in all the samples of cocoa beans collected and analysed. Methamidophos belongs to the class of organophosphorous insecticides with systemic action. Methamidophos is banned for the control of crop insects in some parts of the world (EPA.US.GOV, 2022). This proves that methamidophos had not been used on cocoa production in Ghana, or better still their presence in the environment had diminished.

**Monocrotophos** is an organophosphate insecticide. It is acutely toxic to birds and humans, so it has been banned in the U.S., the E.U., India and many other countries like Ghana for the control of cocoa pest. This may prove why monocrotophos was not detected in all the 10 samples of cocoa beans analysed because it is possibly not used by cocoa farmers recently.

**Pirimiphos-methyl** was detected in only one of the cocoa beans' samples analysed. The residue concentration of Pirimiphos-methyl in the cocoa beans sampled from Boako in the Wiawso Municipal was found to be 0.01 mg/kg. This concentration is within the approved limits of residue concentrations in cocoa beans by the European Union, as the EUs MRL for Pirimiphos-methyl was 0.05 mg/kg. Pirimiphos-methyl is one of the active ingredients in Cocostar (one of the insecticides approved by COCOBOD for use by cocoa farmers). In the late 1990s, imidacloprid (Confidor 200SL) and a cocktail of pirimiphos-methyl and bifenthrin under the trade name Axtellic Talstar were introduced to gradually replace lindane and propoxur as evidence began to emerge that resistance was being built against them by cocoa insects (Okoffo, 2016) [12].

Since then, pirimiphos-methyl has been used by cocoa farmers its presence has been detected in cocoa beans sampled and analysed from different study area in the country. The concentrations of pirimiphos-methyl recorded at all the study sites with detectable residues were below the European Union (EU) MRL of 0.05 mg/kg for cocoa beans (Frimpong *et al*., 2012b; Okoffo, 2016) [19, 12]. The detection of this insecticide in cocoa beans samples in both current and previous studies suggest that pirimiphos-methyl was used by cocoa farmers for the control of cocoa insects, and farmers have been following the recommended dosage during its usage.

**Comparison of the levels of insecticide residue concentrations With European Union's Maximum Residue Limit**

Pirimiphos-methyl scored a mean value of 0.01 for all the 10 samples of cocoa beans analysed from the selected MDAs in the Western North region. This residue concentration of pirimiphos-methyl ranged from not detected to 0.01 mg/kg in the cocoa beans collected from Sefwi Boako in Wiawso municipal. The EUs MRL for pirimiphos methyl is 0.05 mg/kg. this means that the residue concentration of pirimiphos-methyl found in the cocoa beans is within its maximum residue limit (MRL). Therefore, pirimiphos-methyl residue concentrations in the cocoa beans poses no threat to it consumers as its mean concentration were within the approved limits of maximum residue concentration.

These systemic insecticide residues: acetamiprid, clothianidin, imidacloprid, thiamethoxam, acephate, allethrin, dimethoate, methamidophos, monocrotophos, and sulfoxaflor were not detected in all the cocoa beans sampled from Bia East district, Bia West district, Bodi district, Juaboso district, and Wiawso municipal. This means that, it is safe to consume products of cocoa beans from the Western North region as the majority of the insecticide residues tested were not detected in cocoa beans. This implies or suggest that farmers have been applying the recommended dosage of insecticides in the control of pest in their farmers. Also, this will help to avoid possible rejection of cocoa beans from region by the international.

## Conclusions

From the major findings of this study, the following conclusions were drawn:

A minimal representation of the systemic insecticide residues was detected by the partially modified QuEChERS Method MRM by LC-MS/MS in the sampled cocoa beans from the Western North region. This suggests that majority of cocoa farmers from the Western North Region follow the recommended dosage and procedures in insecticides application in the control of mirids, aphids and other insects that attack cocoa plant or the beans.

Also, cocoa beans from the Western North Region can be described as quality, safe to consume and poses no health risk to its consumers as far as insecticide residues are concerned. This implies that the farmers in the region have worked within the recommended procedures and dosage with regards to the use of insecticides on their farms and could be commended. Also, considering levels of insecticides residues in fermented dried cocoa beans against the European (EU) commission regulations on insecticide residues, cocoa beans analysed in this study area will not pose any significant threat to the cocoa industry as far as shipment to Europe is concerned.

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