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Residues of Diazinon and Endosulfan in Cocoa Beans Collected from Three Cocoa Ecological Zones in Nigeria

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Abstract: Cocoa as a cash crop has contributed immensely to the nation's economic growth. It is exported mainly to Europe and America where bulk of the cocoa beans is used for the production of chocolate. Recently, the European Union set up maximum residue limits for various pesticides used on cocoa during pests control. This study was carried out to assess the concentrations of diazinon and endosulfan in cocoa beans obtained from three cocoa ecological zones in Nigeria. GC-MS was used for the pesticide residue analysis. Results showed that, 70%, 10% and 10% of cocoa obtained from Ondo, Cross River and Ogun States respectively had endosulfan (organochlorine) residue. Forty percent (40%) of cocoa beans obtained from Ondo State had diazinon (organophosphate) residue while there was no detectable diazinon in cocoa beans from Cross River and Ogun States. The outcome of this study suggests an urgent need for increased awareness on proper use of pesticides among cocoa farmers.

Key words: Diazinon · Endosulfan · Cocoa · Nigeria · Pesticide residue

INTRODUCTION

The nuisance of pests and disease is a strong factor responsible for the dwindling production of cocoa in Nigeria. Among the various insect pests of *Theobroma cacao*, the brown Mirid, *Sahlbergilla singularis* Haglund is the most harmful insect pest of cocoa tree in Nigeria [1]. Mirid feeds by inserting its mouth parts into the plant and sucking the juices and at the same time, salivary secretions are injected into the tissue which results in plasmolysis of the cells. The cellular lysis results in necrosis, followed by the appearance of lesions [2]. Canker sores develop from lesions due to invasion by cryptogamous parasites causing weakness. The combination of tissue necrosis and cryptogamic attack results in wilting of the plant leading to very low productivity. Yield loss of about 30 - 70% has been attributed to Mirid infestation and damage [3,4]. In order to combat the destructive activities of Mirid, Nigerian cocoa farmers use various brands of insecticides including organochlorine group. Orgnochlorine pesticides have been used extensively worldwide since the early 1950s [5,6] until restriction were introduced in several developed and developing countries due to their persistence in the environment and growing evidence of adverse associated health implications. Many organochlorine pesticides pose substantial short and long-term health risks [7]. They are known to disturb the biological and physiological functions of erythrocytes and lymphocytes [8]. The adverse health effects include a series of chronic end-points including cancer [9,10], neurotoxic [11], immunotoxic [12], developmental [13], endocrine [14], reproductive [15] and neuro- behavioral effects [16]. In recent time, many developed nations discovered an increase in the case of cancer among their citizens. This made the European Union to become conscious of the quality of food products meant for consumption among her citizens. Parts of the measures to monitor food safety and quality was the setting of maximum residue limits (MRL) of pesticides in all agricultural products entering Europe from different parts of the world. This regulation limits the amount of

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pesticides that must be left on agricultural produce after phytosanitation activities. Reports have shown that, some farmers may be using banned pesticides ignorantly because some banned insecticides are repackaged with new names and sold to innocent and unlettered farmers. It has also been reported that, some farmers use pesticides indiscriminately without following the manufacturers' instruction [17]. All these actions ultimately affect the quality of cocoa if the chemicals gain access into the beans through plant roots or directly from the pods through phloem tissue. In order to comply with the maximum residue limit regulation and produce high quality cocoa beans for exportation, it became expedient to assess the level of organophosphate and organochlorine insecticide residue in cocoa beans collected from three cocoa ecological zones in Nigeria in order to know the safety status of Nigerian cocoa beans.

MATERIALS AND METHODS

Selection of the cocoa farms used in this study was based on the density of cocoa production. In Ondo State, cocoa samples were collected from Idanre, Bamikemo, Afun and Owena while in Ogun State, cocoa samples were collected from Sora-Bale, Bodo, Sotiya and Ogunmakin. Ripe cocoa pods were collected from each cocoa farm in a randomized complete block design manner. The pods were transported to the fermentation section of Cocoa Research Institute of Nigeria, Ibadan, Nigeria where the pods were broken with wooden sticks and later fermented for six days. The fermented beans were sun - dried for seven days and later transferred into the oven to remove any absorbed moisture. The oven - drying was done at the temperature of 60°C for six hours until a constant weight was attained after which the samples were taken to IDAEA-CSIC Environmental Chemistry Laboratory, Barcelona, Spain

Chemicals and Reagents: standards of α and β-endosulfan, endosulfan sulfate and diazinon were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Solvents (hexane and dichloromethane SupraSolv) were from Merck (Darmstadt, Germany). Florisil Solid Phase Extraction cartridges (5 g, 25 ml) were purchased from Waters (USA). Nitrogen for drying (99.995% purity) was from Air Liquid (Barcelona, Spain).

Sample Preparation: Prior to extraction, samples were grounded with a mortar and a pestle to fine powder.

One g of sample was placed in centrifuge glass tubes with 30 ml hexane/dichloromethane (1:1) and samples were vortexed and sonicated for 10 minutes in a 360 W Selecta ultrasonic bath (J.P. Selecta, Barcelona, Spain). This procedure was repeated three times and sample was vortexed in between extractions. Samples were centriguged at 3000 rpm for 10 min at 10°C. The supernatant was collected in a clean vial and reduced to less than 1 ml under a gentle stream of nitrogen (TurboVap) before clean-up.

Given the high amount of coextracted compounds, noticed by the viscosity and colour of the extract, the clean-up strategy was developed. Acidification was obviated because it produces the degradation of endosulfans. Then, SPE with florisil cartridge (5 g, 25 ml) was used, since florisil has a great capacity to eliminate the lipids that are present in the sample (in coccoa beans, the % fat is of 50%). Different Florisil cartrigdes, all of 5 g and 25 mL, were used: Waters (Waters, USA); Isolute, ENV (IST, England), Supelco (USA). Best performance was obtained with Florisil SPE cartridges from Waters and further this cartridge was used. Cartridges were conditioned with 30 ml hexane-dichloromethane (1:1) and pesticide elution was done with 30 ml hexane-dichloromethane (1:1). The resulting extract was collected in a clean vial and concentrated under a gentle stream of nitrogen (Turbovap) to circa 0.5 ml. The extract was transferred to a chromatographic vial and evaporated to almost dryness in a Reacti-Therm III from Pierce (Rockford, IL, USA) under a gentle nitrogen stream and finally reconstituted with 500 µl of hexane.

Gas Chromatographic Determination: Gas Chromatography coupled to Mass Spectrometry using electron ionization (GC-EI-MS) was performed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 Network mass spectrometer (Santa Clara, CA, USA). An Agilent HP-5MS (30 m x 0.25 mm i.d. 0.25 µm film thickness) containing 5% phenyl methyl siloxane capillary column was used with helium as the carrier gas at 15 psi at initial flow of 1.1 ml/min. The temperature program was from 65°C (held for 1 min) to 160°C at 12°C/min, to 310°C at 8°C/min and to 325° at 10°C/min (held for 5 min). The total run time was of 34 min. Two µl of sample were injected in splitless mode at 16.41 psi, purge flow of 50 ml/min and purge time of 1 min. Injector, transfer line and ion source temperatures were 280, 250 and 230°C, respectively. Quadrupole temperature was of 150°C.

Table 1: Data of performance of method

Pesticides	T_R(min)	Ions	a	b	Repititivity	R^2	%Recovery
Diazinon	9.37	179-137-304	959803	-39340	10	0.991	70
α-endosul	12.02	237-195-241	642845	-26288	10	0.998	74
β-endosul	13.25	195-241-237	3.0E+06	-66135	3	0.995	92
$EndosSO_4$	14.16	272-274-229	642845	-26288	10	0.996	74

Key: α-endosul - α-endosulfan; β-endosul- β-endosulfan; EndosSO4- Endosulfan sulphate

Acquisition was performed in time scheduled Selected Ion Monitoring (SIM) using 3 ions per compound, as specified in Table 1. External standard quantification was done, although endosulfan d4 and anthracene were used as recovery and internal standards, respectively.

RESULTS

Figure 1 shows the result of diazinon residue in cocoa beans obtained from selected cocoa plantations in Ondo State. Its residue ranged from non- detected to 0.17mg/kg with an average value of 0.12mg/kg. Cocoa samples from Owena 2 had the minimum diazinon residue among the samples with detectable diazinon while samples from Idanre 3 had the maximum diazinon residue. However, cocoa beans from Idanre 2, Owena 1, Bankemo 3 and Owena 3 had no detectable diazinon residue in them.

None of the cocoa beans obtained from Cross River State had detectable diazinon residue in them. Result also showed that, none of the cocoa beans collected from the selected cocoa farms in Ogun State had detectable diazinon residue in them.

Alpha endosulfan residue in the samples ranged from non detectable to 0.74mg/kg with an average value of 0.55mg/kg. Cocoa beans from Idanre 3 had the minimum alpha endosulfan residue among samples with detectable residues while cocoa beans from Idanre 1 had the maximum residue. Samples from Idanre 2, Owena 1 and Owena 3 had no detectable alpha endosulfan. Beta endosulfan residue in cocoa beans ranged from non detectable to 0.72mg/kg with a mean value of 0.08mg/kg. Cocoa beans from Idanre 1had the least beta endosulfan residue among samples with detectable endosulfan while samples from Owena 2 had the highest beta endosulfan residue. Cocoa beans obtained from cocoa farms in Idanre 1, Bankemo 3 and Owena 3 had no detectable beta endosulfan. Total endosulfan was calculated by the addition of alpha and beta endosulfans (Figure). Total endosulfan ranged from 0.94 to 1.22 mg/kg with an average value of 1.06 mg/kg. Samples from Bankemo 2 had the minimum while samples from Bankemo 1 had the maximum total endosulfan in the studied samples.

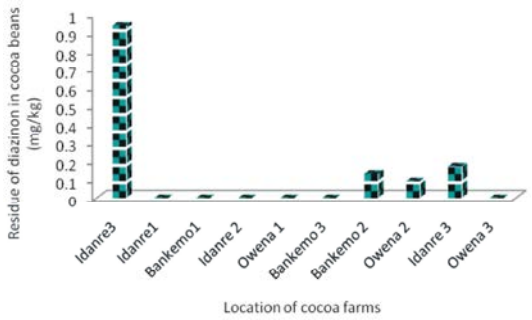

Fig 1: Residue of diazinon in cocoa beans from Ondo State

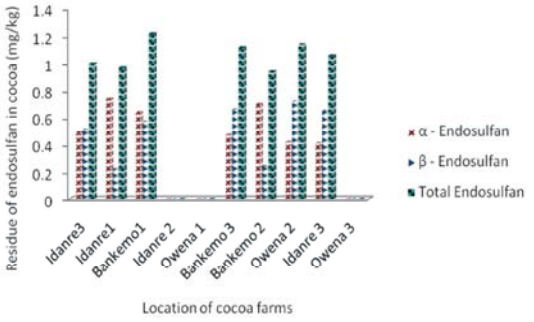

Fig. 2: Residues of endosulfan and its metabolites in cocoa from Ondo State.

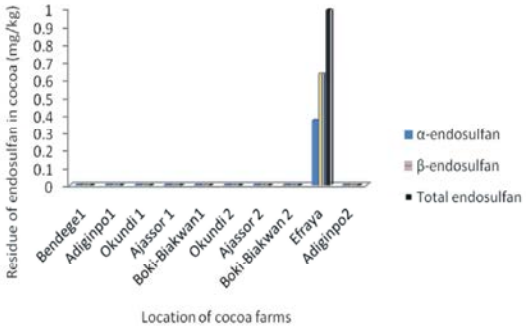

Fig. 3: Residues of endosulfan and its metabolites in cocoa beans from Cross River State

Result (Fig. 3) showed that, only cocoa beans obtained from Efraya had alpha and beta endosulfan residue while the remaining cocoa beans from Cross River State had no detectable endosulfan. Cocoa from Efraya had 0.37 mg/kg alpha endosulfan and 0.63mg/kg beta endosulfan. The total endulfan was however, 0.99 mg/kg.

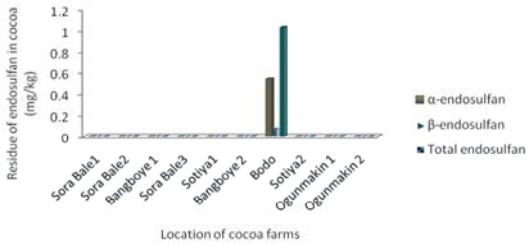

Fig. 4: Residues of endosulfan and its metabolites in cocoa beans from Ogun State

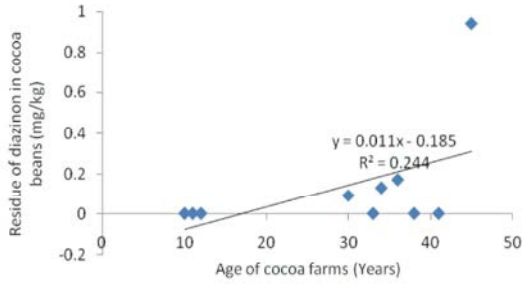

Fig. 5: Relationship between diazinon residue in cocoa and age of selected plantations in Ondo State

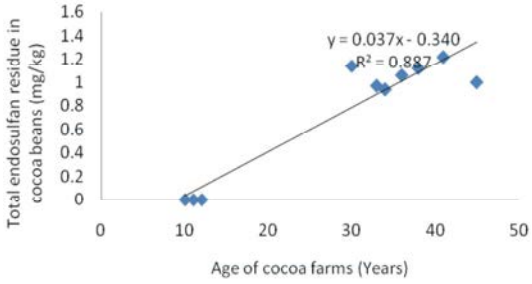

Fig. 6: Relationship between endosulfan residue in cocoa and age of selected plantations in Ondo State.

Result of endosulfan residue in cocoa bean obtained from selected cocoa plantations in Ogun State is presented in Fig. 4. Among the entire cocoa samples analyzed, only samples from Bodo had detectable endosulfan residue while the remaining samples did not have endosulfan residue at detectable level. Samples from Bodo had α -endosulfan residue of 0.54 mg/kg, 0.48mg/kg β- endosulfan and a total endosulfan of 1.027 mg/kg.

The relationship between diazinon concentration in cocoa beans obtained from Ondo State and age of cocoa plantation is presented in Fig.5. The linear regression showed a value of 0.24.

The relationship between total endosulfan residue in cocoa beans obtained from Ondo State and the age of cocoa plantations where the samples were collected is

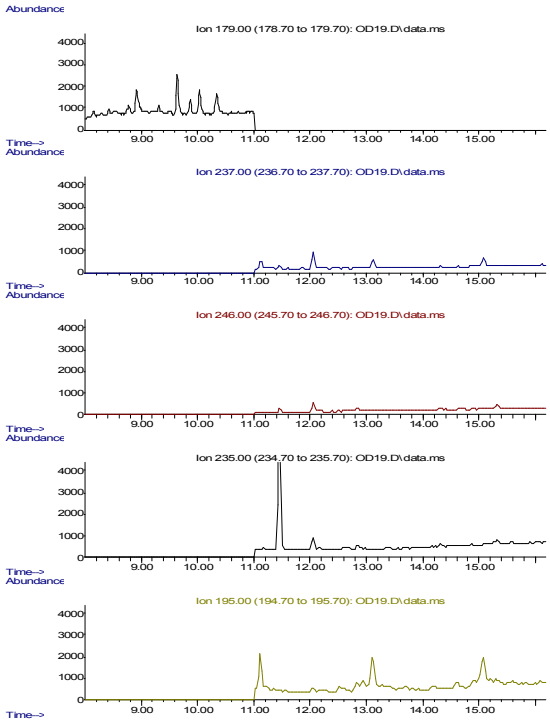

Fig. 7: Representative GC-MS chromatogram of insecticides residue in cocoa beans

presented in Figure 6. The linear regression R^2 was 0.887 which suggests a good fit indicating that, endosulfan application was a regular practice among cocoa farmers in Ondo State. Hence, accumulation of endosulfan in cocoa beans increased with age of cocoa.

DISCUSSION

Diazinon is an organophosphate insecticide which transform to a molecule called diozoxon in living things. Diazinon and the more potent diazoxon [18] kill insects by interfering with the nervous system functions, as do all members of the organophosphate chemical family. Normally, impulses are transmitted chemically from the end of one nerve cell to the beginning of another; one of the chemical transmitters used in animal nervous system is called acetylcholine. After transmitting the nerve impulse, acetylcholine is destroyed by an enzyme called acetylcholinesterase (AchE) in order to clear the way for another transmission. Diazinon attached to AchE and prevent it from destroying acetylcholine causing overstimulation of the nerves. Mammals and insects nervous systems are similar enough that effects of

organphosphates are similar. It is worth noting that not all of diazinon's toxicological effect emerge from its inhibition of AchE. Diazinon and other organophosphates inhibit numerous enzymes with molecular structures that are similar to AchE. Some other problems associated with diazinon toxicology is the disruption of normal functioning of human hormones systems of particular concern is interference with the activity of estrogen often called the female sex hormones. Estrogen has recently been shown to affect the development and growth of cells in the lining of the colon. The result of abnormal growth of these cells is colon cancer. In tests with cultures of cells from a human colon. Low concentration of diazinon had growth promoting effects, suggesting diazinon has interfered with the normal activity of estrogen [19]. Davis [20] reported effects of diazinon on brain cancer in some children in Missouri. A study conducted by Italian researchers used concentration of diazinon equivalent to those found in an Italian food monitoring study. They found that this low level of exposure increased the occurrence in human blood cell cultures of a type of genetic damage called micronuclei. The findings of Matsuoko *et al.* [21] showed that diazinon exposure increased the frequency of abdominal chromosomes in hamster lung cell cultures. Result showed that, of all the studied cocoa samples obtained from selected cocoa farms in Ondo State, 40% had detectable diazinon residue in them while 60% had no detectable diazinon. However, all the cocoa samples with detectable diazinon had diazinon concentrations far higher than 0.02 mg/kg set as maximum residue for diazinon in cocoa beans. The average value of 1.12 mg/kg in the studied cocoa samples is fifty- six times the maximum residue limit. Two studies from Simeon Fraser University found that, medium - term exposure to diazinon caused reduced weight gain, liver injury and reduced level of four chemicals (other than acetyl choline) that are used to transmit nervous system impulse in the brain [22]. Diazinon can gain entrance to human body through inhalation, dermal contact and oral. It has the tendency to accumulate in human system. This was confirmed by a study carried out by [23] among cocoa farmers in South Western Nigeria to determine pesticide residue in human blood serum. The findings showed that, a mean value of 0.089 mg/kg diazinon was detected in blood serum of cocoa farmers who have been exposed to diazinon between 5 and 9years, 0.051 mg/kg in blood serum of farmers who have been exposed to diazinon for a period of 10-14 years and 0.073mg/kg in serum of farmers who have been exposed to diazinon for more than 20 years. Linear regression R^2 was used to determine the relationship between diazinon residue in cocoa beans and the age of plantations where the beans were collected. Results showed that, age of cocoa plantation as a factor, contributed only 24% of the total diazinon in the studied cocoa samples. This suggests that, the use of diazinon as an insecticide in the control of Mirid (*Salhbergella singularis*) by cocoa farmers in Ondo State was not a regular practice and did not follow any regular pattern. This might be the reason why it was only detected in 40% of the total number of samples examined. The entire cocoa beans obtained from selected cocoa plantations in Ondo State with detectable endosulfan (70% of total samples) exceeded the maximum residue limit (0.10 mg kg^{-1}) set by the European Union. The only cocoa sample with detectable α endosulfan residue among the samples from Cross River State contained residue above the maximum residue limit for endosulfan. The only sample with detectable endosulfan among samples from Ogun State also contained endosulfan residue above the maximum residue limit. The range of 0.41 to 0.74 mg kg^{-1} α- endosulfan obtained in samples from Ondo State is higher than 0.002 to 0.042 mg kg^{-1} reported by Scholten *et al.* [24] in their survey of pesticides in 139 cocoa samples collected from fifteen cocoa producing countries. The mean value of β- endosulfan residue (0.51mg kg^{-1}) obtained in cocoa beans from Ondo State, 0.63mg kg^{-1} obtained in samples from Cross River and 0.48 mg kg^{-1} in Ogun State exceeded the 0.10 mg kg^{-1} set by the European Union as maximum residue limit (MRL) for endosulfan. There appeared to be no defined pattern in the ratio of α and β endosulfan residue in the analyzed cocoa beans. However, the range (0.22 to 0.72mg kg^{-1}) of β-endosulfan obtained in samples from the studied cocoa beans was higher than 0.003 to 0.026 mg kg^{-1} reported by Scholten *et al.* [24] in 139 cocoa beans obtained from fifteen cocoa producing countries. Total endosulfan was calculated by the addition of both α and β endosulfan since no endosulfan sulphate was detected in any of the cocoa beans analyzed. The mean total endosulfan residue in cocoa beans obtained from selected cocoa plantations in Ondo State was 1.06mg kg^{-1}. The mean value was almost eleven times the value of maximum residue limit (0.10 mg kg^{-1}) for endosulfan in cocoa beans. The study showed that, 70% of the total cocoa beans assessed for endosulfan residue exceeded the MRL. The total endosulfan obtained in sample from Cross River was 0.99 mg kg^{-1} while that of Ogun State was 1.03mg kg^{-1} the concentration of endosulfan residue in cocoa beans from Ondo State compared with the other two States suggests that, the use of endosulfan products must have been

higher among cocoa farmers in Ondo compared to the farmers in Cross River and Ogun States. This might be connected to cocoa production activities within Ondo State compared to other parts of the country. Cocoa cultivation as a business, started in the South western part of Nigeria and Ondo State happened to be the main centre of cocoa cultivation in South-Western part of Nigeria. According to the report of the National Cocoa Survey carried out in 2006, Ondo State was the highest cocoa producing State in Nigeria. The high cultivation activity of cocoa in Ondo State might have called the attention of pesticide marketers which might have led to influx of various cocoa pesticides into the State. Persistence, frequent and indiscriminate application of pesticide might have led to the level of endosulfan in 70% of the analyzed cocoa beans obtained from Ondo State. The low occurrence (10%) of samples with endosulfan residue among cocoa beans obtained from Cross River and Ogun States suggests that, the use of endosulfan by cocoa farmers in both States was uncommon. Several scientists have detected endosulfan residues in food, vegetables, milk and meat. Darko and Acquaah [25] detected endosulfan in 18 out of 20 samples of beef fat. The mean endosulfan in beef fat in Kumasi was 21.33 mg kg^{-1}. Afful *et al*. [26] reported a range of 0.6 - 71.3 mg kg^{-1} endosulfan in fish samples from the Densu Basin in Ghana. Amoah *et al.* [27] reported that 36% of total lettuce analyzed in Ghana contained endosulfan at a range of 0.04 to 1.3mg kg^{-1} with an average value of 0.4 mg $kg^{-1.}$ This value according to them, exceeded 0.05mg kg^{-1} MRL set for endosulfan in lettuce. Residues were detected in samples from air, water surface and ground water [28] in Benin, Malawi, Nigeria and South Africa. High concentrations of alpha and beta endosulfan isomers as well as endosulfan sulphate have been detected in tree back samples throughout the world, particularly in India and Pacific Rim [29]. The concentration of endosulfan in 70% of Cocoa beans from Ondo State is of great concern. There have been reports of several toxicological effects which the continuous consumption of food contaminated with endosulfan could cause on human health. There are experimental evidence of adverse effects of endosulfan on the male reproductive system, delay sexual maturity and interfering with the sex-hormone synthesis [30]. Endosulfan is a proven endocrine disruptor [31]. It has the potential to induce hypothyroidism [32]. According to Anderson *et al.* [33] endosulfan exhibits estrogenic properties comparable to that of DDT. It competes for estradiol for binding to estrogen receptors, thereby inhibiting hormonal function [34]. Though endosulfan has been banned from being used on cocoa, personal communication with some farmers at the time of sample collection showed that, some farmers who are ignorant of the ban may still have used endosulfan on their plantations many years after its ban. Due to the fact that, endosulfan is an organocholrine insecticide which is persistent in the soil environment coupled with its ability to bio-accumulate in plant tissue, its concentration could still be of significance in cocoa beans years after farmers stop its application in cocoa plantations. Despite the fact that, most of the analyzed cocoa beans from Ondo State exceeded the maximum residue limit set for endosulfan and diazinon, the beans could still be consumed without fear since most chocolate companies get cocoa beans from different sources and the beans are always blended together. In a situation where quantity of cocoa beans with little or no pesticide residue outweighed beans with moderately high residue, the tendency of distribution of the pesticide within the whole mass of raw material which may end up reducing the risk of chronic toxicological effects of pesticide residue in the consumers of cocoa products.

CONCLUSION

The study has shown that, cocoa beans from Cross River and Ogun States are safer than cocoa beans in terms of diazinon and endosulfan residues than cocoa obtained from Ondo State. It showed that, cocoa farmers in Cross River and Ogun States do not use most of the banned pesticides. However, there is need for an intense awareness among cocoa farmers on the health risks associated with the consumption of cocoa products with high concentrations of pesticides that are already banned on cocoa.

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