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Organochlorine pesticide residues in dried cocoa beans obtained from cocoa stores at Ondo and Ile-Ife, Southwestern Nigeria

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ABSTRACT

Levels of organochlorine pesticides (OCPs) were determined in dried cocoa beans obtained from cocoa produce stores at Ondo and Ile-Ife, Southwestern Nigeria. Cocoa beans samples were sun dried to a constant weight, pulverized and soxhlet extracted with dichloromethane to obtain the OCPs. Qualitative identification and quantitative evaluation of the extracted OCPs after clean-up on silica gel were accomplished with the aid of a Gas Chromatography coupled with an Electron Capture Detector (GC-ECD). Levels of OCPs in cocoa beans from Ondo had a mean range of ND (p, p'-DDE) to 82.17 ± 54.53 ng/g (p, p'-DDT) were higher than the OCPs levels in cocoa beans from Ile-Ife with a mean range of 0.37 ± 0.63 ng/g (Endrin) to 57.76 ± 81.48 ng/g (p, p'-DDT). The higher levels of OCPs detected in the cocoa beans from Ondo could be an indication of higher volume of OCPs application by cocoa farmers in Ondo and its environs since cocoa plantations were more concentrated than Ile-Ife environs. Levels of OCPs determined in the cocoa beans were within the Maximum Residue Limit (MRLs) for OCPs set by the World Health Organization/Food and Agricultural Organization. The study established the presence of OCPs in an important crop of Nigeria. Hence, there is the need to keep monitoring ecotoxicological chemical substances in agricultural food products of Nigeria so as to take steps that ensure health safety of end users.

1. Introduction

Organochlorine pesticides which have long been widely used in agriculture and public health as highly effective pest control agents [1,2] have also been found to constitute health hazards to humans, organisms and the environment [3–5]. For example, they are known to cause prostate cancer [6,7], liver cancer [8], diabetes [9], reproductive and developmental defects [10–12] and act as endocrine disruption [13] with acute immunotoxicity [14] and neurotoxicity [15]. This has led to the prescription of tolerance levels such as no-observable-adverse-effect-level (NOAEL) and maximum residue level (MRL) for various pesticides in food and water by the Codex Alimentarius Commission [16].

In Nigeria, before the advent of the oil boom, cocoa production had contributed tremendously to the infrastructural development of the country [17]. The oil boom of the eighties made the government to neglect this agricultural sector which had before then been the country's major foreign exchange earner. This automatically implied a downward trend in cocoa production, thus, causing a substantial fall in the quantity available for export. However, there have been renewed efforts on the part of government and private individuals in the recent times to resuscitate the cocoa industry in Nigeria to its now fledgling status, where it has regained its major economic base for Nigeria [18]. Cocoa is the leading non-oil foreign exchange earner in Nigeria [19]. Nigeria has the capacity to produce over 300,000 t of cocoa, but only produces about 248,000 metric tonnes annually partly because of the scrapping of Cocoa Board in Nigeria. Generally, cocoa contributes over 26% of the Gross Domestic Product of the non-oil export in Nigeria, and 19% contribution to the world market [20]. Nigeria is the world's fourth largest producer of cocoa after Ivory Coast, Indonesia and Ghana, and third largest exporter after Ivory Coast and Ghana. As it were, Ondo state in Southwestern Nigeria is the highest producer of cocoa in Nigeria. In 2007, it produced about 40% of the total cocoa production in the country [21,22].

In order to limit losses from insect pests and diseases, Nigerian cocoa farmers employ the use of a wide range of pesticides, such as copper sulphate, benzene hexachloride (BHC), Aldrex 40 [17]. Others include diazinon, chlorpyrifos, fenitrothion and cuprous oxide. Farmers in Nigeria use various kinds of pesticides such as Gammalin 20 (Lindane), DDT, DD-force, Weed-Off, Termicot, Atrazin,

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Glyphosphate, Metaclors-plus, Alaclhlor 2–4 Amcine and aldrin to formulate local insectides that are applied on farm products [23]. Earlier workers maintained that OCPs are still used in agriculture clandestinely under unknown trade names in developing countries such as Nigeria [24,25].

Organochlorine pesticides continue to experience widespread use by farmers in Nigeria due to the high efficacy and lower cost of the OCPs despite the ban that has been placed on the continuous use of this class of chemicals [26]. The presence of OCPs has been reported in top soils of some agricultural farmlands and settlements in Nigeria [27–29]; in cocoa beans [30]; and cowpea grains and dried yam chips [31,32].

The aim of this study was to carry out a comparative investigation of the quality of dried cocoa beans with respect to occurrence and levels of OCP residues in samples obtained from two prominent cocoa-cultivating areas of Nigeria, namely: Osun and Ondo States. This comparative study is important in evaluating the current status of OCPs usage in the Nigerian environment.

2. Materials and methods

2.1. Reagents used and their sources

The reagents used in this study are dichloromethane (GFS Chemicals, Columbus), n–Hexane (Ultrafine Limited, Marlborough House, London), acetone (GFS Chemicals, Columbus), silica gel 60–200 mesh (Labtech Chemicals), and anhydrous sodium sulphate (Merck, Germany). These reagents were procured through various sales representatives of the producing companies resident in Nigeria. Solvents, such as dichloromethane, n-Hexane and acetone, were doubly distilled to obtain higher purity, while silica gel and anhydrous sodium sulphate were heated in an oven at 120 °C for 12 h to ensure that no adsorbed water element remained as part of the clean-up materials.

2.2. Study area and sample collection

Prior to the sample collection, a preliminary survey of cocoa farmers in the study area was carried out to gather information on types of pesticides commonly used them for pests and diseases in the cocoa plantations. Cocoa beans were sampled from thirteen randomly selected cocoa produce stores (O–1 to O–13) in Ondo and twelve other produce stores (I–1 to I–12) in Ile-Ife. Thus, a total of 25 samples were collected for analysis. Ondo town (in Ondo West Local Government) is known as a major player in the plantation and export of cocoa beans with several plantations at its surburbs, such as Ile-Oluji, Oke-Igbo, Epe, Laje, Litaye, Bagbe, Igunsin, Ayetoro, Igbindo, Lamu, Ajue, Ilekere, and so on. Dried cocoa beans from these suburbs are transported to cocoa produce stores in Ondo with international affiliations for further processing and subsequent export.

Similarly, Ile-Ife in Osun state is an agrarian community with substantial focus on cocoa plantation as one of the major cash crops. The most common category of soils in Ife area is the *Itagunmodi series*, which is well known for its significance in cocoa culture; soils belonging to this series are some of the best cocoa soils in western Nigeria [33]. Several cocoa-producing communities surrounding Ile-Ife, such as Ifetedo, Modakeke, Olode, Owode, Akintola, Mefoworade, Amula and Omifunfun, among others, bring their dried cocoa beans to various cocoa produce stores in Ile-Ife for further processing and onward transmission to local and foreign processing industries.

Samples of cocoa beans collected from various produce stores at Ondo and Ile-Ife were further sun-dried to reduce the moisture contents until constant weights were obtained. The dried cocoa bean samples were manually decorticated and the resultant nibs were ground to powdery form with the aid of Agate pestle and mortar.

2.3. Extraction of organochlorine pesticide residues

A 20 g portion of the ground cocoa bean sample was weighed into a Whatman extraction thimble pre-extracted with n-hexane and dichloromethane (DCM) to remove extraneous organic contaminants that might be adhering to the surface or pores of the thimble. Using the method described elsewhere [34], the sample was soxhlet extracted for an average period of 5 h using DCM as the extracting solvent. The resulting extract was concentrated by distilling off the solvent using a rotary evaporator at about 41 °C to about 3 mL. The concentrated extract was cooled down to room temperature and then concentrated further to about 2 mL under a stream of nitrogen gas of high percentage purity of 99.99%, in preparation for the clean – up procedure.

2.4. Clean-up procedure

A column of about 15 cm (length) × 1 cm (internal diameter) was packed first with glass wool and then with about 7.5 g activated silica gel prepared in a slurry form in DCM. About 5 g of anhydrous sodium sulfate was placed at the top of the column to absorb any water in the sample or the solvent. Pre-elution was done with 15 mL of DCM, without exposing the sodium sulfate layer to air, so as to prevent the drying up of the silica gel adsorbent. The reduced extract was run through the column and allowed to sink below the sodium sulfate layer. Elution was done with 3 × 10 mL portions of the extracting solvent (DCM). The eluate was collected, dried with anhydrous sodium sulfate and evaporated to dryness under a stream of analytical grade nitrogen (99.99%).

2.5. Qualitative identification and quantitative evaluation of the OCPs

The dried eluate was reconstituted with 1 mL 2, 2, 4–trimethylpentane (isooctane). With the aid of a microsyringe, 1 μL of the 1.0 mL purified extract was injected into the injection port of a gas chromatograph coupled with a ^{63}Ni electron capture detector (GC-ECD, Hewlett Packard 7890A series II). The column consisted of a DB-17 fused silica capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness). The temperatures of the injector and detector were 250 °C and 300 °C (held for 5 min), respectively. Oven temperature programme started from 60 °C (1 min) and continued at 20 °C/min to 150 °C and at 5 °C/minute to 280 °C held for 4 min; injected sample volume was 1 μL. The injection was carried out on a splitless injector at 200 °C and the purge activation time was 30 s. The carrier gas was N_2 at 30 mL/min; and the splitless flow rate was 19.6 mL/min. The run time was 23 min. The individual OCPs were identified by comparing the elution time of standard OCPs with those in the samples, while each OCP was quantified by comparing the peak areas of the OCPs in samples with those in standard. Gas chromatographic analysis of the samples was carried out at the Nigerian Institute of Oceanography and Marine Research (NIOMR) Central Laboratory, Victoria Island, Lagos, Nigeria.

2.6. Recovery experiment

Since no certified pesticide reference materials were available during the course of this study, recovery analysis was performed in order to evaluate the precision and efficiency of the analytical procedures using standard addition method. A 20 g sample of pulverized cocoa beans was divided into two. One part was spiked with 10 ppm standard mixture consisting of some of the